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EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments

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ABSTRACT

This draft report details EPA's technical response to the key comments and recommendations included in the 2006 NAS report, "Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment," focusing on the NAS comments regarding TCDD dose-response assessment. After systematically evaluating the epidemiologic studies and rodent bioassays on TCDD, this draft report utilized a TCDD physiologically-based pharmacokinetic model to simulate TCDD blood concentrations, the dose metric used in the dose-response analyses. The draft report develops an oral reference dose (RfD) of 7×10^{-10} mg/kg-day based on two epidemiologic studies that associated TCDD exposures with decreased sperm concentration and sperm motility in men who were exposed during childhood (Mocarelli et al., 2008, 199595) and increased thyroid-stimulating hormone levels in newborn infants (Baccarelli et al., 2008, 197059). EPA also classifies TCDD as carcinogenic to humans, based on numerous lines of evidence, including primarily: multiple occupationally- and accidentally-exposed epidemiologic cohorts showing an association between TCDD exposure and certain cancers or increased mortality from all cancers and extensive evidence of carcinogenicity at multiple tumor sites in both sexes of multiple species of experimental animals. Based on a cancer mortality analysis of an occupational cohort (Cheng et al., 2006, 523122), EPA also develops an oral cancer slope factor of 1×10^{6} per (mg/kg-day) when the target risk range is 10^{-5} to 10^{-7}. While this draft report provides limited sensitivity analyses of several steps in the cancer and noncancer dose-response assessment, it concludes that a comprehensive uncertainty analysis is infeasible at this time.

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LIST OF ABBREVIATIONS AND ACRONYMS

2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,4-D	2,4-dichlorophenoxyacetic acid
AA	ascorbic acid
ACOH	acetanilide-4-hydroxylase
AHH	aryl hydrocarbon hydroxylase
AhR	aryl hydrocarbon receptor
AhR-/-	AhR-deficient
AIC	Akaike Information Criterion
ANL	Argonne National Laboratory
ANOVA	analysis of variance
APE	airborne particulate extract
ASAT	aspartate aminotransferase
AUC	area under the curve
bHLH-PAS	basic helix-loop-helix, Per-Arnt-Sim
B_{max}	equilibrium maximum binding capacity
BMD	benchmark dose
BMDL	benchmark dose lower confidence bound
BMDS	Benchmark dose software
BMI	body mass index
BMR	benchmark response
BPS	balanopreputial separation
BROD	benzyloxy resoufin-O-deethylase
b-TSH	blood thyroid-stimulating hormone
BW	body weight
C	cerebellum
CADM	concentration- and age-dependent elimination model
Cc	cerebral cortex
CI	confidence interval
CSAF	chemical-specific adjustment factor
CSLC	cumulative serum lipid concentration
Cx	connexin
CYP	cytochrome P450
D_a:HED	ratio of administered dose to HED
DEN	diethylnitrosamine
df	degrees of freedom
DLC	dioxin-like compound
DRE/XRE	dioxin/xenobiotic response elements
DRL	differential reinforcement of low rate
DSA	delayed spatial alteration
E_2	17β-estradiol
ED_x	effective dose eliciting x percent response
EGFR	epidermal growth factor receptor

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

EPA	Environmental Protection Agency
ER	estrogen receptor
EROD	7-ethoxyresorufin-O-deethylase
ERα	estrogen receptor alpha
EU	European Union
FFA	free fatty acid
FR	fixed-ratio
FSH	follicle stimulating hormone
FT4	free thyroxine
GD	gestation day
GSH	glutathione stimulating hormone
GSH-Px	glutathione stimulating hormone peroxidase
GST	glutathione-*S*-transferase
H	hippocampus
HCH	hexachlorocyclohexane
HED	human equivalent dose
HQ	hazard quotient
HR	hazard ratio
Hsp90	heat shock protein 90
IARC	International Agency for Research on Cancer
IGF	insulin-like growth factor
IL	interleukin
ILSI	International Life Sciences Institute
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
KABS	oral absorption parameters
LASC	lipid-adjusted serum concentration
LD_{50}	lethal dose eliciting x percent response
LED	lower confidence effective dose
LED_x	lower bound of the 95% confidence interval on the dose that yields an x% effect
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{HED}$	HED estimate based on LOAELs
LOEL	lowest-observed-adverse level
MCH	mean corpuscular hemoglobin
MCMC	Markov Chain Monte Carlo
MCV	mean corpuscular volume
MOA	mode of action
MOE	margin of exposure
MROD	7-methoxyresorufin-O-deethylase
MTD	maximum tolerated dose
NAS	National Academy of Sciences
NIOSH	National Institute for Occupational Safety and Health

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NRC	National Research Council
NTP	National Toxicology Program
OR	odds ratio
OSF	oral slope factor
PA	permeability x area
PAI2	plasminogen activator inhibitor 2
PBMC	peripheral blood mononuclear cells
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PEPCK	phosphoenolpyruvate carboxykinase
PF	adipose tissue:blood partition coefficient
PHAH	polyhalogenated aromatic hydrocarbons
PK	pharmacokinetic
PND	postnatal day
POD	point of departure
pp	phosphotyrosyl protein
PRA	probabilistic risk assessment
PRE	body:blood partition coefficient
PROD	7-pentoxyresorufin-O-deethylase
RAR	retinoic acid receptor
REP	relative potency
RfC	reference concentration
RfD	reference dose
RL	reversal learning
RL	risk level
RR	rate ratios
RR	relative risk
RT-PCR	reverse transcription polymerase chain reaction
RXR	retinoid X receptor
S	saline
SA	superoxide anion
SAhRM	SRM for AhRs
S-D	Sprague-Dawley
SD	standard deviation
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SOD	superoxide dismutase
SRBC	sheep red blood cell
SSB	single-strand break

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

SWHS	Seveso Women's Health Study
T4	thyroxine
TBARS	thiobarbituric acid-reactive substances
TCB	3,3',4,4'-tetrachlorobiphenyl
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCP	2,4,5-trichlorophenol
TEF	toxicity equivalence factor
TEQ	toxicity equivalence
TGFα	transforming growth factor α
TK	toxicokinetic
TNF-α	tumor necrosis factor alpha
TOTTEQ	total toxicity equivalence
TSH	thyroid stimulating hormone
TT4	total thyroxine
TWA	time-weighted average
U.S. NRC	U.S. Nuclear Regulatory Commission
UDP	uridine diphosphate
UDPGT	UDP-glucoronosyl transferase
UED	upper confidence bound for the effective dose
UF	uncertainty factor
UF_A	interspecies extrapolation factor
UF_D	database factor
UF_H	human interindividual variability
UF_L	LOAEL-to-NOAEL UF
UF_S	subchronic-to-chronic UF
UGT	UDP-glucuronosyltransferase
UGT1	uridine diphosphate glucuronosyltransferase I
Vd	volume of distribution
WHO	World Health Organization
ZS@Z	zero slope at zero dose

PREFACE

This report was developed by the U.S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD), National Center for Environmental Assessment (NCEA). Sections of the report, including Section 6 and the updated literature search, were developed through a collaborative effort between NCEA and the Department of Energy's Argonne National Laboratory (ANL).

In 2003, EPA, along with other federal agencies, asked the National Academy of Sciences (NAS) to review aspects of the science in EPA's draft dioxin reassessment entitled, "Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds," and, in 2004, EPA sent the 2003 draft dioxin reassessment to the NAS for their review. In 2006, the NAS released the report of their review entitled, "Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment." The NAS identified three areas in EPA's 2003 draft reassessment that required substantial improvement to support a more scientifically robust risk characterization. These three areas were: (1) justification of approaches to dose-response modeling for cancer and noncancer endpoints; (2) transparency and clarity in selection of key data sets for analysis; and (3) transparency, thoroughness, and clarity in quantitative uncertainty analysis. The NAS provided EPA with recommendations to address their key concerns. This draft report details EPA's response to the key comments and recommendations included in the 2006 NAS report.

In 2008, prior to developing this draft report, EPA, in collaboration with ANL, developed and published a literature database of peer-reviewed studies on TCDD toxicity, including in vivo mammalian dose-response studies and epidemiologic studies. EPA subsequently requested public comment on this database. EPA and ANL then convened a scientific workshop in 2009. The Workshop goals were to identify and address issues related to the dose-response assessment of TCDD and to ensure that EPA's response to the NAS focused on the key issues and reflected the most meaningful science.

This draft report provides a technical response to the 2006 NAS report. It utilizes a TCDD physiologically-based pharmacokinetic model in its development of dose-response analyses of TCDD toxicological and epidemiological literature. This draft report presents new analyses of both the potential cancer and noncancer human health effects that may result from exposures to TCDD. The draft report develops an oral reference dose (RfD) for TCDD. It also presents a new cancer oral slope factor. Federal agencies and White House offices have been provided an opportunity for review and comment on this draft report prior to its public release.

This draft dioxin report is being released for public comment and will also be provided to EPA's Science Advisory Board (SAB) for independent external peer review. The SAB will convene an expert panel composed of scientists knowledgeable about technical issues related to dioxins and risk assessment. The SAB is expected to hold their first public meeting on July 13−15, 2010.

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EXECUTIVE SUMMARY

OVERVIEW

The U.S. Environmental Protection Agency (EPA) is committed to the development of risk assessment information of the highest scientific integrity for use in protecting human health and the environment. Scientific peer review is an integral component of the process EPA uses to generate high quality toxicity and exposure assessments of environmental contaminants. To this end, EPA asked the National Academy of Sciences (NAS) to review its comprehensive human health risk assessment external review draft entitled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* (U.S. EPA, 2003, 537122; "2003 Reassessment"). This current document, *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments,* directly and technically responds to key comments and recommendations pertaining to TCDD dose-response assessment published by the NAS in their review (NAS, 2006, 198441). This document only addresses issues pertaining to TCDD dose-response assessment.

In May 2009, EPA Administrator Lisa P. Jackson announced the "*Science Plan for Activities Related to Dioxins in the Environment*" ("Science Plan") that addressed the need to finish EPA's dioxin reassessment and provide a completed health assessment on this high profile chemical to the American public as quickly as possible.[1] The Science Plan states that EPA will release a draft report that responds to the recommendations and comments included in the NAS review of EPA's 2003 Reassessment, and that, in this draft report, EPA's National Center for Environmental Assessment, Office of Research and Development, will provide a limited response to key comments and recommendations in the NAS report (draft response). This draft response is to focus on dose-response issues raised by the NAS and include analyses of relevant new key studies. The draft response is to be provided for public review and comment and for independent external peer review by EPA's Science Advisory Board. Following completion of this report, EPA is to review the impacts of the response to comments report on its 2003 Reassessment.

[1]Available at http://www.epa.gov/dioxin/scienceplan.

This draft document comprises EPA's report that responds both directly and technically to the recommendations and comments on TCDD dose-response assessment included in the NAS review of EPA's 2003 Reassessment. Because new data are analyzed in this report and toxicity values are derived, this document will follow the IRIS process for review, clearance and completion; however, it is not a traditional IRIS document. Information developed in this document is intended to not only respond to the NAS review, but also to expand EPA's knowledge of TCDD cancer and noncancer dose-response based on the most current literature, existing methods, and adherence to EPA risk assessment guidance documents.

In addition to this document, three separate EPA activities address additional NAS comments pertaining to toxicity equivalence factors (TEFs) and background exposure levels. Information on the application of the dioxin TEFs is published elsewhere by EPA for both ecological (U.S. EPA, 2008, 543774) and human health (U.S. EPA, 2009, 192196) risk assessment. EPA does not directly address TEFs herein, but makes use of the concept of toxicity equivalence (TEQ)[2] as applicable to the analysis of exposure dose in epidemiologic studies and to discussions on the effect of background TEQ on TCDD dose response. Furthermore, information on updated background levels of dioxin in the U.S. population has been recently reported by EPA (Lorber et al., 2009, 543766), addressing the NAS recommendations pertaining to the assessment of human exposures to TCDD and other dioxins.

The NAS identified three key recommendations requiring substantial improvement to support a scientifically robust characterization of human responses to exposures to TCDD. These three key areas are (1) improved transparency and clarity in the selection of key data sets for dose-response analysis, (2) further justification of approaches to dose-response modeling for cancer and noncancer endpoints, and (3) improved transparency, thoroughness, and clarity in quantitative uncertainty analysis. The NAS also encouraged EPA to calculate a Reference Dose (RfD), and provided numerous specific comments on various aspects of EPA's 2003 Reassessment. The three key recommendations specifically pertain to dose-response assessment and uncertainty analysis. Therefore, EPA's response to the NAS in this document is focused on

[2]Toxicity equivalence (TEQ) is the product of the concentration of an individual dioxin like compound in an environmental mixture and the corresponding TCDD TEF for that compound. These products are summed to yield the TEQ of the mixture.

these issues. EPA thoroughly considered the recommendations of the NAS and responds with scientific and technical evaluation of TCDD dose–response data via:

- an updated literature search that identified new TCDD dose-response studies (see Section 2);
- a kickoff workshop that included the participation of external experts in TCDD health effects, toxicokinetics, dose-response assessment and quantitative uncertainty analysis; these experts discussed potential approaches to TCDD dose-response assessment and considerations for EPA's response to NAS; a Workshop Report was developed (U.S. EPA, 2009, 543757, see Appendix A);
- detailed TCDD-specific study inclusion criteria and processes for the selection of key studies (see Section 2.3) and epidemiologic and animal bioassay data for TCDD dose-response assessment (see Section 2.4.1, Appendix B, and Section 2.4.2, respectively);
- kinetic modeling to quantify appropriate dose metrics for use in TCDD dose-response assessment (see Section 3 and Appendices C and D);
- dose-response modeling for all appropriate noncancer and cancer data sets (see Section 4.2/Appendix E and Section 5.2.3/Appendix F, respectively);
- thorough and transparent evaluation of the selected TCDD data for use in the derivation of an RfD and an oral slope factor (OSF) (see Sections 4.2 and 5.2.3, respectively);
- the development of an RfD (see Section 4.3);
- the development of a revised OSF (see Section 5.3) with an updated cancer weight of evidence determination for TCDD based on EPA's 2005 *Cancer Guidelines* (U.S. EPA, 2005, 086237) (see Section 5.1.2);
- consideration of nonlinear dose-response approaches for cancer, including illustrative RfDs for cancer precursor events and tumors (see Section 5.2.3.4) ; and
- discussion of the feasibility and utility of quantitative uncertainty analysis for TCDD dose-response assessment (see Section 6).

Each of the activities listed above is briefly described in this Executive Summary, and is described in detail in the related sections of this document.

PRELIMINARY ACTIVITIES UNDERTAKEN BY EPA TO ENSURE THAT THIS TECHNICAL RESPONSE REFLECTS THE CURRENT STATE-OF-THE-SCIENCE

As part of the development of this document, EPA undertook two activities that included public involvement: an updated literature search and a scientific expert workshop. The adverse

health effects associated with TCDD exposures are documented extensively in epidemiologic and toxicologic studies. As such, the database of relevant information pertaining to the dose-response assessment of TCDD is vast and constantly expanding. Responding directly to the NAS recommendation to use the most current and up-to-date scientific information related to TCDD, EPA, in collaboration with Argonne National Laboratory (ANL), developed an updated literature database of peer-reviewed studies on TCDD toxicity, including in vivo mammalian dose-response studies and epidemiologic studies. An initial literature search for studies published since the 2003 Reassessment was conducted to identify studies published between the year 2000 and October 31, 2008. EPA published the initial literature search results in the Federal Register in November 2008 and invited the public to review the list and submit additional peer-reviewed relevant studies. Additional studies identified by the public and through continued work on this response have been incorporated into the final set of studies for TCDD dose-response assessment (updated through October 2009). EPA believes that the implementation of this rigorous search strategy ensures that the most current and relevant studies were considered for the technical response to NAS and TCDD dose-response assessment included herein.

To assist in responding to the NAS, EPA, in collaboration with ANL, convened a scientific expert workshop ("Dioxin Workshop") in February 2009 that was open to the public. The primary goals of the Dioxin Workshop were to identify and address issues related to the dose-response assessment of TCDD and to ensure that EPA's response to the NAS focused on the key issues, while reflecting the most meaningful science. EPA and ANL assembled expert scientists and asked them to identify and discuss the technical challenges involved in addressing the NAS comments, discuss approaches for addressing these key recommendations, and to assist in the identification of important published and peer-reviewed literature on TCDD. The workshop was structured into seven scientific topic sessions as follows: (1) quantitative dose-response modeling issues, (2) immunotoxicity, (3) neurotoxicity and nonreproductive endocrine effects, (4) cardiovascular toxicity and hepatotoxicity, (5) cancer, (6) reproductive and developmental toxicity, and (7) quantitative uncertainty analysis of dose-response. External co-chairs (i.e., scientists who were not members of EPA or ANL) were asked to facilitate the sessions and then prepare summaries of discussions occurring in each session. The session summaries formed the basis of a final workshop report (U.S. EPA, 2009, 543757, Appendix A of

this document). Some of the key outcomes from the workshop include the following recommendations:

- to further develop study selection criteria for evaluating the suitability of developing dose-response models based on animal bioassays and human epidemiologic studies;
- to use kinetic modeling to identify relevant dose metrics and dose conversions between test animal species and humans, and between human internal dose measures and human intakes;
- to consider newer human or animal (e.g., NTP, 2006, 197605) publications when evaluating quantitative dose-response models for cancer;
- to consider both linear and nonlinear modeling in the cancer dose-response analysis.

The discussions held during the Dioxin Workshop helped inform, guide, and focus EPA's response to NAS.

EPA'S APPROACH TO CONSIDERING TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY STUDIES AND DATA SETS FOR DOSE-RESPONSE MODELING

One of the key NAS recommendations to EPA was to utilize a clear and transparent process for the selection of key studies and data sets for dose-response assessment. EPA agrees with the NAS and believes that clear delineation of the study selection process and decisions regarding key studies and data sets will facilitate communication of critical decisions made in the TCDD dose-response assessment. EPA developed detailed processes and TCDD-specific criteria for the selection of key dose-response studies. These criteria are based on common practices and current guidance for point of departure (POD) identification and RfD and OSF derivation and also consider issues specifically related to TCDD. Following the selection of key studies, EPA employed additional processes to further select and identify cancer and noncancer datasets from these key studies for use in dose-response analysis of TCDD.

For the study evaluation and key data set selection, EPA has undertaken different approaches for the epidemiologic and in vivo animal bioassay studies. The significant differences between animal and human health effects data and their use in EPA risk assessment support development of separate criteria for study inclusion and different approaches to study evaluation. For the vast majority of compounds on EPA's Integrated Risk Information System

(IRIS, U.S. EPA, 2009, 192196), cancer and noncancer toxicity values have been derived using animal bioassay data; thus, some of the TCDD-specific study inclusion criteria for animal bioassay data are based on EPA's common practices and guidance for POD selection and RfD and OSF derivation. Far fewer IRIS toxicity values have been derived from human data, although some examples do exist.[3] The modeling and interpretation of such human data have been conducted on a case-by-case basis because each cohort is uniquely defined and has its own set of exposure conditions, significant confounders, and biases that may need to be considered in dose-response modeling.

Figure ES-1 presents EPA's study evaluation process for the epidemiologic studies considered for this TCDD dose-response assessment, including specific study inclusion criteria (see Section 2.3.1). EPA applied TCDD-specific epidemiologic study inclusion criteria to all epidemiologic studies published on TCDD and dioxin-like compounds (DLCs) that had been identified in the TCDD literature database (see Section 2.4.1, Appendix B). The studies were initially evaluated using five considerations (see Figure ES-1) that provide the most relevant kinds of information needed to consider the feasibility of quantitative human health risk analyses. Then EPA required that the studies meet three study inclusion criteria: 1) the study is published in the peer-reviewed scientific literature and includes an appropriate discussion of strengths and limitations; 2) the exposure is primarily to TCDD, rather than dioxin-like compounds (DLCs), and is properly quantified so that dose-response relationships can be assessed; and 3) the effective dose and oral exposure must be reasonably estimable. To meet the third criterion, information is required on long-term exposures for cancer, and, for noncancer, information is required regarding the appropriate time window of exposure that is relevant for a specific, nonfatal health endpoint. Therefore, the study should include an appropriate latency period between TCDD exposure and the onset of the effect. Only studies meeting these three criteria were included in EPA's TCDD dose-response analyses (see Section 2.4.3).

Figure ES-2 presents EPA's study evaluation process for mammalian bioassays considered for TCDD dose-response assessment, including the specific study inclusion criteria (see Section 2.3.2). EPA applied TCDD-specific in vivo mammalian bioassay study inclusion

[3] Examples of toxicity values on IRIS from human data include benzene, beryllium and compounds, chromium IV, and 1,3-butadiene that have RfDs, Reference Concentrations, Inhalation Unit Risks and/or OSFs all based on occupational cohort data and the methyl mercury RfD that is based on high fish consuming cohorts (U.S. EPA, 2009, 192196).

criteria to all of the bioassay studies of TCDD that had been identified in the TCDD literature database (see Section 2.4.2). After ascertaining that a study had been published in the peer-reviewed literature, EPA applied dose requirements to the lowest tested average daily doses in each study, with specific requirements for cancer (≤1 μg/kg-day) and noncancer (≤30 ng/kg-day) studies to ensure that only low-dose TCDD bioassays would be considered for quantitative assessment. These dose requirements were used to eliminate those studies that would not be selected for development of an RfD or an OSF because the lowest doses tested were too high relative to other TCDD bioassays. EPA also required that the bioassays exposed animals via the oral route to TCDD only and that the purity of TCDD was specified. Finally, the studies were evaluated using four considerations (see Figure ES-2) regarded as providing the most relevant information for development of quantitative human health risk analyses from animal bioassay data. Only the bioassay studies meeting these criteria and considerations were included in EPA's TCDD dose-response analyses (see Section 2.4.3).

Applying the study inclusion criteria for both epidemiologic and mammalian bioassay datasets resulted in a list of key noncancer and cancer studies that were considered for quantitative dose-response analyses of TCDD. Endpoints from these studies that were not considered to be toxicologically relevant were eliminated from consideration (see Section 4.2.1, Appendix G). The study/endpoint dataset combinations from the remaining studies were then subjected to dose-response assessment, and PODs for use in developing RfDs or OSFs were identified. PODs included no-observed-adverse-effect levels (NOAELs), lowest-observed-adverse-effect levels (LOAELs) or lower bound benchmark dose levels (BMDLs). The most sensitive PODs were selected as candidates for derivation of the RfD and OSF.

USE OF KINETIC MODELING TO ESTIMATE TCDD DOSES

NAS recommended that EPA utilize state-of-the-science approaches to finalize the 2003 Reassessment. Although NAS concurred with EPA's use of first-order body burden models in the 2003 Reassessment, analyses of recent TCDD literature and comments by experts at the Dioxin Workshop suggested that the understanding of TCDD kinetics had increased significantly since the release of EPA's 2003 Reassessment. These advances led to the development of several pharmacokinetic models for TCDD (Aylward et al., 2005, 197114; e.g.,

Emond et al., 2004, 197315; Emond et al., 2005, 197317; Emond et al., 2006, 197316) and resulted in EPA's incorporation of TCDD kinetics in the dose-response assessment of TCDD.

The evaluation of internal dose in exposed humans and other species is facilitated by an understanding of pharmacokinetics (i.e., absorption, distribution, metabolism, and excretion). TCDD pharmacokinetics are influenced by three distinctive features: (1) TCDD is highly lipophilic, (2) TCDD is slowly metabolized, and (3) TCDD induces binding proteins in the liver. The overall impact of these factors results in preferential storage of TCDD in adipose tissue, a long half-life of TCDD in blood due to slow metabolism, and sequestration in liver tissue when binding induction becomes significant. As these kinetic features control target tissue levels of dioxin, they become important in relating toxicity in animals to possible effects in humans.

Consideration of pharmacokinetic mechanisms is critical to the selection of the dose metrics of relevance to dose-response modeling of TCDD. Earlier assessments for TCDD, including the 2003 Reassessment, used estimates of body burden as the dose metric for extrapolation between animals and humans. These body burden calculations used a simple one-compartment kinetic model based on the assumption of a first-order decrease in the levels of administered dose as a function of time. However, the assumption of a constant half-life value for the clearance of TCDD from long-term or chronic exposure is not well-supported biologically given the dose-dependant elimination observed in rodents and humans. The dynamic disposition and redistribution of TCDD between blood, fat, and liver as a function of time and dose is better described using biologically-based models. Additionally, these models provide estimates for other dose metrics (e.g., serum, whole blood, or tissue levels) that are more biologically relevant to response than body burden estimated based on an assumption of first-order elimination over time.

EPA considered the following possible dose metrics for TCDD: administered dose, first-order body burden, lipid-adjusted serum concentration (LASC), whole blood concentration, tissue concentration, and functional-related metrics of relevance to the mode of action (MOA) (e.g., receptor occupancy) (see Section 3.3.4.1). After careful evaluation of these dose metrics, EPA chose to use TCDD concentration in whole blood as the dose metric for assessing TCDD dose response in this document. Although LASC is generally considered to be the most relevant metric, whole blood concentration was chosen because of the structure of the PBPK models, in which the target tissue compartments are connected to the whole blood compartment rather than

to the serum compartment; LASC is related to whole blood by a scalar, so use of either is equivalent in the model. Whole blood concentrations also reflect TCDD dose to target tissues and, are biologically-relevant measures of internal dose. EPA used the time-weighted average whole-blood concentration over the relevant exposure periods for all continuous dosing protocols, dividing the area under the time-course concentration curve (AUC) by the exposure duration.[4]

Several biologically-based kinetic models for TCDD exist in the literature. The more recent pharmacokinetic models explicitly characterize the concentration-dependent elimination of TCDD (Carrier et al., 1995, 197618; Carrier et al., 1995, 543780; Emond et al., 2004, 197315; Emond et al., 2005, 197317; Emond et al., 2006, 197316; Aylward et al., 2005, 197114). The biologically-based pharmacokinetic models describing the concentration-dependent elimination (i.e., the pharmacokinetic models of Aylward et al. (2005, 197114) and Emond et al. (2005, 197317; 2006, 197316) are relevant for application to simulate the TCDD dose metrics in humans and animals exposed via the oral route. The rationale for considering the application of the Aylward et al. (2005, 197114) and Emond et al. (2004, 197315; 2005, 197317; 2006, 197316) models was largely based on the fact that both models reflect research results from recent peer-reviewed publications, and both models are formulated with dose-dependent hepatic elimination consistent with the physiological understanding of TCDD kinetics. Dose-response modeling based on body burden of TCDD in adult animals and humans can be conducted with either of the models, provided the duration of the experiment is at least one month, due to limitations in the Aylward et al. (2005, 197114) model. The predicted slope and body burden over a large dose range are quite comparable between the two models (generally within a factor of two).

Results of simulations of serum lipid concentrations or liver concentrations vary for the two models to a larger extent (up to a factor of 7), particularly for simulations of short duration. These differences reflect two characteristics of the Emond et al. (2006, 197316) model: first, quasi-steady-state is not assumed in the Emond et al. (2006, 197316) model; second, the serum lipid composition used in the model is not the same as the adipose tissue lipids. The Aylward

[4]For the Seveso cohort, which had a high single exposure followed by low-level background exposures leading to a gradual decline in the internal TCDD concentrations, EPA estimated dose as the mean of the peak exposure and the average exposure over a defined critical exposure window (see Section 4.2.2).

et al. (2005, 197114) model does not account for differential solubility of TCDD in serum lipids and adipose tissue lipids, nor does it account for the diffusion-limited uptake by adipose tissue. Based on this evaluation, EPA determined that the Emond et al. (2006, 197316) model performed better than the Aylward et al. (2005, 197114) model with respect to the ability to simulate serum lipid and tissue concentrations during exposures that do not lead to the onset of steady-state condition in the exposed organism. Additionally, of the two selected models, the pharmacokinetic model developed by Emond et al. (2006, 197316) is more physiologically-based, as compared to the Aylward et al. (2005, 197114) model, and models the blood compartment directly in the rat, mouse, and human; there are also gestational and life-time nongestational forms of the Emond et al. (2006, 197316) model. In this document, EPA chose the Emond rodent physiologically-based pharmacokinetic (PBPK) model to estimate blood TCDD concentrations based on administered doses (see Section 3.3.4, Appendix C).

To enhance the biological basis of the PBPK model of Emond et al. (2006, 197316), three minor modifications,were made before its use in the computation of dose metrics for TCDD: 1) recalculation of the volume of the "rest of the body compartment" after accounting for volume of the liver and fat compartments; 2) calculation of the rate of TCDD excreted via urine by multiplying the urinary clearance parameter by blood concentration in the equation instead of by the concentration in the rest of the body compartment; and 3) recalibration for the human gastric nonabsorption constant to yield observed oral bioavailability of TCDD (Poiger and Schlatter, 1986) (see Section 3.3.4.4 for details). The modified PBPK model was evaluated against all published data used in the original model. EPA assumed that the same blood TCDD levels that led to effects in animals would also lead to effects in humans; therefore, the Emond human PBPK model was used to estimate the lifetime average daily oral doses (consistent with the chronic RfD and OSF) that would correspond to the blood TCDD concentrations estimated to have occurred during the animal bioassays. EPA used the same Emond human PBPK model to estimate the lifetime average daily doses that would correspond to the TCDD blood or tissue concentrations reported in the epidemiological studies (Appendix D). These estimates are the Human Equivalent Doses (HEDs) that are used to develop candidate RfDs and OSFs for TCDD.

Because TCDD elimination is inducible in the Emond model, ratios of daily averaged intake to long-term blood concentrations are not linear. Because of the nonlinearity of blood concentration and ingested dose in the Emond Human PBPK model, the cancer risk is only

approximately linear with the TCDD blood concentration and low TCDD oral ingestion doses, but is not linear with ingested TCDD at higher doses. Thus, to use these estimates in human health risk assessment, risk-specific TCDD oral intake levels corresponding to the target risk levels should be calculated (see Section 5.2.3.1.2.1).

DERIVATION OF AN RfD FOR TCDD

The NAS specifically recommended that EPA derive an RfD for TCDD. Through a transparent study selection process, EPA identified key studies from both human epidemiologic studies and animal bioassays. To select candidate PODs for its RfD methodology, EPA applied additional processes to the key human epidemiologic studies and animal bioassays. Figure ES-3 (exposure-response array) shows the entire candidate PODs graphically in terms of human-equivalent intake (ng/kg-day). The human study endpoints are shown at the far left of the figure and, to the right, the rodent endpoints are arranged by the following study categories: less than 1 year, greater than 1 year, reproductive, and developmental.

For each noncancer epidemiologic study that EPA selected as key, EPA evaluated the dose-response information developed by the study authors to determine whether the study provided noncancer effects and TCDD-relevant exposure data for a toxicologically-relevant endpoint. If such data were available, EPA identified a NOAEL or LOAEL as a candidate POD. Then, EPA used the Emond human PBPK model to estimate the continuous oral daily intake (ng/kg-day) that would lead to the relevant blood TCDD concentrations associated with the candidate POD. If all of this information was available, then the result was included as a candidate POD.

Through this process, EPA identified health effects from the following four epidemiologic studies to be considered as the basis for the RfD: Eskenazi et al. (2002, 197168)(reproductive—increased length of menstrual cycle), Alaluusua et al. (2004, 197142) (developmental—tooth development), Mocarelli et al. (2008, 199595) (reproductive—decreased sperm concentrations and motility), and Baccarelli et al. (2008, 197059) (developmental—increased thyroid-stimulating hormone levels in neonates). All four studies are from the Seveso cohort, whose members were exposed environmentally to high peak concentrations of TCDD as a consequence of an industrial accident. This complicated the estimation of average daily doses associated with these specific endpoints, however EPA was

able to calculate candidate PODs for derivation of an RfD from each of these human studies (see Section 4.2.3). The Alaluusua et al. (2004, 197142) and Eskenazi et al. (2002, 197168) studies had PODs well above the Mocarelli et al. (2008, 199595) and Baccarelli et al. (2008, 197059); because the LOAEL in Eskenazi et al. (2002, 197168) is almost 2 orders of magnitude higher than the LOAELs for Baccarelli et al. (2008, 197059) and Mocarelli et al. (2008, 199595), it was not considered further as a candidate POD for derivation of the RfD.

Figure ES-4 summarizes the strategy employed for identifying and selecting candidate PODs from the key animal bioassays EPA identified for use in noncancer dose-response analysis of TCDD (see Section 4.2.4). For each noncancer endpoint, EPA first evaluated the toxicological relevance of each endpoint, rejecting those judged not to be relevant for RfD derivation (Section 4.2.1, Appendix G). Next, initial PODs (NOAELs, LOAELs, and BMDLs) based on the first-order body burden metric, and expressed as continuous human-equivalent oral daily doses (HEDs), were determined for all relevant endpoints.

Because there were very few NOAELs, and BMDL modeling was largely unsuccessful due to data limitations, the next stage of evaluation was carried out using LOAELs only. Endpoints not observed at the LOAEL (i.e., reported at higher doses) with BMDLs greater than the LOAEL were eliminated from further analysis, as they would not be considered as candidates for the final POD on either a BMDL or NOAEL/LOAEL basis (i.e. the POD would be higher than the PODs of other relevant endpoints). In addition, all endpoints with HEDs for LOAELs ($LOAEL_{HED}$s) beyond a 100-fold range of the lowest identified $LOAEL_{HED}$ were eliminated from further consideration, as they would not be potential POD candidates either (i.e. the POD would be higher than the PODs of other relevant endpoints). For the remaining endpoints, EPA then determined final potential PODs (NOAELs, LOAELs, and BMDLs) based on TCDD blood concentrations obtained from the Emond rodent PBPK models. HEDs were then estimated for each of these PODs using the Emond human PBPK model. From these HEDs, a POD_{HED} was selected for each study as the basis for the candidate RfD, to which appropriate uncertainty factors were applied following EPA guidelines. The resulting candidate RfDs were then considered in the final selection process for the RfD. Other endpoints occurring at slightly higher doses representing additional effects associated with TCDD exposure (beyond the 100-fold LOAEL range) were evaluated, modeled, and included in the final candidate RfD array

to examine endpoints not evaluated by studies with lower PODs. In addition, BMD modeling based on administered dose was performed on all endpoints for comparison purposes.

For BMD modeling, EPA has used a 10% BMR for dichotomous data for all endpoints; no developmental studies were identified with designs that incorporate litter effects, for which a 5% BMR would be used (U.S. EPA, 2000, 052150). For continuous endpoints in this document, EPA has used a BMR of 1 standard deviation from the control mean whenever a specific toxicologically-relevant BMR could not be defined. Importantly, the 2003 Reassessment defined the ED_{01} as 1% of the maximal response for a given endpoint, not as a 1% change from control. Because RfD derivation is one goal of this document, the noncancer modeling effort undertaken here differs substantially from the modeling in the 2003 Reassessment. Evaluation of BMD modeling performance, goodness-of-fit, dose-response data, and resulting BMD and BMDL estimates included statistical criteria as well as expert judgment of their statistical and toxicological properties. EPA has reported and evaluated the BMD results using the standard suite of goodness-of-fit measures from the benchmark dose modeling software (BMDS 2.1). These include chi-square *p*-values, Akaike's Information Criterion (AIC), scaled residuals at each dose level and plots of the fitted models. In some cases, when restricted parameters hit a bound, EPA used likelihood ratio tests to evaluate whether the improvement in fit afforded by estimating additional parameters could be justified. Goodness-of-fit measures are reported for all key data sets in Appendix E. (See Section 4.2.4.2 for a more complete description of the benchmark dose modeling criteria for model evaluation.)

For selection of the POD to serve as the basis of the RfD, EPA gave the epidemiologic studies the highest consideration because human data are preferred in the derivation of an RfD, given that the underlying epidemiologic and animal bioassay data are of comparable quality. This preference for epidemiologic study data also is consistent with reccomendations of panelists at the Dioxin Workshop (see U.S. EPA, 2009, 543757, Appendix A). Figure ES-5 arrays the candidate RfDs from both the human and animal bioassays. The human studies included in Figure ES-5 (Alaluusua et al., 2004, 197142; Baccarelli et al., 2008, 197059; Mocarelli et al., 2008, 199595) each evaluate a segment of the Seveso civilian population (i.e., not an occupational cohort) exposed directly to TCDD released from an industrial accident. In this document, EPA uses the Baccarelli et al. (2008, 197059) and Mocarelli et al. (2008, 199595)

studies as co-critical studies in deriving the RfD (Section 4.3).[5] In the Seveso cohort exposures were primarily to TCDD, the chemical of concern, with apparently minimal DLC exposures beyond those associated with background intake,[6] making these studies highly appropriate for use in RfD derivation for TCDD. In addition, health effects associated with TCDD exposures were observed in humans, the species of concern whose health protection is represented by the RfD, eliminating the uncertainty associated with interspecies extrapolation. The cohort members who were evaluated included infants (exposed in utero) and adults who were exposed when they were less than 10 years of age. The inclusion of these studies among the RfDs derived also may characterize noncancer health effects associated with TCDD exposures in potentially vulnerable populations, thus accounting for some part of the intraspecies uncertainty in the RfD. Finally, the two virtually identical RfDs from different endpoints in the Baccarelli et al. (2008, 197059) and Mocarelli et al. (2008, 199595) studies provide an additional level of confidence in the use of these data for derivation of the RfD for TCDD.

Although the human data are preferred, Figure ES-5 presents a number of animal studies with RfDs that are lower than the human RfDs. To a large extent, this is expected because a 10-fold interspecies uncertainty factor is generally used to extrapolate from test-animal species to humans, intended to provide a conservative estimate of an RfD that would be derived directly from human data. Two of the rat bioassays among this group of studies—Bell et al. (2007, 197041) and NTP (2006, 197605)—are of particular note. Both studies were recently conducted and very well designed and conducted, using 30 or more animals per dose group; both also are consistent with and, in part, have helped to define the current state of practice in the field. Bell et al. (2007, 197041) evaluated several reproductive and developmental endpoints, initiating TCDD exposures well before mating and continuing through gestation. NTP (2006, 197605) is the most comprehensive evaluation of TCDD chronic toxicity in rodents to date, evaluating dozens of endpoints at several time points in all major tissues. Thus, proximity of the RfDs derived from these two high quality, recent studies, provide additional support for the use of the human data for RfD derivation.

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[5] The candidate RfD for Alaluusua et al. (2004, 197142) was approximately 2 orders of magnitude higher than the RfDs for Mocarelli et al. (2008, 199595) and Baccarelli et al. (2008, 197059), thus, it was not included as a co-critical study for the RfD.

[6] As an example, note the lack of statistically significant effects reported by Baccarelli et al. (2008, 197059; Figure 2 C and D) in regression models based on either maternal plasma levels of non-coplaner PCBs or total TEQ on neonatal TSH levels.

There are several animal bioassay candidate RfDs at the lower end of the RfD range in Figure ES-5 that are more than 10-fold below the human-based RfDs. Two of these studies report effects that are analogous to the endpoints reported in the three human studies and support the RfDs based on human data. Specifically, decreased sperm production in Latchoumydandane and Mathur (2002, 197498) is consistent with the decreased sperm counts and other sperm effects in Baccarelli et al. (2008, 197059), and missing molars in Keller et al. (2007, 198526; 2008, 198531; 2008, 198033) are similar to the dental defects seen in Alaluusua et al. (2004, 197142). Thus, because these endpoints have been associated with TCDD exposures in humans, these animal studies would not be selected for RfD derivation in preference to human data showing similar effects.

Another characteristic of the remaining studies in the lower end of the candidate RfD distribution is that they are dominated by mouse studies (comprising 6 of the 8 lowest rodent-based RfDs). EPA considers the candidate RfD estimates based on mouse data to be much more uncertain than either the rat or human candidate RfD estimates. The EPA considers the Emond mouse PBPK model to be the most uncertain of toxicokinetic models used to estimate the PODs because of the lack of key mouse-specific data, particularly for the gestational component (see Section 3.3.4.3.2.5). The $LOAEL_{HED}$s identified in mouse bioassays are low primarily because of the large toxicokinetic interspecies extrapolation factors used for mice, for which there is more potential for error. The ratio of administered dose to HED (D_a:HED) ranges from 65 to 1,227 depending on the duration of exposure. The D_a:HED for mice is, on average, about four times larger than that used for rats. In addition, each one of the mouse studies has other qualitative limitations and uncertainties that make them less desirable candidates as the basis for the RfD than the human studies.

The most relevant human PODs are based on the Baccarelli et al. (2008, 197059) and Mocarelli et al. (2008, 199595) studies, which exhibited similar LOAELs of 0.024 and 0.020 ng/kg-day, respectively. For Baccarelli et al. (2008, 197059), EPA defined a LOAEL as the group mean of 39 ppt TCDD in neonatal plasma which corresponds to thyroid-stimulating hormone (TSH) values above 5 μU/mL. The World Health Organization (WHO, 1994) established the 5 μU/mL standard as an indicator of potential iodine deficiency and potential thyroid problems in neonates. Increased TSH levels are indicative of decreased thyroid hormone (T4 and/or T3) levels. For TCDD, the toxicological concern is not likely to be iodine uptake

inhibition, but rather increased metabolism and clearance of T4, as evidenced in a number of animal studies (e.g., Seo et al., 1995, 197869). Clinically, a TSH level of >4 μU/mL in a pregnant woman is followed up by an assessment of free T4, and treatment with L-thyroxine is prescribed if T4 levels are low (Glinoer and Delange, 2000). This is to ensure a sufficient supply of T4 for the fetus, which relies on maternal T4 exclusively during the 1st half of pregnancy (Chan et al., 2005; Morreale de Escobar et al., 2000; Calvo et al., 2002). Adequate levels of thyroid hormone also are essential in the newborn and young infant as this is a period of active brain development (Glinoer and Delange, 2000; Zoeller and Rovet, 2004). Thyroid hormone disruption during pregnancy and in the neonatal period can lead to neurological deficiencies.

Baccarelli et al. (2008, 197059) showed, in graphical form, how the TSH distribution in each of three categorical exposure groups (reference, zone A, and zone B—representing increasing TCDD exposure) shifted to higher TSH values with increasing exposure. The individuals comprising the above 5 μU/mL group were from all three categorical exposure groups, not just from the highest exposure group. Therefore, EPA was able to designate a LOAEL independently of the nominal categorical exposure groups for TSH values above 5 μU/mL. Baccarelli et al. (2008, 197059) did not estimate the equivalent oral intake associated with TCDD serum concentrations, rather they provided neonatal serum TCDD concentrations for the groups above and below 5 μU/mL. EPA estimated the maternal intake at the LOAEL from a maternal serum-TCDD/TSH regression model presented in Baccarelli et al. (2008, 197059) by estimating the maternal TCDD lipid adjusted serum concentration (LASC) at which neonatal TSH exceeded 5 μU/mL. EPA then used the Emond PBPK model to estimate the continuous daily TCDD intake that would result in this TCDD LASC. The resulting predicted maternal daily intake rate established the LOAEL (0.024 ng/kg-day). EPA did not defined a NOAEL because it is not clear what maternal intake should be assigned to the group below 5 μU/mL.

For Mocarelli et al. (2008, 199595), EPA defined a LOAEL as the lowest exposed group mean of 68 ppt (1st-quartile) corresponding to decreased sperm concentrations (20%) and decreased motile sperm counts (11%) in men who were 1–9 years old at the time of the Seveso accident (initial TCDD exposure event). Although a decrease in sperm concentration of 20% likely would not have clinical significance for an individual, EPA's concern is that such decreases associated with TCDD exposures could lead to shifts in the distributions of these measures in the general population. Such shifts could result in decreased fertility in men at the

low end of these population distributions. In the group exposed due to the Seveso accident, individuals one standard deviation below the mean are just above the cut-off used by clinicians (20 million/ml) to indicate follow-up for potential reproductive impact in affected individuals, indicating that a number of individuals in the exposed group likely had sperm concentrations less than 20 million/ml; EPA could not obtain the individual data to determine the exact number of men in this category. EPA judged that the impact on sperm concentration and quality reported by Mocarelli et al. (2008, 199595) is biologically significant given the potential for functional impairment as a consequence of potential shifts in the distribution of these male fertility measures in an exposed population.

For Mocarelli et al. (2008, 199595), TCDD LASC levels were measured within approximately one year of the initial exposure event. Because effects were only observed in men who were under 10 years of age at the time of exposure, EPA assumed a maximum 10-year critical exposure window for elicitation of these effects. EPA has estimated a continuous daily oral intake of 0.020 ng/kg-day associated with the designated LOAEL from the lowest exposure group (68 ppt), (see Section 4.2.3.2). The reference group is not designated as a NOAEL because there is no clear zero-exposure measurement for any of these endpoints, particularly considering the contribution of background exposure to DLCs, which futher complicates the interpretation of the reference group response as a true "control" response (see discussion in Section 4.4). However, males less than 10 years old can be designated as a sensitive population by comparison to older males who were not affected.

The two human studies, Baccarelli et al. (2008, 197059) and Mocarelli et al. (2008, 199595), have similar LOAELs of 0.024 and 0.020 ng/kg-day, respectively. Together, these two studies constitute the best foundation for establishing a POD for the RfD, and are designated as co-principal studies. Therefore, increased TSH in neonates (Baccarelli et al., 2008, 197059) and male reproductive effects (decreased sperm count and motility) are designated as cocritical effects. Although the exposure estimate used in determination of the LOAEL for Mocarelli et al. (2008, 199595) is more uncertain than the Baccarelli et al. (2008, 197059) exposure estimate, the slightly lower LOAEL of 0.020 ng/kg-day from Mocarelli et al. (2008, 199595) is designated as the POD.

EPA used a composite UF of 30 for both studies. EPA applied a factor of 10 for UF_L to account for lack of a NOAEL. EPA also applied a factor of 3 ($10^{0.5}$) for UF_H to account for

human interindividual variability because the effects were elicited in sensitive populations. A further reduction to 1 was not made because the sample sizes in these two epidemiologic studies were relatively small, which, combined with uncertainty in exposure estimation, may not fully capture the range of interindividual variability. The resulting RfD for TCDD in standard units is 7×10^{-10} mg/kg-day.

WEIGHT-OF-EVIDENCE STATEMENT FOR CARCINOGENICITY

The NAS recommended that EPA update its cancer classification for TCDD and the weight-of-evidence (WOE) statement to reflect the current state of the science and incorporate the latest EPA Cancer Guidelines (U.S. EPA, 2005, 086237). Several notable new studies addressing TCDD's carcinogenic potential have been published since the release of EPA's 2003 Reassessment, including several new studies of the Seveso epidemiologic cohort and an NTP 2-year cancer bioassay in female rats (NTP, 2006, 197605).

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237) TCDD is characterized as *carcinogenic to humans*, based on the available data as of 2009 (see Section 5.1.2). When evaluating the carcinogenic potential of a compound, EPA employs a WOE approach in which all available information is evaluated and considered. In the case of TCDD, EPA based the classification on numerous lines of evidence, including: multiple occupationally- and accidentally-exposed epidemiologic cohorts showing an association between TCDD exposure and certain cancers or increased mortality from all cancers; extensive evidence of carcinogenicity at multiple tumor sites in both sexes of multiple species of experimental animals; consensus that the mode of TCDD's carcinogenic action in animals involves aryl hydrocarbon receptor (AhR)-dependent key precursor events and proceeds through modification of one or more of a number of cellular processes; the human AhR and rodent AhR are similar in structure and function, and human and rodent tissue and organ cultures respond to TCDD in a similar manner and at similar concentrations; and general scientific consensus that AhR activation is anticipated to occur in humans and may progress to tumors.

Most evidence suggests that the majority of toxic effects of TCDD are mediated by interaction with the AhR. EPA considers interaction with the AhR to be a necessary, but not sufficient, event in TCDD carcinogenesis. Although AhR binding and activation by TCDD is considered to be a key event in TCDD carcinogenesis, the sequence of key events following AhR

activation that ultimately leads to the development of cancer is unknown (See Section 5.1.2.3). Therefore, EPA has determined that TCDD's mode of action, as defined by the 2005 Cancer Guidelines, is unknown. Since the mode of action for TCDD carcinogenesis is not known, EPA has used a low dose linear extrapolation approach in the development of a cancer oral slope factor.

DERIVATION OF CANDIDATE OSFs FROM EPIDEMIOLOGIC STUDIES AND ANIMAL BIOASSAYS

In response to the NAS concerns that EPA evaluate data published since the 2003 Reassessment and better justify its approach to cancer dose-response modeling, EPA has developed candidate OSFs using epidemiologic studies and animal bioassays for TCDD, including both new evaluations of data from the 2003 Reassessment and also the assessment of new studies. The BMR level that has been used for the POD in deriving the cancer OSF is one percent extra risk, which is close to the observable response data for most data sets and, therefore, best represents low dose cancer risks (see Section 5.2.3.2.6.11). EPA has chosen a single BMR for consistency across studies.

There are several well-studied occupationally-exposed epidemiologic cohorts showing an association between TCDD and increased all-cancer mortality, and several epidemiologic cohorts exposed to TCDD as a consequence of industrial accidents showing an association between TCDD and cancer or cancer mortality (see Section 5.2.3.1). The 2003 Reassessment included cancer dose-response analyses based on the following three occupational cohorts: the NIOSH cohort, an occupational cohort subject to chronic TCDD exposures (Steenland et al., 2001, 197433); the Hamburg cohort, an occupational cohort also subject to chronic TCDD exposures (Becher et al., 1998, 197173); and the BASF cohort, an occupational cohort subject to peak TCDD exposures through clean-up following an industrial accident (Ott and Zober, 1996, 198101). In this document, EPA determined that each of these studies met the epidemiologic study inclusion criteria. Thus, after further evaluating the OSFs presented in the 2003 Reassessment for these three studies, EPA accepted those OSF estimates and retained them as candidate OSFs in this document. These OSF estimates are arrayed in Figure ES-6, along with the other OSFs calculated by EPA in this document. EPA also determined that three additional studies met the epidemiologic study inclusion criteria: Cheng et al. (2006, 523122) and Collins

et al. (2009, 197627) (NIOSH cohort) and Warner et al. (2002, 197489) (Seveso cohort). EPA determined that the data presented in Collins et al. (2009, 197627) were not sufficient to derive an OSF, and EPA was unable to derive a credible OSF from the data presented by Warner et al. (2002, 197489) (see discussions in Section 5.2.3.1).

EPA did derive an OSF from Cheng et al. (2006, 523122), as detailed in Text Box ES-1. In Table ES-1, EPA presents estimates of OSFs for specific TCDD intake rates based on target risk levels of 1×10^{-2}, through 1×10^{-7} based on Cheng et al. (2006, 523122). Note that there are two nonlinear steps in the estimation of risk-specific doses from the Cheng et al. model. First, fat-AUC (AUC_{RL}) and the incremental cancer mortality risk (R_D) do not have a linear relationship (Equation 5-4); however, the relationship becomes virtually linear below an incremental risk of 10^{-3} (see Table ES-1). Second, TCDD fat concentration is not linear with oral intake in the Emond human PBPK model (see Section 3); this relationship also is close to linear below the 10^{-5} risk level. The resulting predicted cancer-mortality risk is approximately linear with daily oral intake at low doses.

EPA also identified candidate OSFs for TCDD from key animal bioassays (see Section 5.2.3.2). Based on the inclusion criteria, EPA selected five key rodent cancer bioassays suitable for quantitative dose-response assessment. These included Della Porta et al. (1987, 197405), Kociba et al. (1978, 001818), NTP (1982, 543764), and Toth et al. (1979, 197109) that were evaluated in the 2003 Reassessment, and the new NTP (2006, 197605) rat chronic bioassay. EPA conducted dose-response modeling for each tumor type separately (individual tumor models) as well as for composite tumor incidence (multiple tumor models). The tumor types that EPA analyzed are shown in Table ES-2.

For each in vivo animal cancer study that qualified for TCDD dose-response assessment, EPA selected the species/sex/tumor dataset combinations characterized as having statistically significant increases in tumor incidences, then used the Emond rodent PBPK model to estimate blood concentrations corresponding to each study's average daily administered dose for use in dose-response modeling. $BMDL_{01}$s were then estimated for the blood concentration by two different methodologies: (1) using the multistage cancer model for each species/sex/tumor combination within each study, and (2) using a Bayesian Markov Chain Monte Carlo framework that assumes independence of tumors, modeling all tumors together for each species/sex

Text Box ES-1. OSF Calculations Using Cheng et al. (2006, 523122) Information.

To develop cancer risks for TCDD, EPA used the modeling results of the Cheng analysis, with conversion to oral intake using the Emond human PBPK model as follows. The slope (β) from the Cheng analysis is the slope of the linear relationship between the natural logarithm of the rate ratio (RR) and the cumulative fat TCDD concentration (fat-AUC). Conceptually, the slope (β) is similar to an OSF, except that it is expressed in terms of fat-AUC rather than intake. Also, the slope represents the incremental increase in cancer mortality (expressed as an RR) above the background TCDD exposure experienced by the NIOSH cohort rather than above zero. Using the upper 95% bound on β and assuming that the slope is the same below the NIOSH cohort background exposure level (approximately 5 ppt/yr TCDD fat concentration), EPA calculated risk-specific doses (as daily oral intakes) for TCDD for risk levels of concern to EPA. The risk-specific doses were estimated from the Emond human PBPK model for the lifetime-average TCDD fat concentrations corresponding to the fat-AUC predicted by the Cheng et al. model for each of the risk levels of concern. The steps in this computation are as follows:

- Background cancer mortality risk estimate (R_0). EPA used an R_0 of 0.112 as reported by Cheng et al. (2006, 523122)
- Total cancer mortality risk in the exposed group associated with a specified (extra) risk level (RL) of fatal cancer (TR_{RL}). A TR_{RL} associated with any given extra risk level (e.g., 0.01, 1×10^{-6}) can be calculated using the following relationship for extra risk:

$$ER = \frac{TR_{RL} - R_0}{1 - R_0} \qquad \text{(Eq. ES-1)}$$

- Incremental cancer mortality risk in the exposed population based on a given extra risk (R_D). R_D, is calculated as the difference between the total risk and background risk and expressed in terms of RL and R_0 by combining Equations ES-2 and ES-1.

$$R_D = TR_{RL} - R_0 \qquad \text{(Eq. ES -2)}$$

$$R_D = RL \times (1 - R_0) \qquad \text{(Eq. ES -3)}$$

- Cumulative TCDD concentration in the fat compartment for a given extra risk (AUC_{RL}). AUC_{RL} is then calculated by taking the natural logarithm of Equation 3 from Cheng et al. (2006, 523122), rearranging and substituting for RR[1] (RR = $[R_D + R_0]/R_0$):

$$AUC_{RL} = \ln((R_D + R_0)/R_0)/\beta^* \qquad \text{(Eq. ES -4)}$$

where β^* is the central-tendency regression slope or the 95% upper bound (β_{95}) determined by summing the regression coefficient (β) and the product of 1.96 and the standard error of the regression coefficient, yielding an estimate of 6.0×10^{-6} per ppt-year lipid adjusted serum TCDD, as follows:

$$\beta_{95} = \beta + 1.96 * SE \qquad \text{(Eq. ES -5)}$$

- Continuous daily TCDD intake associated with a given extra risk [D_{RL}]. Because the fat concentrations generated by CADM are not linear with oral exposure at higher doses, a single oral slope factor to be used for all risk levels cannot be obtained; the response is approximately linear with fat concentrations and oral intake at lower doses. Instead, a risk-specific D_{RL} must be estimated by converting the respective AUC_{RL} to the corresponding lifetime daily intake, using an appropriate human toxicokinetic model. EPA has chosen to use the Emond human PBPK model for this purpose because the CADM configuration does not facilitate this process and so that the dose conversions are consistent with those used in the derivation of the RfD. A D_{RL} is obtained from the Emond model by finding the average lifetime daily intake corresponding to the AUC_{RL} in the fat compartment.

combination within each study. The final selected models were subjected to goodness-of-fit tests and visual inspection of fit to the raw data. Thus, for each sex/species combination within each study, EPA generated a $BMDL_{01}$ for each single tumor type and another $BMDL_{01}$ for the combined tumors. Using the Emond human PBPK model, $BMDL_{HED}$s were then calculated for each of the $BMDL_{01}$s, and using a linear extrapolation, OSFs were calculated by $OSF = 0.01/BMDL_{HED}$ The highest OSF for a species/sex combination for either a single tumor type or all combined tumors was selected as a candidate OSF. The OSF candidates from the key animal bioassays are shown in Table ES-2.

DERIVATION OF TCDD ORAL SLOPE FACTOR AND RISK ESTIMATES

EPA was able to derive OSFs for tumor incidence data from five animal cancer bioassays, as well as for cancer mortality data from four epidemiological cohort studies that were selected for TCDD dose-response modeling using the study inclusion criteria (see Section 5.3). These OSFs are arrayed in Figure ES-6. For the animal data, OSFs based on individual tumors were developed for 28 study/sex/endpoint combinations, and the results ranged from 1.8×10^4 to 5.8×10^6 (per mg/kg-day). The OSFs based on combined tumors were developed for seven study/sex combinations, and the results ranged from 3.2×10^5 to 9.4×10^6 (per mg/kg-day). EPA also developed OSFs based on four epidemiologic studies from three cohorts, ranging from 3.75×10^5 to 2.5×10^6 (per mg/kg-day).

EPA has chosen to use the human data over the animal data as recommended by expert panelists at EPA's 2009 Dioxin Workshop (U.S. EPA, 2009, 522927) and in the 2005 Cancer Guidelines (U.S. EPA, 2005, 086237). OSFs derived from the human data are consistent with the animal bioassay results; human OSFs fall within the same range as the animal bioassay OSFs.

Among the human studies, the occupational TCDD exposures in the NIOSH and Hamburg cohorts are assumed to be reasonably constant over the duration of occupational exposure. In contrast, the TCDD exposure pattern for the Seveso and BASF accidents is acute, high dose, followed by low-level background exposure. Such exposure patterns similar to those experienced by the BASF and Seveso cohorts have been shown to yield higher estimates of risk when compared to constant exposure scenarios with similar total exposure magnitudes (Kim et al., 2003, 199146; Murdoch and Krewski, 1988, 548718; Murdoch et al., 1992, 548719).

Thus, EPA has judged that the NIOSH and Hamburg cohort response data are more relevant than the BASF and Seveso data for assessing cancer risks from continuous ambient TCDD exposure in the general population.

The NIOSH (Steenland et al., 2001, 197433; Cheng et al., 2006, 523122) and Hamburg (Becher et al., 1998, 197173) cohort studies report cumulative TCDD levels in the serum for cohort members. The most significant difference among the Cheng et al. (2006, 523122) analysis and those of Steenland et al. (2001, 197433) and Becher et al. (1998, 197173) is the method used to back-extrapolate exposure concentrations based on serum TCDD measurements. Steenland et al. (2001, 197433) and Becher et al. (1998, 197173) back-extrapolated exposures and body burdens using a first-order model with a constant half-life. In contrast, Cheng et al. (2006, 523122) back-extrapolated body burdens using a kinetic modeling approach that incorporated concentration- and age-dependent elimination kinetics.

Although all three of these are high-quality studies, the kinetic modeling used by Cheng et al. (2006, 523122) is judged to better reflect TCDD pharmacokinetics, as currently understood, than the first-order models used by Steenland et al. (2001, 197433) and Becher et al. (1998, 197173). EPA believes that the representation of physiological processes provided by Cheng et al (2006, 523122) is more realistic than the assumption of simple first-order kinetics and this outweighs the attendant modeling uncertainties. Furthermore, the use of kinetic modeling is consistent with recommendations both by the NAS and the Dioxin Workshop panel.

EPA, therefore, has decided to use the results of the Cheng et al. (2006, 523122) study for derivation of the TCDD OSF based on total cancer mortality as calculated by EPA using data and models from the Cheng et al. (2006, 523122) study, as described in Section 5.2.3.1.2. Although the OSF is only strictly defined for exposures above the background exposure experienced by the NIOSH cohort, which was assumed to be 0.5 pg/kg-day TCDD, or 5 pg/kg-day total TEQ, EPA assumes that the slope (risk vs. blood concentration) is the same below those background exposure levels as it is above. Table ES-1 shows the oral slope factors at specific target risk levels (OSF_{RL}s) which range from 1.1×10^{5} to 1.3×10^{6} per (mg/kg-day). EPA recommends the use of an OSF of 1×10^{6} per (mg/kg-day) when the target risk range is 10^{-5} to 10^{-7}.

CONSIDERATION OF NONLINEAR DOSE-RESPONSE APPROACHES FOR CANCER

The NAS focused much of its review on EPA's derivation of a cancer slope factor, commenting extensively on the extrapolation of dose-response modeling below the POD. The NAS questioned EPA's choice of a linear, nonthreshold model for extrapolating risk associated with exposure levels below the POD, concluding that the current scientific evidence was sufficient to justify the use of nonlinear methods when extrapolating below the POD for dioxin carcinogenicity.

While, based on the 2005 Cancer Guidelines, EPA deemed linear extrapolation to be most appropriate for TCDD, EPA carefully considered the NAS recommendation to provide risk estimates using both linear and nonlinear methods. In this document, EPA has evaluated the information available for identifying a threshold and for estimating the shape of the dose-response curve below the POD (see Section 5.2.3.4). EPA presents a hypothetical sublinear dose-response modeling example of rodent carcinogenicity. EPA also presents two illustrative examples of RfD development (i.e., nonlinear method) for carcinogenic effects of TCDD, using data derived from animal bioassays. EPA derives illustrative RfDs for cancer based on combined tumor response and also on hypothesized key events in TCDD's MOA for female rat liver and lung tumors. EPA identifies a number of limitations that prevent making strong conclusions based on the nonlinear dose-response modeling exercises.

FEASIBILITY OF QUANTITATIVE UNCERTAINTY ANALYSIS

EPA also addresses the third key recommendation of the NAS, specifically, improving transparency, thoroughness, and clarity in *quantitative uncertainty analysis* (see Section 6). In summary, NAS suggested that EPA should

- describe and define (quantitatively to the extent possible) the variability and uncertainty for key assumptions used for each key endpoint-specific risk assessment (choices of data set, POD, model, and dose metric),
- incorporate probabilistic models to the extent possible to represent the range of plausible values,
- clearly state it when quantitation is not possible and explain what would be required to achieve quantitation (NAS, 2006, 198441, p. 9).

Although the NAS summarized the shortfalls in the 2003 Reassessment categorically, the elaborations within their report often contain the qualification "if possible" and do not take a position with regard to the feasibility of many suggestions. With appreciation for the extent of information available for dioxin, EPA's goal herein was to examine the feasibility of a data-driven quantitative uncertainty analysis for TCDD dose-response assessment.

In examining feasibility of quantitative uncertainty analysis, EPA recognized that different kinds of uncertainty require different statistical treatment. *Cognitive uncertainty* concerns uncertainty that can be expressed as probabilities and may be operationalized using either frequentist or Bayesian approaches. For example, classical statistical methods yield distributions on model parameters which reflect sample fluctuations, assuming that the model is true. This type of uncertainty can be taken into account in the BMDL estimation. Also, for TCDD epidemiologic data, the dose reconstruction often involves assumptions that may be amenable to data-driven uncertainty analysis if sufficient data can be retrieved; back-extrapolated TCDD levels, biological half-life, body fat, and background levels are example variables that could be included in such an analysis. In addition, a Monte Carlo analysis has been examined to develop quantitative uncertainty distributions for the RfD (e.g., Swartout et al., 1998, 093460). Given a set of animal bioassay data, quantifying dose-response uncertainty may be approached in different ways. The differences reflect different types of uncertainty that are captured. A recent evaluation enumerates the following possible methodologies (Bussard et al., 2009, 543770):

> **Benchmark Dose Modeling (BMD)**: Choose the 'best' model, and assess uncertainty assuming this model is true. Supplemental results can compare estimates obtained with different models, and sensitivity analyses can investigate other modeling issues.
>
> **Probabilistic Inversion with Isotonic Regression (PI-IR)**: Define model-independent 'observational' uncertainty, and look for a model that captures this uncertainty by assuming the selected model is true and providing for a distribution over its parameters.
>
> **Non-Parametric Bayes (NPB)**: Choose a prior mean response (potency) curve (potentially a "non-informative prior") and a precision parameter to express prior uncertainty over all increasing dose-response relations, and update this prior distribution with the bioassay data.

Bayesian Model Averaging (BMA) (as considered here): Choose an initial set of models, and then estimate the parameters of each model with maximum likelihood. Use classical methods to estimate parameter uncertainty, given the truth of the model. Determine a probability weight for each model using the Bayes Information Criterion (BIC), and use these weights to average the model results.

The first of the above methods involves standard classical statistical methods and captures sampling uncertainty conditional on the truth of the model used. The other methods are "exotic" in the sense that they attempt to capture uncertainty that is not conditional on the truth of a given model. In this response document, EPA has not applied such methods, but recognizes that quantitative uncertainty analysis is possible in these cases.

In contrast to cognitive uncertainty, *Volitional uncertainty* concerns uncertainty regarding choices on the best course of action to take; volitional uncertainty cannot be analyzed by sampling from a probability distribution and, thus, is not amenable to a complete quantitative uncertainty analysis. Some of the choices made in TCDD dose-response assessment that are volitional include: choice of occupational cohort data set or bioassay data set; choice of PODs (e.g., ED_{01}, ED_{05}, and ED_{10}); choice of species, strain, or sex within an animal bioassay; and choice of dose metric (e.g., administered doses, blood concentrations, lipid-adjusted serum concentrations). These volitional uncertainties cannot be quantified by sampling an input distribution.

Although EPA has determined that a comprehensive quantitative uncertainty analysis is not feasible because of the limitations discussed above, EPA believes the NAS was requesting that dose-response modeling results be shown for specific choices of interest to TCDD assessment. In response to the NAS concerns, this document provides some limited quantitative comparisons. BMDs, BMDLs, and OSFs from the animal cancer bioassay benchmark dose modeling assuming 1, 5, and 10% extra risk are compared in units of blood concentrations and human equivalent doses (see Tables 5-18 and 5-19, respectively). In addition, central tendency slope estimates and upper bound slope factor estimates based on Cheng et al. (2006, 523122) are presented (see Tables 5-3 and 5-4). For the noncancer effects, key animal study PODs (ng/kg-day) are shown based on different dose metrics: administered dose, first-order body burden HED, and blood concentration (Tables 4-3 and 4-4). EPA has undertaken some limited quantitative uncertainty analyses for the kinetic modeling, presenting a sensitivity analysis and

uncertainty analysis in dose metrics derived for the risk assessment of TCDD and a detailed discussion on the uncertainty in choice of PBPK model-driven dose metrics. (see Sections 3.3.3 and 3.3.5). TCDD kinetic doses from the Emond et al. (2005, 197317; 2006, 197316) PBPK model that is primarily used in the technical analysis in this document are compared with those predicted by the Aylward et al. (2005, 197114) model.

Uncertainty quantification is an emerging area in science. There are many examples of highly vetted and peer-reviewed uncertainty analyses based on structured expert judgment. Under this process, experts in effect synthesize a wide diversity of information in generating their subjective probability distributions. Where considerable data exist for an environmental pollutant, such as for the well-studied TCDD, it is natural to ask whether these extensive data can be leveraged more directly in uncertainty quantification. This is an area where research could be focused. Additional research topics relevant to dioxin that could further inform health assessments include population variability of biokinetic constants and threshold mechanisms for the mass action model. Further data and improved methodologies in these areas, combined with developments illustrated elsewhere in this report, will help reduce or better quantify uncertainties and strengthen EPA's understanding of potential health implications of environmental TCDD exposures.

Table ES-1. Comparison of fat concentrations, risk specific dose estimates and equivalent oral slope factors based on upper 95th percentile estimate of regression coefficient[a] of all fatal cancers reported by Cheng et al. (2006, 523122) for selected risk levels

Risk level (RL)	AUC_{RL} (ppt-yr)	FAT_{RL} (ng/kg)	Risk specific dose[b] (D_{RL}) (ng/kg-day)	Equivalent oral slope factors (OSF_{RL}) per (mg/kg-day)
1×10^{-2}	1.262×10^{4}	1.803×10^{2}	8.79×10^{-2}	1.1×10^{5}
5×10^{-3}	6.432×10^{3}	9.189×10^{1}	3.14×10^{-2}	1.6×10^{5}
1×10^{-3}	1.307×10^{3}	1.867×10^{1}	2.88×10^{-3}	3.5×10^{5}
5×10^{-4}	6.546×10^{2}	9.352×10^{0}	9.56×10^{-4}	5.2×10^{5}
1×10^{-4}	1.311×10^{2}	1.873×10^{0}	1.29×10^{-4}	7.8×10^{5}
5×10^{-5}	6.558×10^{1}	9.368×10^{-1}	5.52×10^{-5}	9.1×10^{5}
1×10^{-5}	1.312×10^{1}	1.874×10^{-1}	8.94×10^{-6}	1.1×10^{6}
5×10^{-6}	6.559×10^{0}	9.370×10^{-2}	4.25×10^{-6}	1.2×10^{6}
1×10^{-6}	1.312×10^{0}	1.874×10^{-2}	8.08×10^{-7}	1.2×10^{6}
5×10^{-7}	6.559×10^{-1}	9.370×10^{-3}	4.00×10^{-7}	1.3×10^{6}
1×10^{-7}	1.312×10^{-1}	1.874×10^{-3}	7.92×10^{-8}	1.3×10^{6}

[a]Based on regression coefficient of Cheng et al. (2006, 523122, Table III), excluding observations in the upper 5% range of the exposures; where reported $\beta = 3.3 \times 10^{-6}$ ppt-years and standard error = 1.4×10^{-6}. Upper 95th percentile estimate of regression coefficient (β_{95}) calculated to be $6.04 \times 10^{-6} = (3.3 \times 10^{-6}) + 1.96 \times (1.4 \times 10^{-6})$; background cancer mortality risk is assumed to be 0.112 as reported by Cheng et al. (2006, 523122).

[b]To calculate the extra cancer risk (ER) and OSF for any TCDD daily oral intake (D):

1. For D in ng/kg-d, look up the corresponding fat concentration (ng/kg = ppt) from the conversion chart (nongestational lifetime dose metrics) in Appendix C.4.1.
2. Calculate the AUC in ppt-yrs by multiplying the fat concentration by 70 years.
3. Calculate Extra Risk (ER) using the following equation:
   ER = [exp(AUC × 6.04E-6) × 0.112 − 0.112] ÷ 0.888.
4. Calculate the OSF $(mg/kg\text{-}d)^{-1}$ = 1E6 × (ER ÷ D).

Example for risk at the RfD: $D = 7 \times 10^{-4}$ ng/kg-d; fat concentration = 6.93 ng/kg;
AUC = 70 years × 6.93 ppt = 485 ppt-year;
ER = exp(485 ppt-year × 6.04E-6 $(ppt\text{-}yr)^{-1}$) × 0.112 – 0.112) ÷ 0.888 = 3.7×10^{-4}
OSF = 1E6 ng/mg × ($3.7 \times 10^{-4} \div 7 \times 10^{-4}$ ng/kg-d) = 5.3×10^{5} $(mg/kg\text{-}d)^{-1}$.

Table ES-2. Tumor points of departure and oral slope factors using blood concentrations

Study	Sex/species: tumor sites	$BMDL_{01HED}$[a] (ng/kg-day)	OSF (per mg/kg-day)
NTP, (1982, 543764)	Male mice: liver adenoma and carcinoma, lung	1.1E−03	9.4E+6
Toth et al., (1979, 197109)	Male mice: liver tumors	1.9E−03	5.2E+6
NTP, (1982, 543764)	Female mice: liver adenoma and carcinoma, thyroid adenoma, subcutaneous fibrosarcoma, all lymphomas	5.3E−03	1.9E+6
NTP, (1982, 543764)	Female rats: liver neoplasitc nodules, liver adenoma and carcinoma, adrenal cortex adenoma or carcinoma, thyroid follicular cell adenoma	5.7E−03	1.8E+6
Kociba et al., (1978, 001818)	Female rats: liver adenoma carcinoma, oral cavity, lung	7.3E−03	1.4E+6
NTP, (1982, 543764)	Male rats: thyroid follicular cell adenoma, adrenal cortex adenoma	9.6E−03	1.0E+6
Della Porta et al., (1987, 197405)	Male mice: Hepatocellular carcinoma	3.1E−02	3.2E+5
NTP, (2006, 197605)	Female rats: liver cholangiocarcinoma, hepatocellular adenoma, oral mucosa squamous cell carcinoma, lung cystic keratinizing epithelioma, pancreas adenoma, carcinoma	2.3E−02	4.4E+5
Kociba et al., (1978, 001818)	Male rats: adrenal cortex adenoma, tongue carcinoma, nasal/palate carcinoma	3.1E−02	3.2E+5

[a]$BMDL_{HED}$s are from the multiple tumor analyses, with the exception of Toth et al. (1979, 197109) and Della Porta et al. (1987, 197405) which are the result of modeling single tumor sites.

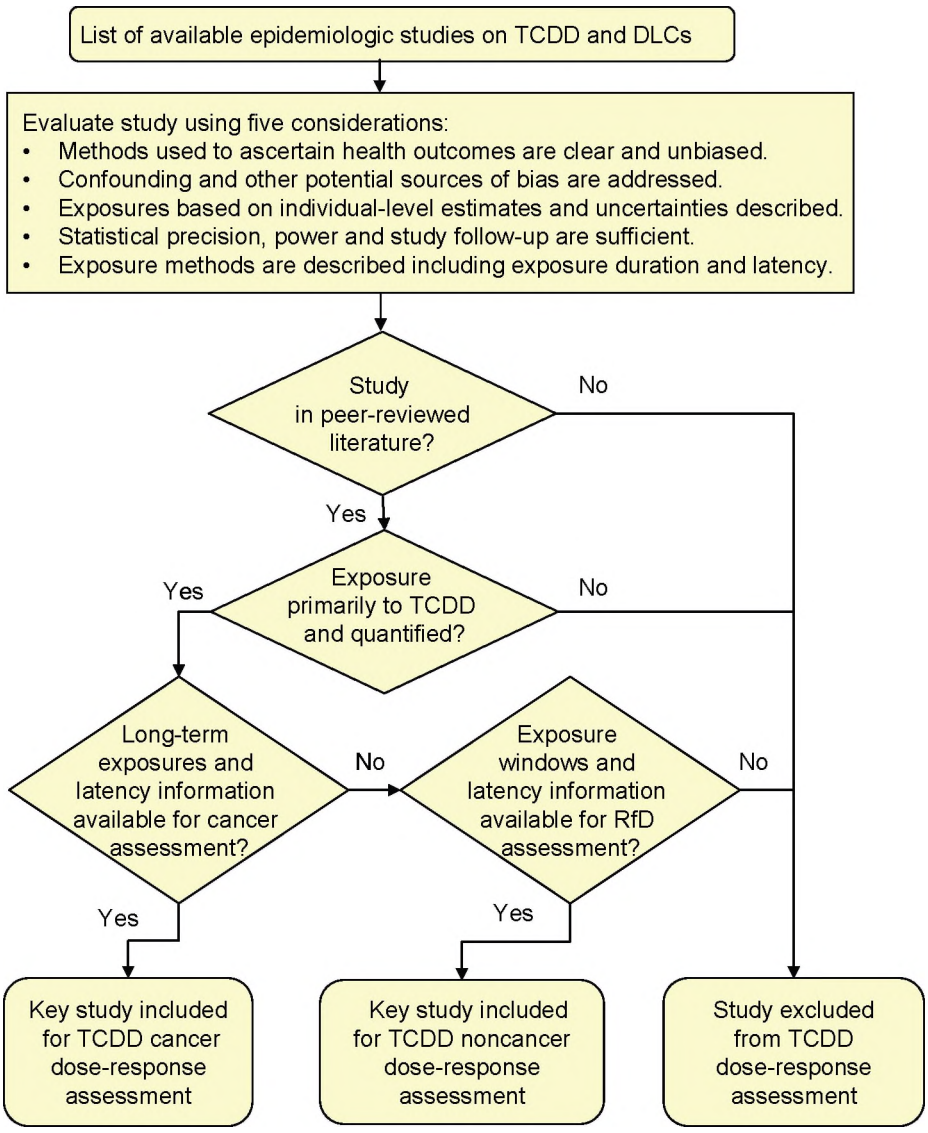


Figure ES-1. EPA's process to evaluate available epidemiologic studies using study inclusion criteria for use in the dose-response analysis of TCDD. EPA applied its TCDD-specific epidemiologic study inclusion criteria to all studies published on TCDD and DLCs. The studies were initially evaluated using five considerations regarded as providing the most relevant kind of information needed for quantitative human health risk analyses. For each study that was published in the peer-reviewed literature, EPA then examined whether the exposures were primarily to TCDD and if the TCDD exposures could be quantified so that dose-response analyses could be conducted. Finally, EPA required that the effective dose and oral exposure be estimable: (1) for cancer, information is required on long-term exposures, (2) for noncancer, information is required regarding the appropriate time window of exposure that is relevant for a specific, nonfatal health endpoint, and (3) for all endpoints, the latency period between TCDD exposure and the onset of the effect is needed. Only studies meeting these criteria were included in EPA's TCDD dose-response analysis.

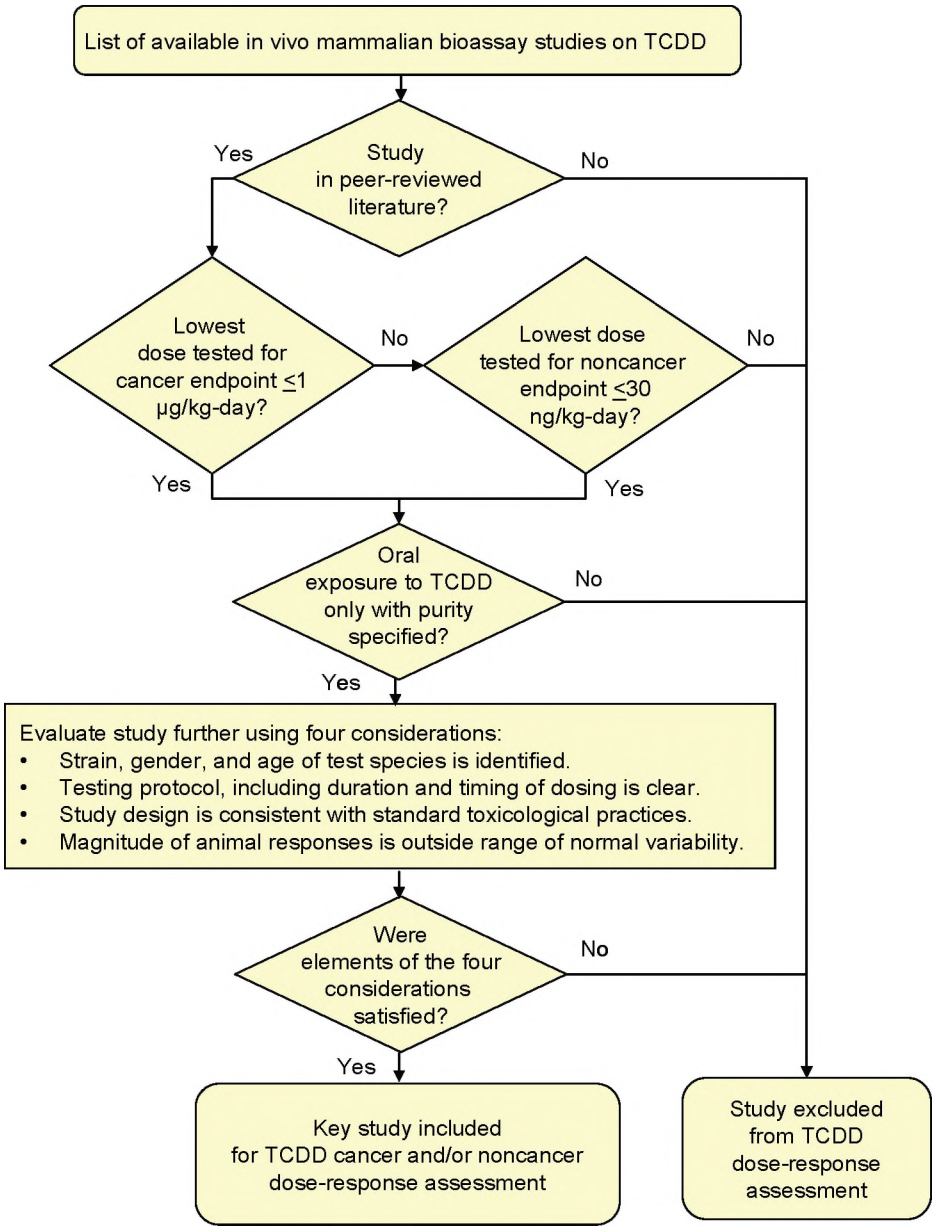


Figure ES-2. EPA's process to evaluate available animal bioassay studies using study inclusion criteria for use in the dose-response analysis of TCDD. EPA evaluated all available in vivo mammalian bioassay studies on TCDD. Studies had to be published in the peer-reviewed literature. Next, to ensure working in the low-dose range for TCDD dose-response analysis, EPA applied dose requirements to the lowest tested average daily doses in each study, with specific requirements for cancer (≤1 µg/kg-day), and noncancer (≤30 ng/kg-day) studies. Third, EPA required that the animals were exposed via the oral route to only TCDD and that the purity of the TCDD was specified. Finally, the studies were evaluated using four considerations regarded as providing the most relevant kind of information needed for quantitative human health risk analyses from animal bioassay data. Only studies meeting all of these criteria and considerations were included in EPA's TCDD dose-response analysis.

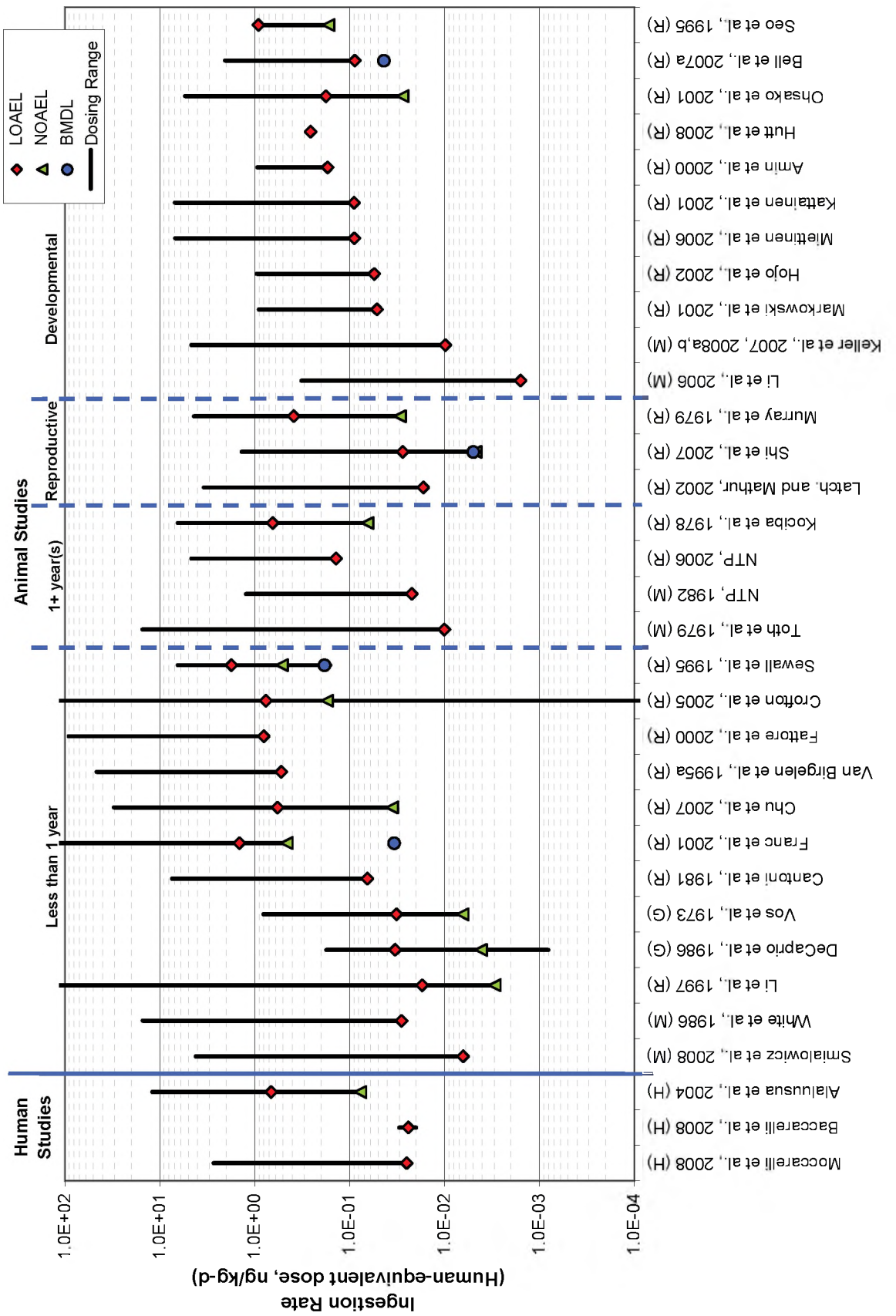


Figure ES-3. Exposure-response array for ingestion exposures to TCDD.

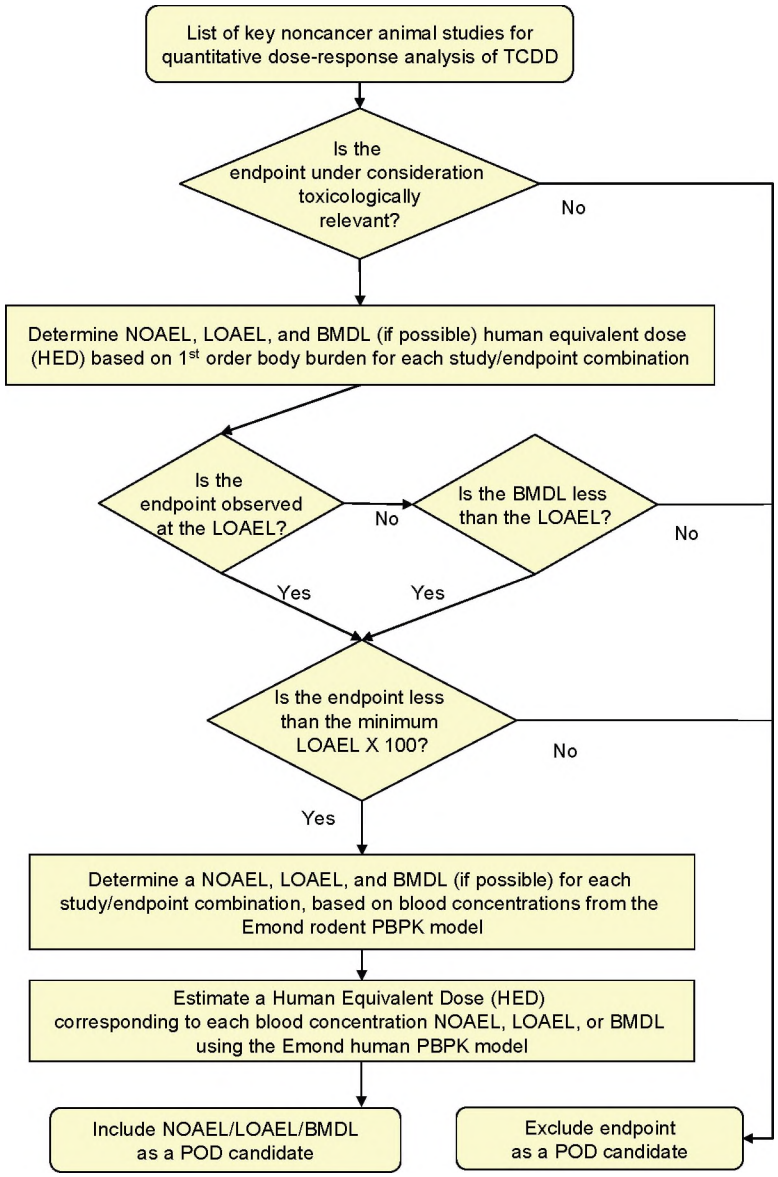


Figure ES-4. EPA's process to select and identify candidate PODs from key animal bioassays for use in noncancer dose-response analysis of TCDD. For each noncancer endpoint found in the studies that qualified for TCDD dose-response assessment using the study inclusion criteria, EPA first determined if the endpoint was toxicologically relevant. If so, EPA determined the NOAEL, LOAEL, and BMDL Human Equivalent Dose (HED) based on 1st-order body burdens for each endpoint. These potential PODs were examined for statistical relevance and included when the endpoint was observed at the LOAEL. If the BMDL was less than the LOAEL, and if the endpoint was less than the minimum LOAEL × 100, EPA then calculated NOAELs, LOAELs, or BMDLs based on blood concentrations from the Emond rodent PBPK model. Then, for all of the candidate PODs, HEDs were estimated using the Emond human PBPK model. Finally, the lowest group of the toxicologically relevant candidate PODs was selected for final use in derivation of an RfD.

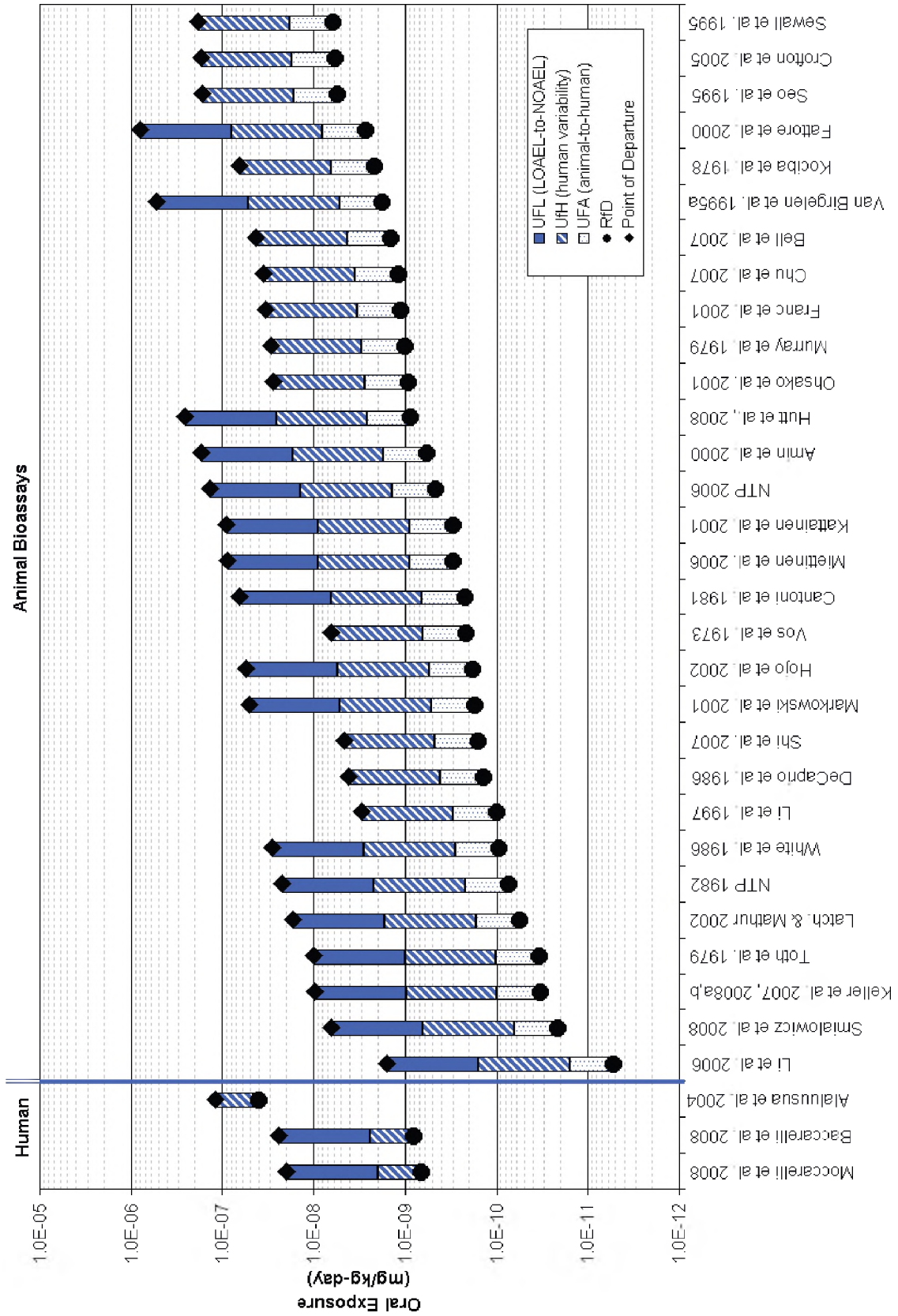


Figure ES-5. Candidate RfD array.

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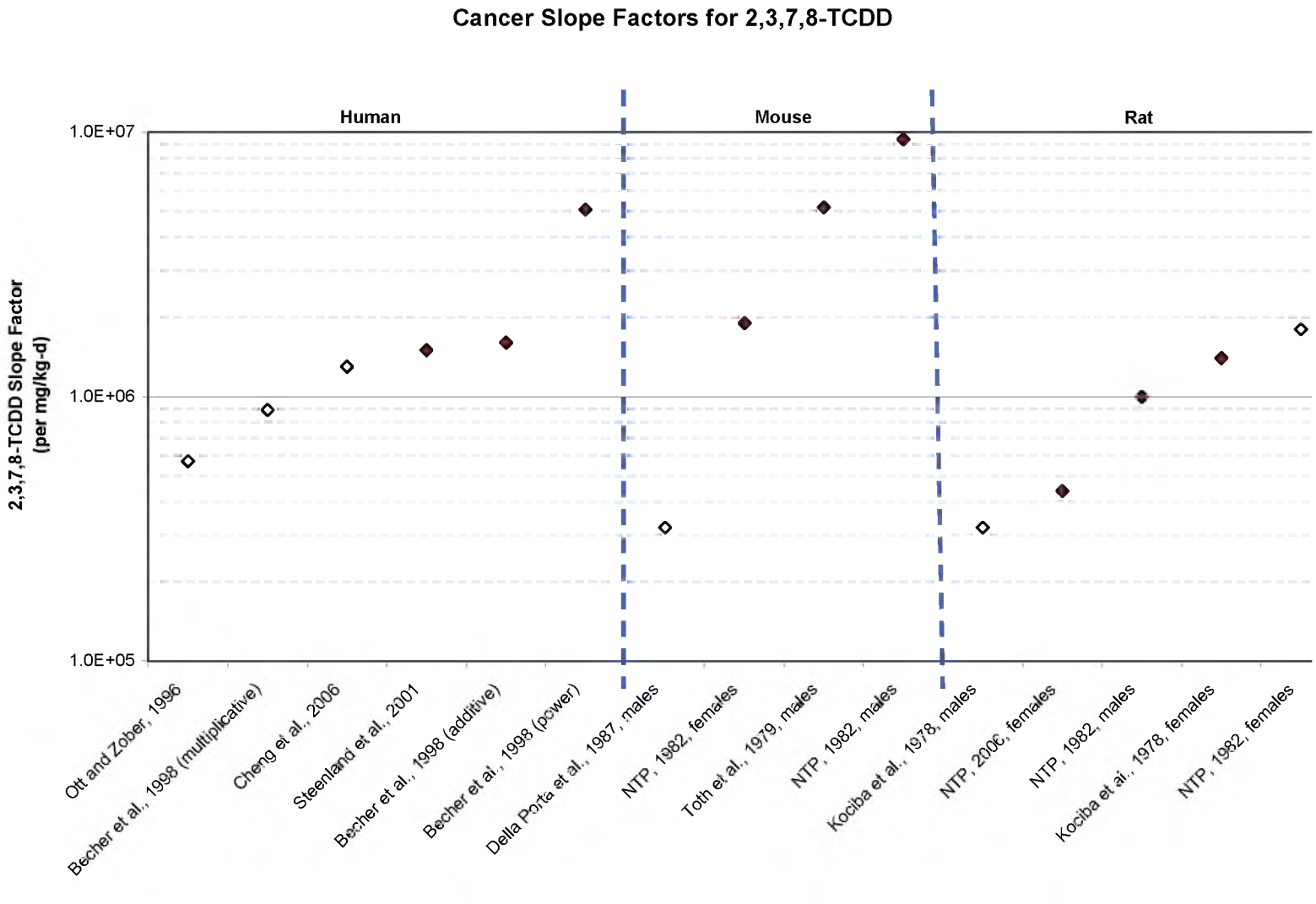


Figure ES-6. Candidate oral slope factor array.

1. INTRODUCTION

Dioxins and dioxin-like compounds (DLCs), including polychlorinated dibenzo-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls are structurally and toxicologically related halogenated dicyclic aromatic hydrocarbons.[7] Dioxins and DLCs are released into the environment from several industrial sources such as chemical manufacturing, combustion, and metal processing; from individual activities including the burning of household waste; and from natural processes such as forest fires. Dioxins and DLCs are widely distributed throughout the environment and typically occur as chemical mixtures. Additionally, they do not readily degrade; therefore, levels persist in the environment, build up in the food chain, and accumulate in the tissues of animals. Human exposure to these compounds occurs primarily through the ingestion of contaminated foods (Lorber et al., 2009, 543766), although exposures to other environmental media and by other routes and pathways do occur.

The health effects from exposures to dioxins and DLCs have been documented extensively in epidemiologic and toxicologic studies. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most toxic members of this class of compounds and has a robust toxicologic database. Characterization of TCDD toxicity is critical to the risk assessment of mixtures of dioxins and DLCs because it has been selected repeatedly as the "index chemical" to serve as the basis for standardization of the toxicity of components in a mixture of dioxins and DLCs. The dose-response information for TCDD is used to evaluate risks from exposure to mixtures of DLCs (Van et al., 1998, 198345; Van den Berg et al., 2006, 543769; also see the World Health Organization's Web site for the dioxin toxicity equivalence factors [TEFs]),[8] therefore, it is imperative to correctly assess the dose response of TCDD and understand the uncertainties and limitations therein.

In 2003, the U.S. Environmental Protection Agency (EPA) produced an external review draft of the multiyear comprehensive reassessment of dioxin exposure and human health effects entitled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* (U.S. EPA, 2003, 537122). This draft report, herein called the

[7]For further information on the chemical structures of these compounds, see U.S. EPA (2003, 537122; 2008, 543774).
[8]Available at http://www.who.int/ipcs/assessment/tef_update/en/.

"2003 Reassessment," consisted of (1) a scientific review of information relating to sources of and exposures to TCDD, other dioxins, and DLCs in the environment; (2) detailed reviews of scientific information on the health effects of TCDD, other dioxins, and DLCs; and (3) an integrated risk characterization for TCDD and related compounds.

In 2004, EPA asked the National Research Council of the National Academy of Sciences (NAS) to review the 2003 Reassessment. The NAS Statement of Task was as follows

> The National Academies' National Research Council will convene an expert committee that will review EPA's 2003 draft reassessment of the risks of dioxins and dioxin-like compounds to assess whether EPA's risk estimates are scientifically robust and whether there is a clear delineation of all substantial uncertainties and variability. To the extent possible, the review will focus on EPA's modeling assumptions, including those associated with the dose-response curve and points of departure; dose ranges and associated likelihood estimates for identified human health outcomes; EPA's quantitative uncertainty analysis; EPA's selection of studies as a basis for its assessments; and gaps in scientific knowledge. The study will also address the following aspects of EPA's 2003 Reassessment: (1) the scientific evidence for classifying dioxin as a human carcinogen; and (2) the validity of the nonthreshold linear dose-response model and the cancer slope factor calculated by EPA through the use of this model. The committee will also provide scientific judgment regarding the usefulness of toxicity equivalence factors (TEFs) in the risk assessment of complex mixtures of dioxins and the uncertainties associated with the use of TEFs. The committee will also review the uncertainty associated with the 2003 Reassessment's approach regarding the analysis of food sampling and human dietary intake data, and, therefore, human exposures, taking into consideration the Institute of Medicine's report Dioxin and Dioxin-Like Compounds in the Food Supply: Strategies to Decrease Exposure. The committee will focus particularly on the risk characterization section of EPA's 2003 Reassessment report and will endeavor to make the uncertainties in such risk assessments more fully understood by decision makers. The committee will review the breadth of the uncertainty and variability associated with risk assessment decisions and numerical choices, including, for example, modeling assumptions, including those associated with the dose-response curve and points of departure. The committee will also review quantitative uncertainty analyses, as feasible and appropriate. The committee will identify gaps in scientific knowledge that are critical to understanding dioxin reassessment (NAS, 2006, 198441, p. 43, Box 1-1).

In 2006, the NAS published its review of EPA's 2003 Reassessment entitled *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NAS, 2006, 198441).

1.1. SUMMARY OF KEY NAS (2006, 198441) COMMENTS ON DOSE-RESPONSE MODELING IN THE 2003 REASSESSMENT

While recognizing the effort that EPA expended to prepare the 2003 Reassessment, the NAS committee identified three key areas that they believe require substantial improvement to support a scientifically robust risk assessment. These three key areas are

- transparency and clarity in selection of key data sets for analysis;
- justification of approaches to dose-response modeling for cancer and noncancer endpoints; and
- transparency, thoroughness, and clarity in quantitative uncertainty analysis.

In their Public Summary, the NAS made the following overall recommendations to aid EPA in addressing their key concerns:

- EPA should compare cancer risks by using nonlinear models consistent with a receptor mediated mechanism of action and by using epidemiological data and the new National Toxicology Program (NTP) animal bioassay data (NTP, 2006, 197605). The comparison should include upper and lower bounds, as well as central estimates of risk. EPA should clearly communicate this information as part of its risk characterization (NAS, 2006, 198441, p. 9).
- EPA should identify the most important data sets to be used for quantitative risk assessment for each of the four key end points (cancer, immunotoxicity, reproductive effects, and developmental effects). EPA should specify inclusion criteria for the studies (animal and human) used for derivation of the benchmark dose (BMD) for different noncancer effects and potentially for the development of RfD (reference dose) values and discuss the strengths and limitations of those key studies; describe and define (quantitatively to the extent possible) the variability and uncertainty for key assumptions used for each key end-point-specific risk assessment (choices of data set, POD [point of departure], model, and dose metric); incorporate probabilistic models to the extent possible to represent the range of plausible values; and assess goodness-of-fit of dose-response models for data sets and provide both upper and lower bounds on central estimates for all statistical estimates. When quantitation is not possible, EPA should clearly state it and explain what would be required to achieve quantitation (NAS, 2006, 198441, p. 9).
- When selecting a BMD as a POD, EPA should provide justification for selecting a response level (e.g., at the 10%, 5%, or 1% level). In either case, the effects of this choice on the final risk assessment values should be illustrated by comparing point estimates and lower bounds derived from selected PODs (NAS, 2006, 198441, p. 9).
- EPA should continue to use body burden as the preferred dose metric but should also consider physiologically based pharmacokinetic modeling as a means to adjust for differences in body fat composition and for other differences between rodents and humans (NAS, 2006, 198441, p. 9).
- Although EPA addressed many sources of variability and uncertainty qualitatively, the committee noted that the 2003 Reassessment would be substantially improved if its risk characterization included more quantitative approaches. Failure to characterize

> variability and uncertainty thoroughly can convey a false sense of precision in the conclusions of the risk assessment (NAS, 2006, 198441, p. 5).

Importantly, the NAS encouraged EPA to calculate an RfD as the 2003 Reassessment does not contain an RfD derivation. The committee suggested that

> …estimating an RfD would provide useful guidance to risk managers to help them (1) assess potential health risks in that portion of the population with intakes above the RfD, (2) assess risks to population subgroups, such as those with occupational exposures, and (3) estimate the contributions to risk from the major food sources and other environmental sources of TCDD, other dioxins, and DLCs for those individuals with high intakes (NAS, 2006, 198441, p. 6).

The NAS made many thoughtful and specific recommendations throughout their review; additional NAS recommendations and comments pertaining to the dose-response assessment of TCDD will be presented and addressed in various sections throughout this document.

1.2. EPA'S SCIENCE PLAN

In May 2009, EPA Administrator Lisa P. Jackson announced the "*Science Plan for Activities Related to Dioxins in the Environment*" ("Science Plan") that addressed the need to finish EPA's dioxin reassessment and provide a completed health assessment on this high profile chemical to the American public as quickly as possible.[9] The Science Plan states that EPA will release a draft report that responds to the recommendations and comments included in the NAS review of EPA's 2003 Reassessment, and that, in this draft report, EPA's National Center for Environmental Assessment, Office of Research and Development, will provide a limited response to key comments and recommendations in the NAS report (draft response to comments report). This draft response is to focus on dose-response issues raised by the NAS and include analyses of relevant new key studies. The draft response is to be provided for public review and comment and for independent external peer review by EPA's Science Advisory Board. Following completion of this report, EPA is to review the impacts of the response to comments report on its 2003 Reassessment.

[9]Available at http://www.epa.gov/dioxin/scienceplan.

This draft document comprises EPA's report that responds both directly and technically to the recommendations and comments on TCDD dose-response assessment included in the NAS review of EPA's 2003 Reassessment. This document focuses on TCDD only. Because new data are analyzed in this report and toxicity values are derived, this document will follow the IRIS process for review, clearance and completion; however, it is not a traditional IRIS document. Information developed in this document is intended to not only respond to the NAS review, but also to expand EPA's knowledge of TCDD cancer and noncancer dose-response based on the most current literature, existing methods, and adherence to EPA risk assessment guidance documents. Following completion of this document, EPA will consider its contents as it reviews the TCDD risk assessment information presented in the 2003 Reassessment and moves forward towards completion of the dioxin reassessment.

1.3. OVERVIEW OF EPA'S RESPONSE TO NAS (2006, 198441) "HEALTH RISKS FROM DIOXIN AND RELATED COMPOUNDS: EVALUATION OF EPA's 2003 REASSESSMENT"

In their key recommendations, the NAS commented that EPA should thoroughly justify and communicate approaches to dose-response modeling, increase transparency in the selection of key data sets, and improve the communication of uncertainty (particularly quantitative uncertainty). They also encouraged EPA to calculate an RfD. These main areas of improvement refer to issues specifically related to TCDD dose-response assessment (and uncertainty analysis); therefore, as noted in the Science Plan, EPA's response to the NAS is particularly focused on these issues.

EPA thoroughly considered the recommendations of the NAS and responds with scientific and technical evaluation of TCDD dose–response data via:

- an updated literature search that identified new TCDD dose-response studies (see Section 2);
- a kickoff workshop that included the participation of external experts in TCDD health effects, toxicokinetics, dose-response assessment and quantitative uncertainty analysis; these experts discussed potential approaches to TCDD dose-response assessment and considerations for EPA's response to NAS (U.S. EPA, 2009, 543757, Appendix A);
- detailed study inclusion criteria and processes for the selection of key studies (see Section 2.3) and epidemiologic and animal bioassay data for TCDD dose-response assessment (see Section 2.4.1/Appendix B and Section 2.4.2, respectively);

- kinetic modeling to quantify appropriate dose metrics for use in TCDD dose-response assessment (see Section 3 and Appendices C and D);
- dose-response modeling for all appropriate noncancer and cancer data sets (see Section 4.2/Appendix E and Section 5.2.3/Appendix F, respectively);
- thorough and transparent evaluation of the selected TCDD data for use in the derivation of an RfD and an oral slope factor (OSF) (see Sections 4.2 and 5.2.3, respectively);
- the development of an RfD (see Section 4.3);
- the development of a revised OSF (see Section 5.3) with an updated cancer weight of evidence determination for TCDD based on EPA's 2005 *Cancer Guidelines* (2005, 086237) (see Section 5.1.2);
- consideration of nonlinear dose-response approaches for cancer, including illustrative RfDs for cancer precursor events and tumors (see Section 5.2.3.4); and
- discussion of the feasibility and utility of quantitative uncertainty analysis for TCDD dose-response assessment (see Section 6).

Each of these activities is described in detail in subsequent sections of this document.

In addition to this document, it should be noted that three separate EPA activities address other TCDD issues, specifically related to the application of dioxin TEFs and to TCDD and DLC background exposure levels. Information on the application of the dioxin TEFs is published elsewhere by EPA for both ecological (U.S. EPA, 2008, 543774) and human health risk assessment (U.S. EPA, 2009, 192196). As a consequence, EPA does not directly address TEFs herein, but makes use of the concept of toxicity equivalence[10] as applicable to the analysis of exposure dose in epidemiologic studies. Furthermore, this document does not address the NAS recommendations pertaining to the assessment of human exposures to TCDD and other dioxins. Information on updated background levels of dioxin in the U.S. population has been recently reported (Lorber et al., 2009, 543766).

1.3.1. TCDD Literature Update

EPA has developed a literature database of peer-reviewed studies on TCDD toxicity, including in vivo mammalian dose-response studies and epidemiologic studies. An initial literature search for studies published since the 2003 Reassessment was conducted by the U.S. Department of Energy's Argonne National Laboratory (ANL) through an Interagency Agreement

[10]Toxicity equivalence (TEQ) is the product of the concentration of an individual DLC in an environmental mixture and the corresponding TCDD TEF for that compound. These products are summed to yield the TEQ of the mixture.

with EPA. ANL used the online National Library of Medicine database (PubMed) and identified studies published between the year 2000 and October 31, 2008. Supporting references published since the release of the 2003 Reassessment were also identified. Supporting studies were classified as studies pertaining to TCDD kinetics, TCDD mode-of-action, in vitro TCDD studies, and TCDD risk assessment approaches. The literature search strategy explicitly excluded studies addressing (1) analytical/detection data and cellular screening assays; (2) environmental fate, transport and concentration data; (3) dioxin-like compounds and toxic equivalents; (4) nonmammalian dose-response data; (5) human exposure analyses only, including body burden data; and (6) combustor or incinerator or other facility-related assessments absent primary dose-response data. EPA published the initial literature search results in the Federal Register on November 24, 2008 (73 FR 70999; November 24, 2008) and invited the public to review the list and submit additional peer-reviewed in vivo mammalian dose-response studies for TCDD, including epidemiologic studies that were absent from the list (U.S. EPA, 2008, 519261). Submissions were accepted by the EPA through an electronic docket, email and hand delivery, and were evaluated for use in TCDD dose-response assessment. The literature search results and subsequent submissions were used during a 2009 scientific workshop, which was open to the public and featured a panel of experts on TCDD toxicity and dose-response modeling (discussed below). Additional studies identified during the workshop and those collected by EPA scientists during the development of this report through October 2009 have been incorporated into the final set of studies for TCDD dose-response assessment.

1.3.2. EPA's 2009 Workshop on TCDD Dose Response

To assist EPA in responding to the NAS, EPA and ANL convened a scientific workshop (the "Dioxin Workshop") on February 18–20, 2009, in Cincinnati, Ohio. The goals of the Dioxin Workshop were to identify and address issues related to the dose-response assessment of TCDD and to ensure that EPA's response to the NAS focused on the key issues and reflected the most meaningful science. The Dioxin Workshop included seven scientific sessions: quantitative dose-response modeling issues, immunotoxicity, neurotoxicity and nonreproductive endocrine effects, cardiovascular toxicity and hepatotoxicity, cancer, reproductive and developmental toxicity, and quantitative uncertainty analysis of dose-response. During each session, EPA asked a panel of expert scientists to perform the following tasks:

- Identify and discuss the technical challenges involved in addressing the NAS comments related to the dose-response issues within each specific session topic and the TCDD quantitative dose-response assessment.
- Discuss approaches for addressing the key NAS recommendations.
- Identify important published, independently peer-reviewed literature—particularly studies describing epidemiologic studies and in vivo mammalian bioassays expected to be most useful for informing EPA's response.

The sessions were followed by open comment periods during which members of the audience were invited to address the expert panels. The session's Panel Co-chairs were asked to summarize and present the results of the panel discussions—including the open comment periods. The summaries incorporated points of agreement as well as minority opinions. Final session summaries were prepared by the session Panel Co-chairs with input from the panelists, and they formed the basis of a final workshop report (U.S. EPA, 2009, 543757, Appendix A of this report). Because the sessions were not designed to achieve consensus among the panelists, the summaries do not necessarily represent consensus opinions; rather reflect the core of the panel discussions. Some of the key discussion points from the workshop that influenced EPA's development of this document are listed below (see Appendix A for detail):

- In the development of study selection criteria, more relevant exposure-level (i.e., dose) decision points using tissue concentrations could be defined.
- A linear approach to body-burden estimation, which was utilized in the 2003 Reassessment (U.S. EPA, 2003, 537122), does not fully consider key toxicokinetic issues related to TCDD—e.g., sequestration in the liver and fat, age-dependent elimination, and changing elimination rates over time. Thus, kinetic/mechanistic modeling could be used to quantify tissue-based metrics. In considering human data, lipid-adjusted serum levels may be preferable over body burden, although the assumptions used in the back calculation of the body burden in epidemiologic cohorts are of concern. In considering rat bioassay data, lipid-adjusted body-burden estimates may be preferable.
- New epidemiologic studies on noncancer endpoints have been published since the 2003 Reassessment that may need to be considered (e.g., thyroid dysfunction literature from Wang et al. (2005, 198734) and Baccarelli et al. (2008, 197059)).
- The 1% of maximal response (ED_{01}) that was utilized in the 2003 Reassessment has not typically been used in dose-response assessment. Some alternative ideas were as follows: (1) the POD should depend on the specific endpoint; (2) for continuous measures, the benchmark response (BMR) could be based on the difference from control and consider

the adversity level; and (3) for incidence data, the BMR should be set to a fixed-risk level.

- The quantitative dose-response modeling for cancer could be based on human or animal data. There are new publications in the literature for four epidemiological cohort studies (Dutch cohort, NIOSH cohort, BASF accident cohort, and Hamburg cohort). The increase in total cancers could be considered for modeling human cancer data. However, non-Hodgkin's lymphoma and lung tumors are the main TCDD-related cancer types seen from human exposure. In reviewing the rat data, the NTP (2006, 197605) data sets are new and can be modeled. Although the liver and lungs are the main target organs, modeling all cancers, as well as using tumor incidence in lieu of individual rats as a measure, should be considered.
- Both linear and nonlinear model functions should be considered in the cancer dose-response analysis because there are data and rationales to support use of either below the POD.
- For quantitative uncertainty analysis, consider the impacts of choices among plausible alternative data sets, dose metrics, models, and other more qualitative choices. Issues to consider include how much difference these choices make and, also, how much relative credence should be put toward each alternative as a means to gauge and describe the landscape of imperfect knowledge with respect to possibilities for the true dose response. This may be difficult to do quantitatively because the factors are not readily expressed as statistical distributions. However, the rationale for accepting or questioning each alternative in terms of the available supporting evidence, contrary evidence, and needed assumptions, can be delineated.

1.3.3. Overall Organization of EPA's Response to NAS Recommendations

The remainder of this document is divided into five sections that address the three primary areas of concern resulting from the NAS (2006, 198441) review. Section 2 describes EPA's approach to the recommendation for transparency and clarity during selection of key data sets—including criteria for the selection of key dose-response studies, evaluations of the important epidemiologic studies and animal bioassays, and a summary of the key studies used for subsequent dose-response modeling. Sections 3, 4, and 5 present EPA's response to the NAS recommendation to better justify the approaches used in dose-response modeling of TCDD. Section 3 discusses the toxicokinetic modeling EPA conducted to support the dose-response analyses. Section 4 presents EPA's approach to noncancer data set selection, dose-response modeling, and derivation of an RfD for TCDD, and contains a qualitative discussion of the uncertainties associated with the RfD. Section 5 presents an updated cancer weight-of-evidence summary, EPA's approach to cancer data set selection, dose-response modeling, derivation of an

OSF for TCDD, and a qualitative discussion of the uncertainties associated with the OSF, including an evaluation of illustrative nonlinear approaches to cancer assessment of TCDD. Finally, Section 6 discusses the feasibility of conducting a quantitative uncertainty analysis of TCDD dose response.

2. TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY DATA SETS FOR DOSE-RESPONSE ANALYSIS

This section addresses transparency and clarity in the study selection process and identifies key data sets for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) dose-response analysis. Section 2.1 summarizes the National Academy of Sciences (NAS) committee's comments specifically regarding this issue. Section 2.2 presents U.S. Environmental Protection Agency's (EPA's) response to those comments and describes EPA's approach to ensuring transparency and clarity in the selection of studies for subsequent dose-response analyses. Section 2.3 describes the TCDD-specific study inclusion criteria and evaluation process EPA used in this document for determining the eligibility of both epidemiologic and experimental animal studies for TCDD dose-response analysis. Section 2.4.1 summarizes epidemiologic data and evaluates the suitability of these data for TCDD dose-response analyses. Section 2.4.2 summarizes animal bioassay data that have met the study inclusion criteria for TCDD dose-response assessment. Finally, Section 2.4.3 identifies key TCDD epidemiologic and animal bioassay studies that were determined using the study inclusion criteria. Study/endpoint combination data sets for developing TCDD toxicity values for noncancer and cancer effects are further evaluated in Sections 4 and 5 of this document, respectively.

2.1. SUMMARY OF NAS COMMENTS ON TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY DATA SETS FOR DOSE-RESPONSE ANALYSIS

The NAS committee proposed that EPA develop a clear and readily understandable methodology for evaluating and including epidemiologic and animal bioassay data sets in dose-response evaluations. The NAS committee recommended the development and application of transparent initial criteria to judge whether or not specific epidemiologic or animal bioassay studies be included in TCDD dose-response analysis.

Specific NAS comments on the topic of study evaluation and inclusion criteria include

> EPA should specify inclusion criteria for the studies (animal and human) used for derivation of the benchmark dose (BMD) for different noncancer effects and potentially for the development of RfD values and discuss the strengths and limitations of those key studies (NAS, 2006, 198441, p. 27).

> …in its [EPA's] evaluation of the epidemiological literature of carcinogenicity, it did not outline eligibility requirements or otherwise provide the criteria used to assess the methodological quality of other included studies (NAS, 2006, 198441, p. 56).

> With regard to EPA's review of the animal bioassay data, the committee recommends that EPA establish clear criteria for the inclusion of different data sets (NAS, 2006, 198441, p. 191).

> …the committee expects that EPA could substantially improve its assessment process if it more rigorously evaluated the quality of each study in the database (NAS, 2006, 198441, p. 56).

> EPA could also substantially improve the clarity and presentation of the risk assessment process for TCDD…by using a summary table or a simple summary graphical representation of the key data sets and assumptions…(NAS, 2006, 198441, p. 56).

2.2. EPA'S RESPONSE TO NAS COMMENTS ON TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY DATA SETS FOR DOSE-RESPONSE ANALYSIS

EPA agrees with the NAS committee regarding the need for a transparent and clear process for selecting studies and key data sets for TCDD dose-response analyses. The delineation of the study selection process and decisions regarding key data sets will facilitate communication regarding critical decisions made in the TCDD dose-response assessment. In keeping with the NAS committee's recommendation to use a transparent process and improve clarity and presentation of the risk assessment process for TCDD, Figure 2-1 overviews the approach that EPA has used in this document to develop a final list of key cancer and noncancer studies for quantitative dose-response analysis of TCDD. The steps in Figure 2-1 are further explained below.

Literature search for in vivo mammalian and epidemiologic TCDD studies (2000−2008): EPA conducted a literature search to identify peer-reviewed, dose-response studies for TCDD that have been published since the 2003 Reassessment. This search included in vivo mammalian and epidemiological studies of TCDD from 2000 to 2008. Additional details describing the conduct of this literature search are presented in Section 1.3.1 of this document.

Federal Register Notice—Web publication of literature search for public comment: In November 2008, EPA published a list of ~500 citations from results of this literature search (U.S. EPA, 2008, 519261) and invited the public to review this preliminary list of dose-response citations for use in TCDD dose-response assessment. EPA requested that interested parties identify and submit peer-reviewed studies for TCDD that were absent

from this list. Two parties identified additional references that were not included in the 2008 Federal Register notice and submitted additional references for EPA to consider. These references were included in the final TCDD literature database considered by EPA for TCDD dose-response analysis.

Initial study inclusion criteria development for TCDD in vivo mammalian bioassays: EPA developed an initial set of draft criteria for evaluating the extensive TCDD database of in vivo mammalian bioassays. These initial inclusion criteria had three purposes. First, they provided a transparent and rigorous evaluation of the scientific quality of each study in EPA's database, a deficiency in the 2003 Reassessment identified by the NAS committee. Second, given the vast TCDD mammalian bioassay database, they provided a transparent method for initially screening studies to be considered for TCDD dose-response analyses. Third, they served as a starting point for discussions of study inclusion criteria by expert panelists who were convened by EPA for its scientific workshop on TCDD dose-response analysis (the Dioxin Workshop), described next (also see the workshop report in Appendix A, U.S. EPA [2009b]).

Dioxin Workshop and expert refinement of TCDD in vivo mammalian bioassay inclusion criteria: In February 2009, EPA convened "A Scientific Workshop to Inform EPA's Response to NAS Comments on the Health Effects of Dioxin in EPA's 2003 Dioxin Reassessment." The goals of this 3-day public and scientific workshop were to identify and address issues related to the dose-response assessment of TCDD. Sessions at the workshop examined toxicities associated with TCDD, issues related to developing dose-response estimates based on these data and associated uncertainties. At the workshop, EPA presented the draft set of study inclusion criteria for evaluating the extensive TCDD in vivo mammalian bioassay literature and asked workshop panelists to discuss these criteria and make recommendations for their revision. Further details on this workshop are presented in Section 1.3.2 of this document, and the complete report from this workshop is available in Appendix A (U.S. EPA, 2009b), including detailed summaries of the panels' comments on the inclusion criteria in relation to the various toxic endpoints that were discussed.

Final development of inclusion criteria for TCDD in vivo mammalian studies: Based on discussions at the Dioxin Workshop, the initial draft inclusion criteria for evaluating the TCDD mammalian bioassay literature were revised and are presented in Section 2.3.2 (see Figure 2-3). An initial criterion is that studies for consideration must be publically available and published in a peer-reviewed scientific journal. Because the methodology EPA uses to develop reference doses (RfDs) and cancer oral slope factors (OSFs) relies on identification of studies reporting potential adverse effects at low doses (relative to the overall database), another important criterion shown in Section 2.3.2 identifies a maximum value for the lowest TCDD dose tested in a bioassay. This maximum value was used to eliminate those studies that could not be selected for development of an RfD or an oral slope factor because tested doses were too high relative to other TCDD bioassays.

Development of inclusion criteria for epidemiologic studies: Following the Dioxin Workshop, EPA determined that an evaluation process was also needed for inclusion of epidemiologic studies for TCDD dose-response assessment. These criteria were

developed and are detailed in Section 2.3.1 (see Figure 2-2). Analogous to animal bioassay data, epidemiologic studies for consideration must also be publically available and published in a peer-reviewed scientific journal. In addition to assessing the methodological considerations relative to epidemiologic cohorts and studies (e.g., statistical power and precision of estimates, consideration of latency periods), key criteria for use of a study in TCDD dose-response modeling were that the exposure be primarily to TCDD and that the effective dose and oral exposure are reasonably estimable.

Final literature collection (October 2009): Additional literature was collected as it was identified by EPA following the Dioxin Workshop through October 2009 to ensure the consideration of all recently published data for this report.

Studies screened using inclusion criteria: The two sets of TCDD-specific study inclusion criteria presented in Section 2.3 were used to evaluate all studies included in the 2003 Reassessment, studies identified in the 2000–2008 literature search, studies identified through public comment and submission, and studies collected in 2009 as identified by EPA during the development of this document. Section 2.4 presents results of EPA's evaluation of epidemiologic and mammalian bioassay literature for both cancer and noncancer endpoints.

Final list of key cancer and noncancer studies for quantitative dose-response analysis of TCDD: Application of the study inclusion criteria concludes in Section 2.4 with development of a list of key noncancer and cancer studies that were considered for quantitative dose-response analyses of TCDD in Sections 4 and 5, respectively. In those sections, points of departure (PODs) are developed and evaluated for all biologically relevant study/endpoint combinations from these final key study lists, and key data sets and PODs for the development of TCDD toxicity values are identified.

2.3. STUDY INCLUSION CRITERIA FOR TCDD DOSE-RESPONSE ANALYSIS

One of the three major recommendations made by the NAS (2006, 198441) committee was that EPA should provide greater clarity and transparency on the selection of studies that were used in the quantitative dose-response modeling of TCDD in the 2003 Reassessment. In this section, EPA describes TCDD-specific study inclusion criteria that have been developed to evaluate epidemiologic studies and animal bioassays for TCDD dose-response assessment. These criteria reflect EPA's goal of developing an RfD and a cancer OSF for TCDD through a transparent study selection process; they are intended to be used by EPA for TCDD dose-response assessment only. These criteria were applied to each of the ~500 studies listed in *Preliminary Literature Search Results and Request for Additional Studies on 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Dose-Response Studies* (U.S. EPA, 2008, 519261); studies identified and submitted by the public and by participants in the Dioxin

Workshop (U.S. EPA, 2009, 522927); studies included in the 2003 Reassessment, and other relevant published studies collected by EPA scientists through October 2009.

EPA has undertaken different approaches for epidemiologic versus in vivo animal bioassay study evaluation and key data set selection. The significant differences between animal and human health effects data and their use in EPA risk assessment support development of separate criteria for study inclusion and different approaches to study evaluation. For the vast majority of compounds on EPA's Integrated Risk Information System (IRIS), cancer and noncancer toxicity values have been derived using animal bioassay data; therefore, approaches to dose-response modeling and POD selection from in vivo mammalian bioassays have been standardized and codified (U.S. EPA, 2000, 052150). The study criteria shown below and in Figure 2-3 for animal bioassay data reflect EPA's preferences for TCDD-specific study inclusion, some of which are based on common practices and guidance for POD selection and RfD and OSF derivation. Far fewer IRIS toxicity values have been derived from human data, although some examples do exist. For example, benzene, beryllium and compounds, chromium IV, and 1,3-butadiene have RfDs, Reference Concentrations, Inhalation Unit Risks and/or OSFs based on occupational cohort data and the methyl mercury RfD is based on high fish consuming cohorts (U.S. EPA, 2009, 543757). The modeling and interpretation of such human data have been conducted on a case-by-case basis because each cohort is uniquely defined and has its own set of exposure conditions, significant confounders, and biases that may need to be considered in dose-response modeling. For TCDD, not all data are from occupational cohorts, but include cohorts exposed for relatively short time periods to high concentrations as a consequence of industrial accidents, a scenario that has not commonly been used to establish EPA toxicity values.

Because of these differences in data characteristics, divergent selection approaches are used in this document to present and evaluate the epidemiologic studies (see Section 2.3.1) and the in vivo animal bioassays (see Section 2.3.2). In Section 2.4.1, all of the available epidemiologic studies on TCDD are summarized and evaluated for suitability for dose-response modeling using the TCDD-specific study inclusion criteria below and shown in Figure 2-2; only studies meeting the inclusion criteria are presented as key studies in Section 2.4.3 (see Tables 2-4 and 2-5 for the cancer and noncancer endpoints, respectively). In Section 2.4.2, because summarizing and showing the evaluation of the thousands of available animal bioassays on

TCDD was prohibitive, only studies first meeting the in vivo animal bioassays study inclusion criteria below (and shown in Figure 2-3) are summarized. These studies are also presented as key studies in Section 2.4.3 (see Tables 2-6 and 2-7 for cancer and noncancer endpoints, respectively).

2.3.1. Study Inclusion Criteria for TCDD Epidemiologic Studies

This section identifies the process EPA used to select epidemiologic studies for defining candidate PODs for TCDD dose-response modeling. These criteria are based on EPA's approaches for deriving OSFs and RfDs. A discussion of the considerations used in selecting epidemiologic data for quantitative dose-response modeling is valuable, particularly given EPA's preference to use high-quality human studies over animal studies because such human studies are regarded as providing the most relevant information needed for quantitative human health risk analyses (U.S. EPA, 2005, 086237). As described by Hertz-Picciotto (1995, 065678), key components needed for the use of an epidemiologic study as a basis for quantitative risk assessment include issues regarding exposure assessment (a well-quantified exposure assessment with exposures linked to individuals) and study quality ("strong biases," for example with respect to inclusion criteria for membership in the cohort and follow-up procedures "ruled out or unlikely" and "confounding controlled or likely to be limited"). The strength of the association, either within the full study or within a high exposure subgroup, can also be considered in the evaluation of suitability for dose-response modeling (Hertz-Picciotto, 1995, 065678). Stayner et al. (1999, 198654), however, note that even weak associations could be useful in terms of providing an estimate of a potential upper bound for a quantitative risk estimate.

EPA's method for applying the TCDD study inclusion criteria to epidemiologic data is detailed below and in Figure 2-2. Based on the framework discussed above, EPA evaluated the available epidemiologic cohorts and studies based on the five following considerations:

1. The methods used to ascertain health outcomes are clearly identified and unbiased, with high sensitivity and specificity.
2. The risk estimates generated from the study are not susceptible to important biases arising from an inability to control for potential confounding exposures or other sources of bias arising from either study design or statistical analysis.

3. The study demonstrates an association between TCDD and an adverse health effect (assuming minimal misclassification of exposure and absence of important biases) with some suggestion of an exposure-response relationship.

4. The exposure assessment methodology is clearly described and can be expected to provide adequate characterization of exposure, with assignment of individual-level exposures within a study (e.g., based on biomarker data, or based on a job-exposure-matrix approach). Limitations and uncertainties in the exposure assessment are considered.

5. The size and follow-up period of a cohort study are large enough and long enough, respectively, to yield sufficiently precise estimates for use in development of quantitative risk estimates and to ensure adequate statistical power to limit the possibility of not detecting an association that might be present (i.e., to avoid Type II Errors due to failing to reject the null hypothesis when the null hypothesis is true). Similar considerations regarding sample size and statistical precision and power apply to case-control studies.

Three specific study inclusion criteria were used to select studies for further evaluation and potential TCDD quantitative dose-response assessment

1. The study is published in the peer-reviewed scientific literature and includes an appropriate discussion of strengths and limitations.

2. The exposure is primarily to TCDD, rather than dioxin-like compounds (DLCs), and is properly quantified so that dose-response relationships can be assessed. All epidemiologic cohorts will have background exposures to DLCs through the food chain and these exposures are not included in this criterion.

3. The effective dose and oral exposure must be reasonably estimable. The measures of exposure must be consistent with the current biological understanding of dose. For TCDD dose-response assessment, it is critical that reported dose is consistent with a dose that is likely to be toxicologically relevant. The timing of the measurement of effects (i.e., the response) also must be consistent with current biological understanding of the effect and its progression.

   For cancer endpoints, EPA assumes that cumulative TCDD dose estimates are toxicologically relevant measures. Thus, cancer studies must provide information about long-term TCDD exposure levels. Further, EPA reasons that measures of cancer occurrence or death need to allow for examination of issues of latency between the end of effective exposure and cancer detection or death.

   For noncancer endpoints, exposure estimates and analysis must allow for examination of issues of latency and other issues regarding the appropriate time window of exposure relevant for specific endpoints. Also, to be consistent with the RfD methodology, the response must be to a nonfatal endpoint.

Those studies that met these three inclusion criteria (see Sections 2.4.1, 2.4.3, and Appendix B) were then subjected to further consideration for quantitative dose-response analyses.

2.3.2. Study Inclusion Criteria for TCDD In Vivo Mammalian Bioassays

This section identifies the criteria EPA applied to select nonhuman in vivo mammalian studies for defining candidate PODs for use in TCDD dose-response modeling. These inclusion criteria are based on EPA's approaches for deriving OSFs and RfDs from bioassay data (U.S. EPA, 2005, 086237). EPA agrees with the NAS committee regarding the utility of an oral RfD and the need for reevaluation of the OSF for TCDD, specifically in light of data that have been published since the 2003 Reassessment was released. RfDs and OSFs are generally derived using data sets that demonstrate the occurrence of adverse effects, or their precursors, in low-dose range for that chemical. RfDs and OSFs are derived from a health protective perspective for chronic exposures. Thus, when a group of studies is available on a chemical for which a number of effects are observed at various doses across those studies, the studies using the lowest exposures that show effects will typically drive the RfD and OSF derivations, all other considerations being equal. Studies conducted at higher exposures relative to other available studies are used as supporting evidence for the final RfD or OSF since they were conducted at doses too high to impact the numeric derivations of toxicity values. EPA expresses RfDs and OSFs in terms of average daily doses, usually as mg/kg-day and per mg/kg-day, respectively. Thus, the study inclusion criteria for the animal bioassay data presented in this section include requirements that average daily exposures in the studies are within a low dose range where, relative to other studies, they could be considered for development of a toxicity value. These low-dose requirements do not imply that TCDD studies conducted at higher doses are of poor quality, simply that they are not quantitatively useful in the development of toxicity values because other studies with lower exposures will drive the RfD and OSF derivations under current EPA practice. Because EPA has identified ~2,000 studies on TCDD that may be considered for this purpose, the development and application of these study inclusion criteria has been critical to moving the risk assessment process forward.

EPA's method for applying study inclusion criteria for mammalian bioassays is detailed below and in Figure 2-3. The first study inclusion criterion is that the study is published in the peer-reviewed scientific literature. Then, two specific study inclusion criteria were used to select

studies for further evaluation and potential TCDD quantitative dose-response analyses and identification of candidate PODs:

1. The lowest dose level tested is ≤1 μg/kg-day for cancer studies and ≤30 ng/kg-day for noncancer studies.
2. The study design consists of orally administered TCDD-only doses, and specifies the purity and matrix used to administer the doses.

Then, EPA evaluated the remaining in vivo animal studies based on the following four considerations.

1. The study tests mammalian species, identifying the strain, gender, and age of the tested animals.
2. The study clearly documents testing protocol, including dosing frequency, duration, and timing of dose administration relative to age of the animals.
3. The overall study design is consistent with standard toxicological principles and practices. The control group or groups are appropriate, given the testing protocol, and are well characterized. Clinical and pathological examinations conducted during the study are endpoint-appropriate, particularly for negative findings.
4. The magnitude of animal responses is outside the range of normal variability exhibited by control animals (e.g., greater than or less than one standard deviation).

Those studies that met the aforementioned considerations and inclusion criteria (see Sections 2.4.2 and 2.4.3) were then subjected to dose-response analysis.

The criteria for dose requirements, although somewhat arbitrary, are intended to be reasonable cutoffs that restrict the number of studies that would need to be modeled while ensuring that all study/data set combinations that could be candidates for the cancer slope factor or RfD were modeled. Thus, the dose range under consideration allows for liberal ranges of no-observed-adverse-effect levels (NOAELs), lowest-observed-adverse-effect levels (LOAELs), and benchmark dose lower confidence bound (BMDLs) for assessment of both cancer and noncancer effects.

For cancer studies, the dose requirements were selected based on an initial evaluation of available average daily doses administered in TCDD animal bioassays in which adverse effects were observed. For example, in cancer studies, a sample of the relatively low ranges of tested

average daily doses include 1-1,000 ng/kg-day (Toth et al., 1979), 1−100 ng/kg-day (Kociba et al., 1978), 1.43−286 ng/kg-day (NTP, 1982, 543764) and 2.14−71.4 ng/kg-day (NTP, 2006, 197605) with statistically significant increases in tumor incidence via pair-wise or trend tests found in all of these studies. The entire range of each these studies is ≤1 µg/kg-day. The linearized multistage model used by EPA to estimate OSFs is most appropriately applied to studies from which PODs can be estimated as closely as possible to the experimental data. Thus, given the dose ranges in these studies that are available for modeling, the restriction to ≤1 µg/kg-day for cancer was considered to be a reasonable cutoff.

For noncancer studies, dose ranges are more complex and vary according to study endpoint. Examples of the lowest administered doses that might be considered as NOAELs or LOAELs in POD determinations for noncancer endpoints include 1 ng/kg-day (Toth et al., 1979, 197109), 1.43 ng/kg-day (Cantoni et al., 1981, 197092), 1.07 ng/kg-day (Smialowicz et al., 2008, 198341) 1.43 ng/kg-day (NTP, 1982, 543764) and 2.14 ng/kg-day (NTP, 2006, 197605). Most of the lowest tested doses in the TCDD studies have been designated as LOAELs (see Section 4.1). Given the available database, it is likely that the same composite uncertainty factor (e.g., of 300; 3 for UF_A [interspecies], 10 for UF_H [intraspecies], and 10 for UF_L [LOAEL to NOAEL]) would be applied to any animal noncancer LOAEL used to derive an RfD for TCDD. This implies that any study that has a LOAEL of 30 ng/kg-day or more would result in a candidate RfD that is more than an order of magnitude higher than the example doses of 1−2 ng/kg-day shown here. BMDLs that might be derived from such data also would not be expected to be lower than these example doses of 1−2 ng/kg-day. Thus, a tested dose ≤30 ng/kg-day is considered to be a reasonable cutoff where the lowest tested dose would never be used as a POD to derive an RfD given that much lower tested doses (associated with adverse effects) are available from other studies of acceptable quality.

2.4. EVALUATION OF KEY STUDIES FOR TCDD DOSE RESPONSE

2.4.1. Evaluation of Epidemiological Cohorts for Dose-Response Assessment

This section summarizes and evaluates studies for potential use in TCDD dose-response assessment using the study evaluation considerations and inclusion criteria for epidemiologic data (see Section 2.3.1). Those studies that meet the study inclusion criteria are are listed later in this Section in Tables 2-4 and 2-5, for cancer and noncancer, respectively, and are considered in

the dose-response modeling conducted later in this document (see Sections 4 and 5). The following sections are organized by epidemiologic cohort. Following a brief summary of each cohort, its associated studies are then summarized chronologically, assessed for methodological considerations relative to epidemiologic cohorts and studies (e.g., statistical power and precision of estimates, consideration of latency periods) and evaluated for suitability for TCDD dose-response assessment.

2.4.1.1. *Cancer*

In the 2003 Reassessment, EPA selected three cohort studies from which to conduct a quantitative dose-response analysis: the National Institute for Occupational Safety and Health (NIOSH) cohort (Steenland et al., 2001, 197433), the BASF cohort (Ott and Zober, 1996, 198408), and the Hamburg cohort (Becher et al., 1998, 197173). Although these studies were deemed suitable for quantitative dose-response analysis, the criteria EPA used to reach this conclusion were unclear. In this section, the study selection criteria and methodological considerations presented in Section 2.3 are systematically applied to evaluate a number of studies to determine their suitability for inclusion in dose-response modeling. In addition to the three cohorts used in previous TCDD quantitative risk assessment, considerations are applied to other relevant TCDD epidemiological data sets that were identified through a literature review for epidemiological studies of TCDD and cancer. Study summaries and suitability for quantitative dose-response analysis evaluations are discussed below.

2.4.1.1.1. *Cancer cohorts.*

2.4.1.1.1.1. ***The NIOSH cohort.***

In 1978, the NIOSH undertook research that identified workers employed by U.S. chemical companies that made products contaminated with TCDD between 1942 and 1982. TCDD was generated in the production of 2,4,5-trichlorophenol and subsequent processes. This chemical was used to make 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which was a major component of the widely-used defoliant, Agent Orange. The NIOSH cohort is the largest cohort of occupational workers studied to date and has been the subject of a series of investigations spanning more than two decades. It is important to note that this cohort consists mostly of male workers that were exposed to TCDD via daily occupational exposure, as compared to an acute

accidental exposure scenario seen with other cohorts. The investigations have progressed from a comparison of the mortality patterns of the cohort to the U.S. general population to dose-response modeling using serum-derived estimates of TCDD that have been back-extrapolated several decades. Analyses of cancer data from the NIOSH cohort that are addressed in this section include Fingerhut et al. (1991, 197375), Steenland et al. (1999, 197437; 2001, 197433), Cheng et al. (2006, 523122), and Collins et al. (2009, 197627).

2.4.1.1.1.1. Fingerhut et al. (1991, 197375).

2.4.1.1.1.1.1. *Study summary.*

The investigation of Fingerhut and her colleagues published nearly two decades ago attracted widespread attention (Fingerhut et al., 1991, 197375). This retrospective study examined patterns of cancer mortality for 5,172 workers who comprised the NIOSH cohort, which combined workers from the company-specific cohorts of Dow Chemical (Ott et al., 1987, 064994)(Cook, 1981) and the Monsanto Company (Zack and Gaffey, 1983, 548783; Zack and Suskind, 1980, 065005). These workers were employed at 12 plants producing chemicals contaminated with TCDD. Almost all workers in the cohort (97%) had production or maintenance jobs with processes involving TCDD contamination. On average, workers were employed for 2.7 years specifically in processes that involved TCDD contamination, and overall, were employed for 12.6 years. The mortality follow-up began in 1940 and extended until the end of 1987. Vital status was determined using records from the Social Security Administration, the Internal Revenue Service, or the National Death Index. The ascertainment of vital status in the cohort was nearly complete, with less than 1% of the cohort not followed up until death or the end of the study period.

Comparisons of mortality were made relative to the U.S. male general population and expressed using the standardized mortality ratio (SMR) metric and 95% confidence intervals (CIs). Life-table methods were used to generate person-years of risk accrued by cohort members at each plant. Person-years and corresponding deaths were tabulated across age, race, and year of death strata, which permitted the SMRs to be examined for potential confounding from these three characteristics. No unadjusted SMRs were presented in the paper. Cross-classification of person-years and deaths was also done across several exposure-related groupings, including duration of employment, years since first exposure, years since last exposure, and duration of

exposure. Employment duration was categorized as <5, 5− <10, 10− <15, 15− <20, 20− <25, 25− <30, and ≥30 years. The variable "years since first exposure" (<10, 10− <20, and ≥20 years) was used to evaluate associations in relation to different latency periods. The analysis was jointly stratified by duration of employment and for varying latency intervals to evaluate whether cohort members with higher cumulative TCDD levels had higher cancer mortality rates than those cohort members with lower cumulative levels.

Overall, the cohort of workers had slightly elevated cancer mortality than the general population (SMR = 1.15, 95% CI = 1.02−1.30). Comparisons to the general population, however, yielded no statistically significant excess for any site-specific cancer. Cancer mortality was examined for the subset of workers that worked for at least one year and had a latency interval of at least 20 years (n = 1,520). The 1-year cut-point was selected based on analyses of serum levels in a subset of 253 workers which revealed that every worker employed for at least one year had a lipid-adjusted serum level that exceeded the mean (7 ppt). Relative to the U.S. general population, statistically significant excesses in cancer mortality were observed for all cancers (SMR = 1.46, 95% CI = 1.21−1.76), cancers of the respiratory system (SMR = 1.42, 95% CI = 1.03−1.92), and for soft tissue sarcoma (SMR = 9.22, 95% CI = 1.90−26.95) among this subset of 1,520 male workers. The elevated SMR for soft tissue sarcoma, however, was based on only three cases in this subset.

SMRs also were generated across joint categories of duration of exposure and period of latency for deaths from all cancer sites (combined), and cancer of the trachea, bronchus, and lung. Increased SMRs were observed in strata defined by longer exposure and latency, but no statistically significant linear trends were found.

2.4.1.1.1.1.1.2. *Study evaluation.*

This cohort was the largest of four the International Agency for Research on Cancer (IARC) considered in its 1997 classification of TCDD as a Group 1 human carcinogen (IARC, 1997, 537123). Duration of employment in processes that involved TCDD contamination was used as a surrogate measure of cumulative exposure. In using this exposure metric, Fingerhut et al. (1991, 197375) assumed that TCDD exposures were equivalent at all production plants. Doses for individual cohort members were not reconstructed for these analyses, although they were in subsequent analyses of this cohort.

Workers in this cohort also were exposed to other chemicals, which could lead to bias due to confounding if these exposures were associated with both TCDD exposure and the health outcomes being examined. At one plant, workers were exposed to 4-aminobiphenyl. Previous investigators also reported that workers at another plant were exposed to 2,4,5-T and 2,4-dichlorophenoxyacetic acid (2,4-D) (Bond et al., 1988, 197183; Bond et al., 1989, 064967; Ott et al., 1987, 064994). Although this study did not examine the impact of confounding by other occupational coexposures, subsequent analyses of this cohort showed that associations between cumulative TCDD and all cancer mortality persisted after excluding workers exposed to pentachlorophenols from the analyses (Steenland et al., 1999, 197437). Removal of workers who died from bladder cancer also did not substantially change the dose-response association between TCDD and cancer mortality from all other sites combined. This finding suggests that exposures to 4-aminobiphenyl did not confound the association between cancer mortality and TCDD exposure. Overall, there is little evidence of confounding by these co-exposures among this cohort, however, exposure to other possible confounders, such as dioxin-like compounds, was not examined.

The study collected no information on smoking behavior of the workers, and therefore, the SMRs do not account for any differences in the prevalence of smoking that might have existed between the workers and the general population. For several reasons, however, the inability to take into account smoking is unlikely to have been an important source of bias. First, mortality from other smoking-related causes of death such as nonmalignant respiratory disease were not more common in the cohort than in the general population (SMR = 0.96, 95% CI = 0.54−1.58). Second, stratified analyses of workers with at least a 20-year latency (assuming this subset shared similar smoking habits) revealed that excesses were apparent only among those who were exposed for at least 1 year. Specifically, when compared to the general population, the SMR among workers exposed for at least 1 year with a latency of 20 years was 1.46, (95% CI = 1.21−1.76) while those exposed for less than 1 year had an SMR of 1.02 (95% CI = 0.76−1.36). Third, for comparisons of cancer mortality between blue-collar workers and the general population, smoking is unlikely to explain cancer excesses of greater than 10−20% (Siemiatycki et al., 1988, 198556). Finally, the investigators found no substantial changes in the results for lung cancer when risks were adjusted for smoking histories obtained in 1987 from 223 workers employed at two plants. These data were used to adjust for the expected

number of lung cancer deaths expected in the entire cohort (Fingerhut et al., 1991, 197375). Following this adjustment, a small change was observed in the SMR for lung cancer in the overall cohort from 1.11 (95% CI = 0.89−1.37) to 1.05 (95% CI = 0.85−1.30). Similarly, only a slight change in the SMR for lung cancer in the higher exposure subcohort was noted from an SMR of 1.39 (95% CI = 0.99−1.89 to 1.37 (95% CI = 0.98−1.87).

The use of death certificate information from the National Death Index is appropriate for identifying cancer mortality outcomes. For site-specific cancers such as soft tissue sarcoma, however, the coding of this underlying cause of death is more prone to misclassification (Percy et al., 1981, 004891). Indeed, a review of tissues from four men concluded to have died from soft-tissue sarcoma determined that two deaths had been misclassified (Fingerhut et al., 1991, 197375). A review of hospital data revealed that two other individuals had soft tissue sarcomas that were not identified by death certificate information. The use of death certificate information to derive SMRs for cancer as a whole is likely not subject to significant bias; the same might not hold true, however, for some site-specific cancers such as soft tissue sarcoma.

Using the SMR metric to compare an occupational cohort with the general population is subject to what is commonly referred to as the "healthy worker effect" (Choi, 1992, 594250; Li and Sung, 1999, 198427). The healthy worker effect is a bias that arises because those healthy enough to be employed have lower morbidity and mortality rates than the general population. The healthy worker effect is likely to be larger for occupations that are more physically demanding (Aittomaki et al., 2005, 197139; Checkoway et al., 1989, 027173), and the healthy worker effect is considered to be of little or no consequence in the interpretation of cancer mortality (McMichael, 1976, 073484; Monson, 1986, 001410). Few cancers are associated with a prolonged period of poor health that would affect employability long before death. Also recognized is that, as the employed population ages, the magnitude of the healthy worker effect decreases as the absolute reduction in mortality becomes relatively smaller in older age groups (McMichael, 1976, 073484). The mortality follow-up of occupational cohorts generally spans several decades, which should minimize the associated healthy worker effect in such studies. Bias could also be introduced in that workers who are healthier might be more likely to stay employed and therefore accrue higher levels of exposure. In the NIOSH cohort, however, mortality was ascertained for those who could have left the workforce or retired by linking subjects to the National Death Index. Although internal cohort comparisons can minimize the

potential for the healthy worker effect for the reasons presented above, for cancer outcomes, the SMR statistic is a valuable tool for characterizing whether occupational cohort are more likely to die of cancer than the general population. Moreover, stratified analyses across categories of duration of exposure, or latency periods within a cohort can yield important insights about which workers are at greatest risk. Perhaps most important, subsequent analyses of the NIOSH cohort that presented risk estimates derived from external comparisons using the SMR were remarkably consistent with rate ratios derived using an internal referent (Steenland et al., 1999, 197437).

2.4.1.1.1.1.1.3. *Suitability of data for TCDD dose-response modeling.*

This cohort meets most of the identified considerations for conducting a quantitative dose-response analysis for mortality from all cancer sites combined. The NIOSH cohort is the largest cohort of TCDD-exposed workers, exposure characterization at an individual level is possible but not available in this particular study, and the follow-up period is long enough to evaluate latent effects. Although there is no direct evidence of any important sources of bias, confounding may be present due to a lack of consideration of dioxin-like compounds. For the purpose of quantitative dose-response modeling, it is important to note that subsequent studies of this cohort adopted methods that greatly improved the characterization of TCDD exposure in this cohort and increased the follow-up interval (Cheng et al., 2006, 523122; Steenland et al., 2001, 197433). As such, for all practical purposes, due consideration for dose-response modeling should focus on the more recently developed data sets.

For quantitative dose-response modeling for individual cancer sites, the data are much more limited. A statistically significant positive association with TCDD was noted only for soft-tissue sarcoma among those with more than 1 year of exposure and 20 years of latency (SMR = 9.22, 95% CI = 1.90−26.95). However there were only three deaths from soft tissue sarcoma among this exposed component of the cohort, and four deaths in total in the overall cohort. Also, misclassification of outcome for soft-tissue sarcoma through death registries is well recognized and supported with additional review of tissue from two of the men. Specifically, tissues from the four men who died of soft-tissue sarcoma revealed that only two of these cases were coded correctly.

Although subsequent analyses of the NIOSH cohort did not show evidence of confounding by other occupational exposures, the design of this initial publication of the NIOSH

cohort did not allow for examination of exposures to other possible confounders, such as dioxin-like compounds. Duration of exposure was used as a surrogate for cumulative TCDD exposure; therefore, effective doses could not be estimated. Therefore, dose-response modeling was not conducted for this study.

2.4.1.1.1.1.2. Steenland et al. (1999, 197437).

2.4.1.1.1.1.2.1. *Study summary.*

A subsequent analysis of the NIOSH cohort extended the follow-up interval of Fingerhut et al. (1991, 197375) by 6 years (i.e., from 1940−1993) and improved characterization of TCDD exposure (Steenland et al., 1999, 197437). A key distinction from the work of Fingerhut et al. (1991, 197375) was the exclusion of several workers that had been included in the previous mortality analyses. The authors excluded 40 workers who were either female, had never worked in TCDD-exposed departments, or had missing date of birth information. An additional 238 workers were excluded as occupational data for characterizing duration of exposure were lacking, preventing their use in a subcohort dose-response analysis. This subcohort was further reduced by excluding workers from four plants (n = 591) because the information on the degree of TCDD contamination in work histories was limited, preventing the characterization of TCDD levels by job type. Thirty-eight additional workers were excluded from the eight remaining plants because TCDD contamination could not be estimated. Finally, 727 workers were excluded because they had been exposed to pentachlorophenol. In total, exposures were assigned to 3,538 (69%) members of the overall cohort, a cohort substantially reduced from the 5,172 on which Fingerhut et al. (1991, 197375) reported. Steenland et al. (1999, 197437) also evaluated the mortality experience of a subcohort of 608 workers with chloracne who had no exposure to pentachlorophenol.

For each worker, a quantitative exposure score for each day of work was calculated based on the concentration of TCDD (μg/g) present in process materials, the fraction of the day worked, and a qualitative contact level based on estimates of the amount of TCDD exposure via dermal absorption or inhalation. The authors derived a cumulative measure of TCDD exposure by summing the exposure scores across the working lifetime history for each worker. The authors validated this cumulative exposure metric indirectly by comparing values obtained for workers with and without chloracne. Such a validation is appropriate, given that chloracne is

considered a clinical sign of exposure to high doses of dioxin (e.g., Ott et al., 1993, 594322). The median exposure score among those with chloracne was 11,546 compared with 77 among those without (Steenland and Deddens, 2003, 198587).

Cancer mortality was compared using two approaches. As in Fingerhut et al. (1991, 197375), external comparisons were made to the U.S. general population using the SMR statistic. The authors adjusted the SMR statistics for race, age, and calendar time. They also applied life-table methods to characterize risks across the subcohort of 3,538 workers with exposure data by categorizing the workers into seven cumulative exposure groups. The cut-points for these categories were selected so that the number of deaths in each category was nearly equal to optimize study power. Life-table analyses were extended further to consider a 15-year lag interval, which in a practical sense means that person-years at risk would not begin to accrue until 15 years after the first exposure occurred. The person-years and deaths that occurred in the first 15 years were included in the lowest exposure grouping. The Cox proportional hazards model was used to characterize risk within the cohort. Cox regression was used to provide an estimate of the hazard ratios and the 95% CIs for ischemic heart disease, all cancers combined, lung cancer, smoking related cancers, and all other cancers. The authors also performed Cox regression analyses using the seven categories of exposure, adjusting the regression coefficients for year of birth and age. The regression models were run for both unlagged and lagged (15 years) cumulative exposure scores.

Overall, when compared with the U.S. general population, a slight excess of cancer mortality (from all sites) was noted in the 5,132 cohort study population (SMR = 1.13, 95% CI = 1.02−1.25). This result did not substantially differ from the earlier finding that Fingerhut et al. (1991) published (SMR = 1.15, 95% CI = 1.03−1.30). Site-specific analyses revealed statistically significant excesses relative to the U.S. general population for bladder cancer (SMR = 1.99, 95% CI = 1.13−3.23) and for cancer of the larynx (SMR = 2.22, 95% CI = 1.06−4.08). In the chloracne subcohort (n = 608), SMRs of 1.25 (95% CI = 0.98−1.57) and 1.45 (95% CI = 0.98−2.07) were found for all cancer sites and for lung cancer, repectively, relative to the general population. The authors also found statistically significant excesses for connective and soft tissue sarcomas (SMR = 11.32, 95% CI = 2.33−33.10) and for lymphatic and hematopoietic malignancies (SMR = 3.01, 95% CI = 1.43−8.52).

External comparisons made by grouping workers into septiles of cumulative TCDD exposure and generating an SMR for each septile using the U.S. population as the referent group suggested a dose-response relationship. For all cancer sites combined, workers in the highest exposure score category had an SMR of 1.60 (95% CI = 1.15−1.82); increases also were observed in the sixth (SMR = 1.34) and fifth (SMR = 1.15) septiles. The two-sided p-value associated with the test for trend for cumulative TCDD exposure was statistically significant ($p = 0.02$). A similar approach for lung cancer revealed virtually the same pattern. The incorporation of a 15-year latency for the analyses of all cancer deaths, in general, produced slightly higher SMRs across the septiles, although a slight attenuation of effect was noted in the highest septile ($SMR_{unlagged} = 1.60$ vs. $SMR_{lagged} = 1.54$). For a 15-year lag, the lung cancer SMRs were mixed compared to the unlagged results with some septile exposure categories increasing and others decreasing relative to the lowest exposure group.

For the internal cohort comparisons using Cox regression analyses higher hazard ratios were found among workers in the higher exposure categories than in the lowest septile. The linear test for trend, however, was not statistically significant ($p = 0.10$). The associations across the septiles for the unlagged exposure for the internal cohort comparisons were not as strong as for the external cohort comparisons. The opposite was true, however, for cumulative exposures lagged 15 years.

Relative to the lowest septile, stratified analyses revealed increased hazard ratios in the upper septiles of the internal cohort comparisons for both smoking- and nonsmoking-related forms of cancer. The test for linear trend was statistically significant for all other cancers (after smoking-related cancers were excluded). These analyses suggest that the overall cancer findings were not limited to an interaction between TCDD and smoking. Additional sensitivity analyses by the authors indicated the findings for smoking-related cancers were largely unaffected by the exclusion of bladder cancer cases. This observation suggests that the exposure to 4-aminobiphenyl, which occurred at one plant and might have contributed to an increased number of bladder cancers, did not substantially bias the dose-response relationship between TCDD and all cancers combined.

The investigators also evaluated the dose-response relationship with a Cox regression model separately for each plant using internal cohort comparisons and found some heterogeneity. This finding is not unexpected particularly given the relatively small number of cancer deaths at

each plant, and given that exposures were quite low for one plant at which no positive association was found. The variability among plants was taken into account by modeling plant as a random effect measure in the Cox model, which produced little change in the slope coefficient ($\beta = 0.0422$ vs. $\beta = 0.0453$).

2.4.1.1.1.1.2.2. *Study evaluation.*

This study represents a valuable extension of that by Fingerhut et al. (1991, 197375). Internal comparisons were performed to help minimize potential biases associated with using an external comparison group (e.g., healthy worker effect, and differences in other risk factors between the cohort and the general population). That similar dose-response relationships were found for internal and external comparison populations suggests that the bias due to the health worker effect in the cohort might be minimal for cancer mortality. More importantly, the construction of the cumulative exposure scores provides an improved opportunity to evaluate dose-response relationships compared with the length of exposure and duration of employment metrics that Fingerhut et al. (1991, 197375) used.

A potential limitation of the NIOSH study was the inability to account for cigarette smoking. If cigarette smoking did contribute to the increased cancer mortality rates in this and other cohorts, increased cancer mortality from exposure to TCDD would be expected only for smoking-attributable cancers. This study demonstrates associations with TCDD for both smoking- and nonsmoking-related cancers, including a stronger association for nonsmoking-related cancers. Therefore, the data provide evidence that associations between TCDD and cancer mortality are not likely due to cigarette smoking.

The findings regarding latency should be interpreted cautiously as the statistical power in the study to compare differences across latency intervals was limited. Caution also should be heeded, given that latency intervals can vary on an individual basis as they are often dose-dependent (Guess and Hoel, 1977, 197464). The evaluation of whether TCDD acts as either an initiating or promoting agent (or both) is severely constrained by the reliance on cancer mortality data rather than incidence data. This constraint is due to the fact that survival time can be quite lengthy and can vary substantially across individuals and by cancer subtype. For example, the 5-year survival among U.S. males for all cancer sites combined ranged between 45 and 60% (Clegg et al., 2002, 594267). When only mortality data are available, evaluating the

time between when individuals are first exposed and when they are diagnosed with cancer is nearly impossible.

Starr (2003, 594271) suggested that Steenland et al. (1999, 197437) focused too heavily on the exposures that incorporated a 15-year period of latency and that those who experienced high exposures would inappropriately contribute person-years to the lowest exposure group "irrespective of how great the workers' actual cumulative exposure scores may have been." Most cancer deaths would, however, typically occur many years postemployment. Given that the follow-up interval of the cohort was long and the average exposure duration was 2.7 years, at the time of death, person-years for those with high cumulative exposures would be captured appropriately. The median 5-year survival for all cancers is approximately 50% (Clegg et al., 2002, 594267), so applying a minimum latency of 5 years when using cancer mortality rather than cancer incidence data is needed to assure that the exposure metric is capturing exposures that occur before diagnoses. Increasing this latency period, for example to 10 or 15 years, would eliminate consideration of exposures that occur in the period between tumor occurrence and tumor detection (diagnosis), and allows for an appropriate focus on exposures that act either early or late in the pathogenic process. If the association of TCDD with cancer is causal, effects might become apparent only at high exposures and with adequate latency. As such, IARC has concluded that a latency interval of 15 years could be too short (IARC, 1997, 537123). EPA considers the Steenland et al. (1999, 197437) presentation to be balanced in that they provided the range in lifetime excess risk estimated across the various models used. The authors' finding that the models with a 15-year lag provided a statistically significant improvement in fit based on the chi-square test statistic should not be readily dismissed.

2.4.1.1.1.1.2.3. *Suitability of data for TCDD dose-response modeling.*

This study meets most of the epidemiological considerations for conducting a quantitative dose-response analysis for mortality from all cancer sites combined. This study excludes a large number of workers who were exposed to pentachlorophenol, thus eliminating the potential for bias from this exposure and used an improved methodology for assigning TCDD exposures to the workers. However, given that exposures to other dioxin-like compounds were not described, it is unclear if the exposures among this cohort were primarily to TCDD. Therefore, dose-response modeling was not pursued for this study, but was for the subsequent

NIOSH study by Steenland et al. (2001, 197433), which did examine exposure to dioxin-like compounds.

2.4.1.1.1.1.3. Steenland et al. (2001, 197433).

2.4.1.1.1.1.3.1. *Study summary.*

In 2001, Steenland et al. published a risk analysis using the NIOSH cohort that for the first time incorporated serum measures in the derivation of TCDD exposures for individual workers. The authors applied the same exclusion criteria to the entire cohort of workers across the 12 plants in the Steenland et al. (1999, 197437) study, which left 3,538 workers for which risk estimates could be calculated. Cumulative TCDD serum levels were estimated on an individual basis for all 3,538 workers by developing predictive models that used a subset of 170 workers for which both serum measures and TCDD exposures scores were available (Steenland et al., 2001, 197433). Unlike previous analyses of the NIOSH cohort that considered several different mortality outcomes, the analyses presented in Steenland et al. (2001, 197433) focused exclusively on mortality from all cancers sites combined. The authors observed 256 cancer deaths in the cohort during the follow-up interval that extended from 1942 until the end of 1993. All risks estimated in the Steenland et al. (2001, 197433) study were based on internal cohort comparisons.

Characterization of TCDD exposure levels among the workers was based on serum measures obtained in 1988 from 199 workers who were employed in one of the eight plants. The researchers restricted the development of the model to include only those workers whose measured serum levels were deemed to be greater than the upper range of background levels (10 ppt), which resulted in 170 workers.

The authors developed a regression model that could estimate the level of TCDD at the time of last exposure for the 170 workers. The model was developed based on the estimated half-life of TCDD, the known work history of each worker, a pharmacokinetic model for the storage and excretion of TCDD, and exposure scores for each job held by each worker over time. The resulting equation follows

$$y_{last\ exposure} = y_{1988}\ exp(\lambda \Delta t) \qquad \text{(Eq. 2-1)}$$

The first-order elimination rate constant (λ) was based on a half-life of 8.7 years previously reported for the Ranch Hands cohort (Michalek et al., 1996, 198893). The background rate of TCDD exposure was assumed to be 6.1 parts per trillion (ppt), which was based on the median level in a sample of 79 unexposed workers in the NIOSH cohort (Piacitelli et al., 1992, 197275). This value was subtracted when TCDD values were back-extrapolated, and then added again after the back-extrapolation was completed. A background level of 5 ppt also was used in some of the analyses with minimal demonstrable effects on the results. Sensitivity analyses also were incorporated to consider a 7.1-year half-life estimate that had been developed for the earlier Ranch Hands study (Pirkle et al., 1989, 197861).

After back-extrapolating to obtain TCDD serums levels at the time of last exposure, the investigators estimated cumulative (or "area under the curve") TCDD serum levels for every cohort member. This estimation procedure was the same method Flesch-Janys et al. (1998, 197339) applied to the Hamburg cohort to derive a coefficient for relating serum levels to exposure scores. The "area under the curve" approach integrates time-specific serum levels over the employment histories of the individual workers. The slope coefficient was estimated using a no-intercept linear regression model. This model is based on the assumption that a cumulative score of zero is associated with no serum levels above background.

Cox regression was also used to model the continuous measures of TCDD. A variety of exposure metrics were considered that took into account different lags, nonlinear relationships (e.g., log-transform and cubic spline), as well as threshold and nonthreshold exposure metrics. Categorical analyses were used to evaluate risks across TCDD exposure groups, while different shapes of dose-response curves were evaluated through the use of lagged and unlagged continuous TCDD measures. Categorical analyses of TCDD exposure were conducted using the Cox regression model to derive estimates of relative risk (RR) as described by hazard ratios and 95% CIs. The reference group in this analysis was those workers in the lowest septile cumulative exposure grouping (<335 ppt-years). The septiles were chosen based on cumulative serum levels that considered no lag and also a 15-year lag.

The investigators also conducted dose-response analyses using the toxicity equivalence (TEQ) approach. The TEQ is calculated as the sum of all exposures to dioxins and furans weighted by the potency of each specific compound. In this study, TCDD was assumed to be account for all dioxin exposures in the workplace. For background TEQ levels, the investigators

used a value of 50 ppt in the dose-response modeling. This is based on the assumption that TCDD accounted for 10% of the toxicity of all dioxins and furans (WHO, 1988, 594278), and is equivalent to using a background level of 5 ppt/yr that was used in the derivation of cumulative serum TCDD levels. A statistically significant dose-response pattern was observed for all cancer mortality and TCDD exposure based on log of cumulative TEQs with a 15-year lag. A comparison of the overall model chi-square values indicated that the fit of this model was not as good as that for TCDD.

The hazard ratios among workers grouped by categories of cumulative TCDD exposure (lagged 15 years) suggested a dose-response relationship. Steenland et al. (2001, 197433) found statistically significant excesses in the higher exposure categories compared to the lowest septile. The RR was 1.82, 95% CI = 1.18−2.82 for the sixth septile (7,568−20,455 ppt-years) and 1.62, 95% CI = 1.03−2.56) for the seventh septile (>20,455 ppt-years). Cox regression indicated that log TCDD serum concentrations (lagged 15 years) was positively associated with cancer mortality ($\beta = 0.097$, standard error (β) = 0.032, $p < 0.003$). A statistically significant improvement in fit was observed when a 15-year lag interval was incorporated into the model compared to a model with no such lag [Model χ^2 with 4 degrees of freedom (df) = 7.5]. Results were similar when using a half-life of 7.1 years rather than 8.7 years. The excess lifetime risk of death from cancer at age 75 for TCDD intake (per 1.0-picogram per kilogram [pg/kg] of body weight (BW) per day) was about 0.05−0.9% above a background lifetime risk of cancer death of 12.4%. The results from the best-fitting models provide lifetime risk estimates within the ranges derived using data from the Hamburg cohort (Becher et al., 1998, 197173).

In both categorical and continuous analyses of TCDD based on a linear exposure metric, the dose-response pattern tailed off at high exposures suggesting nonlinear effects. This phenomenon could be due to saturation effects (Stayner et al., 2003, 054922) or, alternatively, could have resulted from increased exposure misclassification of higher exposures (Steenland et al., 2001, 197433). As the authors highlighted, some of the highest exposures might have been poorly estimated as they occurred in workers exposed to short-term high exposures during the clean-up of a spill. The choice of a linear model to develop data from a single time point can also result in exposure misclassification in those individuals that have differences in the length of exposure (Emond et al., 2005, 197317). Misclassification would be less likely at low concentrations where dose-dependent elimination is minimal.

2.4.1.1.1.1.3.2. *Study evaluation.*

An important consideration in the Steenland et al. (2001, 197433) study was the use of a small subset of workers (n = 170) to infer exposures for the remainder of the cohort. This subset comprised surviving members of the cohort (in 1988), and therefore, their age distribution would have differed from the rest of the cohort. Furthermore, these workers were employed at a single plant, at which the work histories were less detailed than at other plants; thus, the development of the exposure scores differed between this plant and that of the others. Also, many of the workers at this plant had the same job title and were employed during the same calendar period. The use of serum data from this subset adds a level of uncertainty that is not readily characterized. Despite this limitation, the use of these sera data to derive cumulative measures for all cohort workers has merit given the strong correlation observed between the exposure scores, and TCDD serum levels estimates at the time of last exposure (Spearman r = 0.90).

The authors performed an extensive series of sensitivity analyses and considered several alternative exposure metrics to the simple linear model. The lifetime excess risk above background was nearly twice as high for the log cumulative serum measures with a 15-year lag when compared to the piecewise linear models with no lag. An important observation was that the exposure metric based on cumulative serum (lagged 15 years) did not fit the data as well as the cumulative exposure score used in earlier analyses (Steenland et al., 1999, 197437). A priori, one would expect that a better fit would be obtained with serum-based measures because serum levels are a better measure of relevant biological dose. As the authors noted, inaccuracies introduced in estimating the external-based exposure scores could have contributed to a poorer fit of the data. Alternatively, exposure misclassification error could be introduced if serum samples based on the 170 workers were not representative of the entire cohort. Although the serum-based measures did not fit the data as well as the exposures scores, the authors regarded them as providing a reasonable fit based on an improvement in log likelihood of 3.99 (between the log cumulative serum model and the log cumulative exposure score model). Moreover, the serum-based measures enabled better characterization of risk in units (pg/kg-day) that can be used in regulation exposures.

2.4.1.1.1.1.3.3. *Suitability of data for TCDD dose-response modeling.*

This study meets all of the epidemiological considerations for conducting a quantitative dose-response analysis for mortality from all cancer sites combined. As mentioned previously, the NIOSH cohort is the largest assembled to date for which TCDD-related risks of cancer mortality can be estimated. The use of serum-based measures provides an objective measure of TCDD exposure. Repeated measures in other study populations have provided reasonable estimates of the half-life of TCDD, which permitted back-extrapolation of exposures.

The authors have made extensive efforts to evaluate a wide variety of nonlinear and linear models with varying lengths of latency and log transformations. The model chi-square test statistics were fairly similar for the log cumulative serum (15-year lag) (Model $\chi^2_{(4df)} = 11.3$) model and the piecewise linear model (no lag) (Model $\chi^2_{(5df)} = 12.5$). These models, however, produced results with twofold differences in lifetime excess risks. These differences underscore the importance of characterizing uncertainty in modeling approaches when conducting dose-response analysis.

The Steenland et al. (2001, 197433) study characterizes risk in terms of pg/kg of body weight per day. Given that tolerable daily intake dioxin levels are typically expressed in pg/kg of body weight (WHO, 1988, 594278), the presentation of risks in terms of these units is an important advance from the earlier analyses that used exposure scores (Steenland et al., 1999, 197437). Many of the Steenland et al. (2001, 197433) findings are consistent with earlier work from this cohort, which is not surprising given that exposures scores were used to derive serum-based levels for the cohort. The findings of excess lifetime risks obtained for the best- fitting model are also consistent with those derived from the Hamburg cohort (Becher et al., 1998, 197173). This study meets the epidemiological considerations noted previously as there is no evidence that the study is subject to bias from confounding due to cigarette smoking or other occupational exposures. Given the considerable efforts to measure effective dose to TCDD among the study participants, this study also meets the requisite dose-response modeling criteria and will be used in quantitative dose-response analyses of cancer mortality.

2.4.1.1.1.1.4. Cheng et al. (2006, 523122).

2.4.1.1.1.1.4.1. *Study summary.*

Cheng et al. (2006, 523122) undertook a subsequent quantitative risk assessment of 3,538 workers in the NIOSH cohort using serum-derived estimates of TCDD. This dose-response analysis was published after the 2003 Reassessment document was released. The goal of this study was to examine the relationship between TCDD and cancer mortality (all sites combined) using a new estimate of dose that estimated TCDD as a function of both exposure intensity and age using a kinetic model. This physiologically based pharmacokinetic model has been termed the "concentration- and age-dependent elimination model" (CADM) and was developed by Aylward et al. (2005, 197014). This model describes the kinetics of TCDD following oral exposure to humans by accounting for key processes affecting kinetics by simulating the total concentration of TCDD based on empirical consideration of hepatic processes (see Section 3.3). An important feature of this kinetic model is that it incorporates concentration- and age-dependent elimination of TCDD from the body; consequently, the effective half-life of TCDD elimination varies based on exposure history, body burden, and age of the exposed individuals. The study was motivated by the reasoning that back-calculations of TCDD using a first-order elimination model and a constant half-life of 7–9 years underestimated exposures to TCDD among workers. This underestimate, in turn, would result in overestimates of the carcinogenic potency of TCDD.

As with the earlier Steenland et al. (2001, 197433) analyses, the cohort follow-up period was extended from 1942 until the end of 1993 and work histories were linked to a job exposure matrix to obtain cumulative TCDD scores. Two cumulative serum lipid exposure metrics (in ppt-years) were constructed using the data obtained from the sample of 170 workers. The first replicated the metric used in a previous analysis of the cohort (Steenland et al., 2001, 197433) and was based on a first-order elimination model with an 8.7-year half-life (Michalek et al., 1996, 198893). The second metric was based on CADM and had two first-order elimination processes (Aylward et al., 2005, 197114). This metric assumes that the elimination of TCDD in humans occurs at a faster rate when body concentrations are high and at slower rates in older individuals (Aylward et al., 2005, 197114; Aylward et al., 2005, 197014). The model was optimized using individuals for which serial measures of serum TCDD were available. These measures were obtained from 39 adults with initial serum levels between 130 and 144,000 ppt

(Aylward et al., 2005, 197014). This group included 36 individuals who had been exposed in the Seveso accident and 3 exposed in Vienna, Austria. In practice, for serum levels greater than 1,000 ppt, the effective half-life would be less than 3 years, and for serum TCDD levels less than 50 ppt, the effective half-life would be more than 10 years (Aylward et al., 2005, 197014). Results from the model indicate that men eliminate TCDD faster than women do as demonstrated previously by Needham et al. (1994, 200030). These age- and concentration-dependent processes were assumed to operate independently on TCDD in hepatic and adipose tissues, and TCDD levels in liver and adipose tissue were assumed to be a nonlinear function of body concentration. Cheng et al. (2006, 523122) calibrated CADM using a dose of 156 ng per unit of exposure score and assumed a background exposure rate of 0.01 ng/kg-month. The average TCDD ppt-years derived from CADM with a 15-year lag was 4.5−5.2 times higher than with the first-order elimination model. The two metrics, however, were highly correlated based on a Pearson correlation coefficient of 0.98 ($p < 0.001$). Comparisons of fit between the CADM and first-order elimination model were made using R^2 values and presented in Aylward et al. (2005, 197014).

Cheng et al. (2006, 523122) compared the mortality experience of NIOSH workers to the U.S. general population using the SMR statistic. SMR statistics also were generated separately for each of the 8 plants and for all plants combined. Cox regression models were used to analyze internal cohort dose-response. These models used age as the time variable, and penalized smoothing spline functions of the CADM metric also were considered. The possible confounding effects of other occupational exposures and other regional population differences were assessed by repeating analyses after excluding one plant at a time. Lagged and unlagged TCDD exposures were analyzed separately, and stratified analyses compared risk estimates for smoking- and nonsmoking-related cancers. Cheng et al. (2006, 523122) adjusted the slope estimates derived from the Cox model for potential confounding effects of race and year of birth.

Overall, a statistically significant excess in all cancer mortality in the cohort occurred relative to the general population (SMR = 1.17, 95% CI = 1.03−1.32). The plant-specific SMRs ranged from 0.62−1.87, with a statistically significant excess evident only for plant 10 (SMR = 1.87, 95% CI = 1.35−2.52). For lung cancer mortality, the overall SMR was not statistically significant (SMR = 1.11, 95% CI = 0.89−1.37). A statistically significant excess for lung cancer also was found for plant 10 (SMR = 2.35, 95% CI = 1.44−3.64). The SMRs between

smoking- (SMR = 1.22, 95% CI = 1.01−1.45) and nonsmoking-related cancers (SMR = 1.12, 95% CI = 0.94−1.33) were comparable.

For the internal cohort analyses of serum-derived measures, the authors were able to replicate the one-compartmental model used previously (Steenland et al., 2001, 197433). As had been noted by Steenland et al. (2001, 197433), an inverse-dose-response pattern was seen for individuals with high exposures (above 95th percentile); this type of pattern is often seen in occupational studies (Stayner et al., 2003, 054922). Excluding these data produced a stronger association between TCDD and all-cause mortality. In fact, only when the upper 2.5% or 5% of observations was removed did a statistically significant positive association become evident with the untransformed data. Similarly, when the model incorporated a lag of 15 years, a statistically significant association was noted only for the untransformed TCDD ppt-years with the upper 5% of observations removed. Stratified analyses revealed little difference between smoking- and nonsmoking-related cancers, and the removal of one plant at a time from the analyses of TCDD ppt-years changes did not substantially change the slope.

2.4.1.1.1.1.4.2. *Study evaluation.*

The authors reported that CADM provided an improved fit over the one-compartmental model, but presented no evidence regarding any formal test of statistical significance. A comparison of R^2 values presented in Aylward et al. (2005, 197014), however, does reveal that the R^2 value increased from 0.27 (first-order compartmental model with an 8.7-year half-life) to 0.40 for CADM. TCDD exposures estimated using CADM were approximately fivefold higher than the one-compartmental model estimates among cohort members with higher levels of exposure. Differences in exposure estimates between the two metrics were less striking among individuals with lower TCDD exposures. The net effect was that CADM produced a 6- to 10-fold decrease in estimated risks compared to estimates previously reported (Steenland et al., 2001, 197433). Nonetheless, the estimates produced by CADM span more than two orders of magnitude under various assumptions. Further uncertainties arise from between-worker variability of TCDD elimination rates, possible residual confounding, and the variability associated with the use of data obtained from other cohorts. Nevertheless, the use of the CADM model to estimate TCDD exposure is considered a significant advantage over the previous first-order body burden calculations.

2.4.1.1.1.1.4.3. *Suitability of data for TCDD dose-response modeling.*

The value of including the NIOSH cohort data has already been established based on investigations published by Steenland et al. (1999, 197437; 2001, 197433). The decision to include data from the quantitative dose-response analysis that Cheng et al. (2006, 523122) conducted relates to the added value that the CADM exposure estimates would provide. The earlier modeling work of Aylward et al. (2005, 197014) provided some support for a modest improvement of the fit of CADM over the first-order compartmental model, and they also confirmed previous studies that found that TCDD elimination rates varied by age and sex. Recent work by Kerger et al. (2006, 198651) also demonstrates that the half-life for TCDD is shorter among Seveso children than the corresponding half-life for adults, and that body burdens influence the elimination of TCDD in humans. That estimates of half-lives among men have been remarkably consistent, with mean estimates ranging between 6.9 and 8.7 years (Flesch-Janys et al., 1996, 197351; Michalek et al., 2002, 199579; Needham et al., 2005, 594295; Pirkle et al., 1989, 197861), however, is noteworthy. Based on the underlying strengths of the NIOSH cohort data and efforts by Cheng et al. (2006, 523122) to improve estimates of effective dose, these data support further dose-response modeling.

2.4.1.1.1.1.5. Collins et al. (2009, 197627).

2.4.1.1.1.1.5.1. *Study summary.*

In a recent study, Collins et al. (2009, 197627) investigated the relationship between serum TCDD levels and mortality rates in a cohort of trichlorophenol workers exposed to TCDD. These workers were part of the NIOSH cohort having accounted for approximately 45% of the person-years in an earlier analysis (Bodner et al., 2003, 197135). The investigators completed an extensive dioxin serum evaluation of workers employed by the Dow Chemical plant in Midland, Michigan, that made 2,4,5-trichlorophenol (TCP) from 1942 to 1979 and 2,4,5-T from 1948 to 1982. Collins et al. (2004, 197267) developed historical TCDD exposure estimates for all TCP and 2,4,5-T workers. This study represents the largest group of workers from a single plant ever studied for the health effects of TCDD. Little information on how vital status was ascertained, either in this paper or in the Bodner et al. (2003, 197135) report of mortality in this cohort. Although the authors indicate that death certificates were obtained from

the states in which the employees died, whether vital status was ascertained from company records or through record linkage to the National Death Index is unclear.

The follow-up interval for these workers covered the period between 1942 and 2003. Thus, the study included 10 more years of follow-up than earlier investigations of the entire NIOSH cohort. Serum samples were obtained from 280 former workers collected during 2004−2005. A simple one-compartment first-order pharmacokinetic model and elimination rates as estimated from the BASF cohort were used (Flesch-Janys et al., 1996, 197351). The "area under the curve" approach was used to characterize workers' exposures over the course of their working careers and provided a cumulative measure of exposure. Analyses were performed with and without 165 of the 1,615 workers exposed to pentachlorophenol to evaluate the impact of these exposures.

External comparisons of cancer mortality rates to the general U.S. population were made using SMRs. Internal cohort comparisons of exposure-response relationships were made using the Cox regression model. This model used age as the time variable, and was adjusted for year of hire and birth year. Only those causes of death for which an excess was found based on the external comparisons or for which previous studies had identified a positive association were selected for dose-response analyses.

A total of 177 cancer deaths were observed in the cohort. For the external comparison with the U.S. general population, overall, no statistically significant differences were observed in all cancer mortality among all workers (SMR = 1.0, 95% CI = 0.8−1.1). Results obtained after excluding workers exposed to pentachlorophenol were similar (SMR = 0.9, 95% CI = 0.8−1.1). Excess mortality in the cohort were found for leukemia (SMR = 1.9, 95% CI = 1.0−3.2) and soft tissue sarcoma (SMR = 4.1, 95% CI = 1.1−10.5). Although not statistically significant SMRs for other lymphohemopoietic cancers included non-Hodgkin's lymphoma SMR = 1.3; 95%CI = 0.6, 2.5) and Hodgkin's disease (SMR = 2.2; 95% CI = 0.2, 6.4).

Internal cohort comparisons using the Cox regression model were performed for all cancers combined, lung cancer, prostate cancer, leukemia, non-Hodgkin's lymphoma, and soft-tissue sarcoma. Whether the internal comparisons excluded those workers exposed to pentachlorophenol is not entirely clear from the text or accompanying table, but presumably they do not. The RR was 1.002 (95% CI = 0.991−1.013) for all cancer mortality per 1 ppb-year increase in cumulative TCDD exposure was not statistically significant. Except for soft tissue

sarcomas, no statistically significant exposure-response trends were observed for any cancer site. For soft tissue sarcoma, analyses were based on only four deaths.

2.4.1.1.1.1.5.2. *Study evaluation.*

A key limitation of this study is that SMRs were not derived for different periods of latency for the external comparison group analysis. The original publication on the NIOSH cohort found that SMRs increased when a 20-year latency period was incorporated (Fingerhut et al., 1991, 197375), and similar patterns have been observed in other occupational cohorts (Manz et al., 1991, 199061; Ott and Zober, 1996, 198101) and among Seveso residents (Consonni et al., 2008, 524825). Additionally, dose-response analyses showed marked increases in slopes with a 15-year latency period (Cheng et al., 2006, 523122; Steenland and Deddens, 2003, 198587). In this context, the absence of an elevated SMR for cancer mortality is consistent with previous findings of the NIOSH cohort. While the cohort did have sufficient follow-up, no evaluation of possible latent effects was presented and this is a major limitation of this study. Further, the evaluation of the exposure metrics should be expanded from what was presented in Collins et al. (2009, 197627) due to the previous analyses of the same workers finding positive associations between cancer mortality and TCDD (Steenland et al., 2001, 197433).

Unfortunately, the Collins et al. (2009, 197627) study did not include a categorical analysis of TCDD exposure and cancer mortality. This categorical analysis would have enabled an evaluation of whether a nonlinear association exists between TCDD exposure and cancer risk. The analyses of both Cheng et al. (2006, 523122) and Steenland et al. (2001, 197433) suggest an attenuation of effects at higher doses, and several investigations have considered log-transformed associations as a means to address nonlinearity. Also, the earlier plant-specific dose-response analyses of Steenland et al. (2001, 197433) are not consistent with the findings for the Midland plant that Collins et al. (2009, 197627) presented. These differences could be due to differences in the construction of exposure metrics, additional follow-up, or lagging of exposures.

2.4.1.1.1.1.5.3. *Suitability of data for dose-response modeling.*

The Collins et al. (2009, 197627) study uses serum levels to derive TCDD exposure estimates and does not appear to be subject to important biases. The reliance on data from one

plant offers some advantages over the multiplant analyses, as heterogeneity in exposure to other occupational agents would be lower. The number of individuals who provided serum samples (n = 280) is greater than the 170 individuals used to derive TCDD estimates for the NIOSH cohort. The authors found a statistically signficant dose-response trend for soft tissue sarcoma mortality and TCDD exposures. Therefore, this study is considered for quantitative dose-response analysis.

2.4.1.1.1.2. *The BASF cohort.*

In 1953, dioxin contamination occurred as a result of an autoclave accident during the production of trichlorophenol at the BASF plant in Ludwigshafen, Germany. A second dioxin incident occurred in 1988 that was attributed to the blending of thermoplastic polyesters with brominated flame retardants. Of the two events, the one on November 13, 1953, was associated with more severe acute health effects, including chloracne that resulted in immediate hospitalizations for seven workers. These adverse events were not linked to TCDD until 1957 when TCDD was identified as a byproduct of the production of trichlorophenol and was shown to induce chloracne (Zober et al., 1994, 197572). Zober and colleagues (1998, 594300) noted that with the 1988 accident, affected individuals did not exhibit clinical symptoms or chloracne, but rather were identified through "analytical measures." In both instances, efforts were made to limit the potential for exposure to employees.

2.4.1.1.1.2.1. Thiess and Frentzel-Beyme (1977, 594302) and Thiess et al. (1982, 064999).

2.4.1.1.1.2.1.1. *Study summary.*

A study of the mortality of workers employed at the BASF plant was first presented in 1977 (Thiess and Frentzel-Beyme, 1977, 594302) with subsequent updates in both 1982 (Thiess et al., 1982, 064999), and in 1990 (Zober et al., 1990, 197604). In the first published paper (Thiess et al., 1982, 064999), 74 employees involved in the 1953 accident were traced and their death certificate information extracted. Of these, 66 suffered chloracne or severe dermatitis. Observed deaths were compared to the expected number using three external reference groups: the town of Ludwigshafen (n = 180,000), the district of Rhinehessia-Palatinate (n = 1.8 million), and the Federal Republic of Germany (n = 60.5 million). Another comparison group was assembled by selecting age-matched employees taken from other cohorts under study. This

additional comparison was aimed at avoiding potential biases associated with healthy worker effect when using an external referent.

During a follow-up interval of up to 26 years (1953−1979), 21 individuals died. Of these, seven deaths were from cancer. The expected number of cancer deaths derived for the three external comparison groups ranged between 4.1 and 4.2, producing an SMR of 1.7 (p-values ranged between 0.12 and 0.14). Excess mortality was found for stomach cancer based on the external comparisons ($p < 0.05$); however, this was based on only three cases. No other statistically significant excesses were found with the external comparisons made to the other cohorts of workers.

2.4.1.1.1.2.1.2. *Study evaluation.*

In the Thiess et al. (1982, 064999) study, no TCDD exposures were derived for the workers, thus no dose-reconstruction was performed. The findings from this study are limited by the small size of the cohort. The 74 workers followed in this cohort represent the smallest number of workers across the occupational cohorts (Becher et al., 1998, 197173; Fingerhut et al., 1991, 197375; Hooiveld et al., 1998, 197829; McBride, 2009, 198490; McBride et al., 2009, 197296; Michalek and Pavuk, 2008, 199573; Steenland et al., 2001, 197433) that have investigated TCDD exposures and cancer mortality. Mechanisms of follow-up were excellent as all individuals were traced, and death certificates were obtained from all deceased workers.

Although the study does compare the mortality experience to other occupational cohorts, the paper provides insufficient information to adequately interpret the associated findings. For example, a description of these occupations is lacking making it impossible to determine whether these cohorts were exposed to other occupational carcinogens that might have confounded the associations between TCDD exposure and cancer mortality.

2.4.1.1.1.2.1.3. *Suitability of data for TCDD dose-response modeling.*

Subsequent data assembled for the BASF cohort provide more detailed exposure characterization and also include information for 243 male workers employed at the plant. As such, this study did not meet the considerations for further dose-response analysis.

2.4.1.1.1.2.2. Zober et al. (1990, 197604).

2.4.1.1.1.2.2.1. *Study summary.*

Zober et al. (1990, 197604) also examined the mortality patterns of 247 individuals involved in the 1953 accident at the BASF plant. As detailed in their paper, the size of the original cohort was expanded by efforts to locate all individuals who were exposed in the accident or during the clean-up. Three approaches were followed in assembling the cohort. Sixty-nine cohort members were identified from the company physician's list of employees exposed as a result of the accident (Subcohort C1). Sixty-six of these workers were included in the original study population of workers Thiess et al. (1982, 064999) examined. Eighty-four other workers who were potentially exposed to TCDD due to their involvement in demolitions or operations were added to the cohort. This group included 43 firemen, 18 plant workers, 7 bricklayers, 5 whitewashers, 4 mechanics, 2 roofers, and 5 individuals in other occupations (Subcohort C2). The cohort was further augmented through the Dioxin Investigation Program, which sought to locate those who were involved in the 1953 accident and were still alive in 1986. Current and former workers enrolled in the study were asked to identify other current or former coworkers (including deceased or retired) who might have been exposed from the accident. This third component of 94 workers (Subcohort C3) included 27 plant workers, 16 plumbers, 10 scaffolders, 10 professionals, 7 mechanics, 6 transportation workers, 5 bricklayers, 5 laboratory assistant, 3 insulators, and 5 individuals in other occupations. A medical examination was performed for those identified through the Dioxin Investigation Program, and blood measures were obtained for 28 of these workers.

External comparisons of the workers' mortality experience to the general population of the Federal Republic of West Germany were made using SMRs. Person-years were tabulated across strata defined by calendar period, sex, and age group. Sixty-nine deaths including twenty-three from cancer were detected among the workers during the 34-year follow-up period (November 17, 1953 through December 31, 1987). Cause-specific death rates for these same strata were available for the Federal Republic of West Germany. Stratified analyses were conducted to examine variations in the SMRs according to years since first exposure (0–9, 10–19, and ≥20 years) for each of the three subcohorts, as well as 114 workers with chloracne.

Although it was consistent in magnitude with findings from the NIOSH cohort, a statistically significant SMR for all cancer mortality was not observed (SMR = 1.17,

90% CI = 0.80−1.66). The SMRs for each of the three subcohorts varied substantially. For Subcohorts C1, C2, and C3, the SMRs were 1.30 (90% CI = 0.68−2.26), 1.71 (90% CI = 0.96−2.83), and 0.48 (90% CI = 0.13−1.23), respectively. The SMRs increased dramatically when analyses were restricted to those with 20 or more years since first exposure in Subcohort C1 (SMR = 1.67, 90% CI = 0.78−3.13) and Subcohort C2 (SMR = 2.38, 90% CI = 1.18−4.29). Meanwhile, in a subgroup analysis of those with chloracne, for the period of 20 or more years after first exposure, a statistically significant excess in cancer mortality was noted (SMR = 2.01; 90% CI = 1.22−3.15).

2.4.1.1.1.2.2.2. *Study evaluation.*

An important limitation of the study is the manner in which the cohort was constructed. Subcohort C3 was constructed by identifying individuals who were alive in 1986. This resulted in 97 active and retired employees who participated in the program, with 94 included in the analysis. Although these individuals did identify other workers who might have also retired or died, inevitably, some individuals who had died were not included in the cohort. This would serve to underestimate the SMRs that were generated with external comparisons to the German population. Indeed, cancer mortality rates in this subcohort were about half of what would have been expected based on general population rates (SMR = 0.48, 90% CI = 0.13−1.23). Additionally, more than half of Subcohort C2 were firemen (43 of 84), who would likely have been exposed to other carcinogens as a consequence of their employment. Quantitative analyses of epidemiologic data for firefighters have demonstrated increased cancer risk for several different forms of cancer (Youakim, 2006, 197295). Therefore, potential confounding from other occupational exposures of the firefighters could have contributed to the higher SMR in Subcohort C2 cohort and is a concern. Data on cigarette smoking were not available either. No excess for nonmalignant respiratory disease was found, however, suggesting this might not be an important source of bias.

2.4.1.1.1.2.2.3. *Suitability of data for TCDD dose-response modeling.*

As with the Thiess et al. (1982, 064999) publication, worker exposure was not estimated. Lack of exposure estimates precludes a quantitative dose-response analysis using these data. Also, the study design is not well suited to characterization of risk using the SMR statistic.

Mortality is also likely under-ascertained in the large component of the cohort that was constructed through the identification of surviving members of the cohort.

2.4.1.1.1.2.3. Ott and Zober (1996, 198101).

2.4.1.1.1.2.3.1. *Study summary.*

Ott and Zober (1996, 198101) extended the analyses of the BASF cohort to include estimates of individual-level measures of TCDD. The researchers also investigated associations with cancer mortality and identified incident cancer cases. The cohort follow-up period of 39 years extended until December 31, 1992, adding 5 years to a previous study (Zober et al., 1990, 197604). Ott and Zober (1996, 198101) identified incident cases of cancer using occupational medical records, death certificates, doctor's letters, necropsy reports, and information from self-reported surveys sent to all surviving cohort members. Self-reported cancer diagnoses were confirmed by contacting the attending physician.

This study characterized exposure by two methods: (1) determining chloracne status of the cohort members and (2) estimating cumulative TCDD (μg/kg) levels. In 1989, serum measures were sought for all surviving members of the 1953 accident, and serum TCDD levels were quantified for 138 individuals. These serum levels were used to estimate cumulative TCDD concentrations for all 254 members of the accident cohort. Ott et al. (1993, 594322) published a description of the exposure estimation procedure, which was a regression model that accounted for the circumstances and duration of individual exposure. The average internal half-life of TCDD was estimated to be 5.8 years based on repeated serum sampling of 29 individuals. The regression model allowed for this half-life to vary according to the percentage of body fat, and yielded half-lives of 5.1 and 8.9 years among those with 20% and 30% body fat, respectively. Previous analyses of this cohort had used a half-life of 7.0 years (Ott et al., 1993, 594322).

TCDD half-life has been reported to increase with percentage of body fat in both laboratory mammals (Geyer et al., 1990, 197700) and humans (Zober and Papke, 1993, 197602). Ott and Zober (1996, 198101) contend that observed correlations with chloracne severity and cumulative estimates of TCDD exposure indirectly validated this exposure metric. Specifically, the mean TCDD concentration for those without chloracne was 38.4 ppt; for those with moderate and severe forms of chloracne, the mean was 420.8 ppt and 1,008 ppt, respectively.

Unlike for the NIOSH cohort, individual-level data were collected for other cancer risk factors. These factors included body mass index at time of first exposure, history of occupational exposure to β-naphthylamine and asbestos, and history of smoking. Smoking data were available for 86% of the cohort. SMRs were based on the external referent population of West Germany. For cancer incidence, Ott and Zober (1996, 198101) generated standardized incidence ratios (SIRs) using incidence rates for the state of Saarland (1970−1991) as the external referent. They calculated SMRs (and SIRs) for three categories of cumulative TCDD levels: <0.1 μg/kg, 0.1−0.99 μg/kg and ≥1 μg/kg. The Cox regression model was used to characterize risk within the cohort using a continuous measure of TCDD. These analyses considered the potential confounding influence of age, smoking, and body mass index using a stepwise regression modeling approach. The Cox modeling employed a stratified approach using the date of first exposure to minimize possible confounding between calendar period and exposure. The three first exposure groups were exposure within the first year of the accident, exposure between 1 year after the accident and before 1960, and exposure after 1959. The Cox regression estimates were presented in terms of conditional risk ratios (i.e., hazard ratios adjusted for body mass index, smoking and age).

Although no statistically significant excesses relative to the general population were detected for all cancer mortality, there was some suggestion of an exposure-response relationship. In the 0.1−0.99 μg/kg and ≥1 μg/kg exposure groups, the all cancer SMRs were 1.2 (95% CI = 0.5−2.3) and 1.6 (95% CI = 0.9−2.6), respectively. Higher SMRs for cancer (all sites combined) were also found with an increased interval since exposure first occurred. Specifically, when observed versus expected counts of cancer were compared in the time interval 20 years after first exposure, the SMR in the highest exposure group (≥1 μg/kg) was 1.97 (95% CI = 1.05−5.36). An excess in lung cancer also was noted with the same lag in this exposure group (SMR = 3.06, 95% CI = 1.12−6.66). For cancer incidence, a statistically significant increased SIR for lung cancer was observed in the highest exposure category (SIR = 2.2, 95% CI = 1.0−4.3), but no other statistically significant associations were detected for any other cancer site. No cases of soft-tissue sarcoma were found among the cohort members in this analysis.

Based on internal cohort comparisons, Cox regression models also were used to generate hazard ratios as measures of relative risk for TCDD exposures following adjustment for

smoking, age and body mass index. A statistically significant association between TCDD dose (per µg/kg) and cancer mortality was detected (RR = 1.22, 95% CI = 1.00−1.50), but not for cancer incidence (RR = 1.11, 95% CI = 0.91−1.35). Statistically significant findings were observed for stomach cancer mortality (RR = 1.46, 95% CI = 1.13−1.89) and incidence (RR = 1.39, 95% CI = 1.07−1.69).

The Ott and Zober (1996, 198101) study also compared the relationship between TCDD exposure categories and cancer mortality from all sites combined according to smoking status. Associations were noted between increased exposure to TCDD and mortality from cancer among smokers, but not among nonsmokers or former smokers.

2.4.1.1.1.2.3.2. *Study evaluation.*

The Ott and Zober (1996, 198101) study characterizes exposure to TCDD at an individual level. Therefore, unlike in past studies involving this cohort, these data can provide an opportunity for conducting quantitative dose-response modeling. As with the more recent studies involving the NIOSH cohort, serum samples were obtained from surviving cohort members and then used to back-extrapolate TCDD values for all cohort members. In the BASF cohort, however, serum data were available for a much higher percentage of cohort members (54%) than in the NIOSH cohort (5%). An additional study strength was the collection of questionnaire data, which allowed for the potential confounding from cigarette smoking and body mass index to be examined.

The Ott and Zober (1996, 198101) study also evaluates the relationship between TCDD and cancer incidence. Most cohort studies of TCDD-exposed workers have relied solely on mortality outcomes. The availability of incidence data better allows for period of latency to be described, and moreover, to characterize risks associated with cancers that typically have long survival periods. The authors provide few details on the expected completeness of ascertainment for incident cancer cases, which makes determining any associated bias difficult. They do, however, suggest that nonfatal cancers are more likely to have been missed in the earlier part of the follow-up. The net result of differential case ascertainment over time makes evaluating differences in risk estimates across different periods of latency impossible.

The small sample size of the cohort (n = 243 men) likely limited the statistical power to detect small associations for some of the exposure measures. This also effectively limited the

ability to analyze dose-response relationships quantitatively, particularly across strata such as time since exposure. For site-specific analyses, the cancer site with the most cancer deaths was the respiratory system ($n = 11$). Thus, quantitative dose-response analysis using these cohort data would be limited to the evaluation of all cancer sites combined.

The most important limitation of this study is related to the construction of the third component of the cohort. As mentioned earlier, this cohort was assembled by actively seeking out surviving members of the cohort in the mid-1980s. The mortality experience of this cohort is much lower than that of the general population over the entire follow-up, a result that is expected given that the individuals were known to be alive as of 1986. The net result is likely an underestimate of the SMR.

2.4.1.1.1.2.3.3. *Suitability of data for TCDD dose-response modeling.*

This study was included in the quantitative dose-response modeling for the 2003 Reassessment (U.S. EPA, 2003, 537122). The characterization of exposure data and availability of other risk factor data at an individual level are appropriate for use in quantitative dose-response analyses.

2.4.1.1.1.3. *The Hamburg cohort.*

The Hamburg cohort has been the subject of several cancer risk assessments. As with the NIOSH and BASF cohorts, analyses have progressed from basic comparisons of mortality experience to general population rates to more sophisticated internal cohort analyses involving the reconstruction of TCDD exposures using serum measures. This cohort consists of approximately 1,600 workers who were employed in the production of herbicides at a plant in Hamburg, Germany during 1950−1984 (Becher et al., 1998, 197173; Flesch-Janys et al., 1995, 197261). The herbicides produced included 2,4,5-T, β-hexachlorocyclohexane and lindane. The production of TCP and 2,4,5-T was halted in 1954 following a chloracne outbreak. The plant ceased operations in 1984. Approximately 20 different working areas were identified, which, in turn, were grouped into five main areas based on putative TCDD exposure levels. One working area was deemed to be extremely contaminated, having TCDD exposures at least 20-fold higher than in other areas. In this section, the studies undertaken in this cohort that have examined cancer mortality are summarized.

2.4.1.1.1.3.1. Manz et al. (1991, 199061).

2.4.1.1.1.3.1.1. *Study summary.*

Manz et al. (1991, 199061) investigated patterns of mortality in the Hamburg cohort. The study population consisted of 1,583 workers (1,184 men, 399 women) who were employed for at least three months between 1952 and 1989. Casual workers were excluded as they lack sufficient personal identifying information thereby not allowing for associations with mortality outcomes to be examined. Vital status was determined using community-based registries of inhabitants throughout West Germany. Cause of death until the end of 1989 was determined from medical records for all cancer deaths and classified based on the ninth revision of the International Classification of Diseases (WHO, 1978, 594329). Although Manz et al. (1991, 199061) present some data on cancer incidence for the cohort, the data are incomplete as information was available on only 12 cases; 93 cancer deaths were observed in the cohort.

In this study, the authors used information on production processes to group workers into categories of low, medium, or high exposure to TCDD. This information was based on TCDD concentrations in precursor materials, products, waste, and soil from the plant grounds, measured after the plant closed in 1984. The distribution of workers into the low, medium, and high exposure groups was 186, 901, and 496, respectively. The authors examined the validity of the three exposure categories using a separate group of 48 workers who provided adipose tissue samples. The median exposure of the 37 volunteers in the high group was 137 and 60 ng/kg in the remaining 11. Information about chloracne in the cohort was incomplete, and, therefore, was not used as a marker of TCDD exposure. Other surrogate measures of exposure were considered in this study, including duration of exposure and year of first employment. For the latter measure, employment that began after 1954 was assumed to result in much lower exposures given that production of 2,4,5-T and TCP stopped in 1954.

External comparisons of cancer mortality were made by calculating SMRs using the general population of West Germany as a referent. Comparisons of mortality in the cohort also were made to a separate cohort of 3,417 gas supply workers to avoid bias from a healthy worker effect. Vital status and cause of death in the gas supply workers were determined using the same methods as used in the Hamburg cohort. SMRs were calculated relative to both referent populations (West Germany and gas supply workers) across low, medium, and high TCDD exposure groups. The comparison of mortality to the gas supply workers, however, extended

only until the end of 1985, whereas, comparisons to the general population extended until 1989. Stratified analyses were undertaken to calculate SMRs for each of the three exposure groups for categories of duration of employment (<20 versus ≥20 years) and date of entry into the cohort (≤1954 vs. >1954).

When compared to the general population, overall cancer mortality was elevated in male cohort members (SMR = 1.24, 95% CI = 1.00−1.52) but not in females (SMR = 0.80, 95% CI = 0.60−1.05). A two-fold increase in female breast cancer mortality was noted although it did not achieve statistical significance at the alpha level of 0.05 (SMR = 2.15, 95% CI = 0.98−4.09). The SMR among men was further increased when analyses were restricted to workers who were employed for at least 20 years (SMR = 1.87, 95% CI = 1.11−2.95). Analyses restricted to those in the highest exposure group produced an even higher SMR for those with at least 20 years of employment (SMR = 2.54, 95% CI = 1.10−5.00). Statistically significant excesses in risk were detected among those who first worked before 1954, but not afterward. Furthermore, a dose-response trend was observed across increasing exposure categories in the subset of workers employed before 1954. The SMRs using the cohort of gas supply workers as the referent group for the low, medium, and high groups in this subset were 1.41 (95% CI = 0.46−3.28), 1.61 (95% CI = 1.10−2.44), and 2.77 (95% CI = 1.59−4.53), respectively. This finding is consistent with what was known about TCDD exposures levels at the plant, namely, that TCDD concentrations were much higher between 1951 and 1954, with subsequent declining levels after 1954.

Generally speaking, patterns of excess mortality were similar when the cohort of gas workers was used as a reference group. The overall SMR for men was 1.39 (95% CI = 1.10−1.75); and was 1.82 (95% CI = 0.97−3.11) when analyses were restricted to workers with 20 or more years of employment. A dose-response trend also was observed across exposure categories when analyses were restricted to those employed for at least 20 years. In particular, with these analyses, no cancer deaths were observed among those in the lowest exposure group, while the SMRs in the middle and high exposure groups were 1.36 (95% CI = 0.50−2.96) and 3.07 (95% CI = 1.24−6.33).

SMRs also were generated for several site-specific cancers relative to the West German general population and the gas worker cohort. No statistically significant excesses were observed using the general population reference. In contrast, statistically significant excesses

were observed for lung cancer (SMR = 1.67, 95% CI = 1.09−2.44) and hematopoietic system cancer (SMR = 2.65, 95% CI = 1.21−5.03) relative to the gas workers cohort.

2.4.1.1.1.3.1.2. *Study evaluation.*

The Manz et al. (1991, 199061) findings indicate an excess of all cancer mortality among the workers with the highest exposures, particularly those who worked for at least 20 years and were employed before 1954. The findings across categories of exposure within the subsets of workers employed for at least 20 years and before 1954, particularly using the cohort of gas supply workers, are consistent with a dose-response relationship. These elevated cancer mortality rates found among those employed before 1954 were likely due to higher TCDD exposures. Other carcinogenic coexposures, such as benzene, asbestos, and dimethyl sulfate, could have occurred among this population. Given that no substantial changes in the production processes at the Hamburg plant occurred after 1954, comparable levels of these coexposures would be expected before and after 1954. Exposures to these other chemicals varied across different departments/groups; therefore, confounding was unlikely since a strong association between concentrations of these chemicals and TCDD exposures was not evident. No information, however, was presented on potential exposure to other dioxin-like compounds which may confound the associations that were detected.

Detailed information on workers' smoking behaviors was not collected. Limited evidence indicated, however, that smoking prevalence between the Hamburg cohort and the gas supply workers cohort was quite similar. A nonrepresentative sample of 361 workers in the Hamburg cohort and the sample of 2,860 workers in the gas supply cohort indicated that the self-reported smoking prevalence was 73% and 76%, respectively. This suggests that the two cohorts are comprised predominantly of smokers. The similarity in overall smoking prevalence indicates that comparisons of cancer mortality between the two groups are not unduly influenced by an inability to adjust for smoking.

2.4.1.1.1.3.1.3. *Suitability of data for TCDD dose-response modeling.*

The data compiled for the Manz et al. (1991, 199061) study do satisfy many of the considerations for conducting quantitative dose-response analysis; health outcomes appear to be ascertained in an unbiased manner, and exposure was characterized on an individual-level basis.

However, as demonstrated in later studies, there was a large dioxin-like compound component that was not quantified or assessed in this study. Dose-response associations between TCDD and cancer mortality were detected, with stronger associations observed with increased periods of latency and for those who first worked when TCDD was at higher levels.

The size of the cohort, although not as large as the NIOSH cohort, does offer sufficient statistical power to evaluate TCDD-related risk for cancers from all cancer sites. The data are limited, however, for characterizing cancer risks among women; only 20 cancer deaths occurred in the 399 women included in the cohort. It is unlikely that the findings are biased by confounding due to cigarette smoking since dose-response patterns were strengthened when comparisons were made to the cohort of gas supply workers rather the general population referent where smoking rates were likely lower. The inability to account for other occupational exposure when TCDD exposures were much higher (pre-1955) could result in confounding if these other exposures were related to TCDD and the health outcomes under consideration. This data set would be suitable for quantitative dose-response modeling if the exposure characterization of the cohort could be improved using biological measures of dose.

2.4.1.1.1.3.2. Flesch-Janys et al. (1995, 197261).

2.4.1.1.1.3.2.1. *Study summary.*

In 1995, Flesch-Janys et al. (1995, 197261) published an analysis of the male employees from the Hamburg cohort that extended the follow-up to 40 years (1952−1992). Inclusion of these three additional years of follow-up resulted in a sample size of 1,189 male workers.

The authors estimated a quantitative exposure variable for concentrations of TCDD in blood at the end of exposure (i.e., when employment in a department ended) and above German median background TCDD levels. The TCDD exposure assessment defined 14 production departments according to TCDD levels in various products in the plant, in waste products, and in various buildings. The time (in years) each worker spent in each department then was calculated. Concentrations of TCDD were determined in 190 male workers using serum (n = 142) and adipose tissue samples (n = 48). The authors used a first-order kinetic model to calculate TCDD levels at the end of exposure for the 190 workers with available polychlorinated dibenzo-p-dioxin (PCDD) and -furan (PCDF) at various time points. Half-lives were calculated from an elimination study of 48 workers from this cohort, and the median TCDD background

level was estimated at 3.4 ng/kg blood fat from the German population (Flesch-Janys et al., 1994, 197372; Päpke et al., 1994, 198279). Using the one-compartment, first-order kinetic model, the half-life of TCDD was estimated to be 6.9 years (Flesch-Janys, 1997, 197305). Increased age and higher body fat percentage were associated with increased TCDD half-life, while smoking was associated with a higher decay rate for most of the congeners examined (Flesch-Janys et al., 1996, 197351). Cumulative TCDD exposures were estimated by summing exposures over the time spent in all production departments and were expressed in terms of ng/kg of blood fat. The authors also applied a metric of total toxicity equivalence (TOTTEQ) as the weighted sum of all congeners where weights were TEQs that denoted the toxicity of each congener relative to TCDD.

Similar to previous analyses on this cohort, comparisons were made using an external referent group of workers from a gas supply company (Manz et al., 1991, 199061). In contrast to previous analyses where SMR statistics were generated using this "external" reference, however, Flesch-Janys et al. (1995, 197261) used Cox regression. The Cox regression models treated the gas worker cohort as the referent group, and six exposure groups were defined by serum-derived cumulative TCDD estimates. The groups were determined by using the first four quintiles with the upper two exposure categories corresponding to the ninth and tenth deciles of the cumulative TCDD. Internal cohort comparisons used those workers in the lowest quintile as the referent group, as opposed to the cohort of gas workers. A similar approach was used to model TEQs. No known TCDD exposures occurred in the gas workers, so they were assigned exposures based on the median background levels in the general population. RRs were calculated based on exposure above background levels; in other words, background levels were assumed to be equivalent across all workers and also for those employed by the gas supply company. The RRs derived using the Cox model were adjusted for total duration of employment, age, and year when employment began.

The Cox regression with the cohort of gas workers as the referent exposure group yielded a linear dose-response relationship between cumulative TCDD exposure and cancer mortality for all sites combined ($p < 0.01$). The RRs for all-cancer mortality were 1.59, 1.29, 1.66, 1.60, 1.70, and 3.30. For four of the six categories (excluding the referent group), the RRs were statistically significant ($p < 0.05$); in the highest TCDD exposure category (344.7−3,890.2 ng/kg) the RR was 3.30 (95% CI = 2.05−5.31). Similar findings were evident with TOTTEQ. A dose-response

pattern for all cancer mortality ($p < 0.01$) based on the internal cohort comparisons was also detected.

The authors performed an additional analysis to evaluate the potential confounding role of dimethylsulfate. Although no direct measures of dimethylsulfate were available, the investigators repeated analyses by excluding 149 workers who were employed in the department where dimethylsulfate was present. A dose-response pattern persisted for TCDD ($p < 0.01$), and those in the highest exposure group (344.7−3,890.2 ng/kg of blood fat) had a RR of 2.28 (95% CI = 1.14−4.59).

2.4.1.1.1.3.2.2. *Study evaluation.*

The Flesch-Janys et al. (1995, 197261) study used serum-based measures to determine cumulative exposure to TCDD at the end of employment for all cohort members. They used the standard one-compartment, first-order kinetic model and samples obtained from 190 male workers. This quantitative measure of exposure permits an estimation of a dose-response relationship.

Confounding for other occupational exposures is unlikely to have biased the results. A dose-response relationship persisted after excluding workers exposed to dimethylsulfate. Other potential exposures of interest included benzene and isomers of hexachlorocyclohexane. Exposure to these agents, however, was highest in the hexachlorocyclohexane and lindane department, where TCDD exposures were lower. Confounding was unlikely due to exposure to these chemicals, since a strong association between concentrations of these chemicals and TCDD exposures was not evident (due to considerable variability in concentrations across different departments/groups). As outlined earlier, the study findings are unlikely to be biased for cigarette smoking as cigarette smoking in the cohort was similar to that in the comparison population. Moreover, more recent analyses of serum-based TCDD exposure measures found no correlation with smoking status in this cohort (Flesch-Janys et al., 1995, 197261)—a necessary condition for confounding.

The authors used an exposure metric that described cumulative TCDD exposure of workers at the time they were last exposed. As a result, the authors were unable to characterize risks associated with this metric for different periods of latency despite a sufficient follow-up

period. Subsequent analyses constructed time-dependent measures of cumulative TCDD and accounted for excretion of TCDD during follow-up.

In contrast to most risk assessments of TCDD exposure, this study modeled the relationship between other dioxin-like compounds and the risk of cancer mortality using the TOTTEQ metric.

2.4.1.1.1.3.2.3. *Suitability of data for TCDD dose-response modeling.*

The data used in this study satisfy most of the considerations developed for performing a quantitative dose-response analysis. However, latency period was not examined in this study. Dose-response analyses were, therefore, limited to a subsequent study of this cohort (Becher et al., 1998, 197173), which did examine latency.

2.4.1.1.1.3.3. Flesch-Janys et al. (1998, 197339).

2.4.1.1.1.3.3.1. *Study summary.*

Flesch-Janys et al. (1998, 197339) undertook another analysis on this cohort that incorporated additional sera data for 275 workers (39 females and 236 males). The follow-up period was the same as that used in the 1995 analyses, with mortality follow-up extending until December 31, 1992. Analyses were based on 1,189 males who were employed for at least 3 months from January 1, 1952 onward. The authors continued this dose-response analysis to address limitations in their previous work. One limitation was that the previous method did not account for the elimination of TCDD while exposures were being accrued during follow-up. A second limitation was that the amount of time workers spent in different departments was not considered. In the 1998 study, the "area under the curve" approach was used because it accounts for variations in concentrations over time and reflects cumulative exposure to TCDD. The authors used a first-order kinetic model to link blood levels and working histories to derive department-specific dose rates for TCDD. The TCDD background level of 3.4 ng/kg blood fat for the German population was used (Päpke et al., 1994, 198279). The dose rates were applied to estimate the concentration of TCDD at every point in time for all cohort members. A cumulative measure expressed as ng/kg blood fat multiplied by years was calculated and used in the SMR analysis. SMRs were calculated using general population mortality rates for the German population between 1952 and 1992. No lag period was incorporated into the derivation

of the SMRs. The SMRs were estimated for the entire cohort and for exposure groups based on quartiles obtained from the area under the curve. Linear trend tests were also performed. The overall SMR for cancer mortality in the cohort was 1.41 (95% CI = 1.17−1.68). This SMR value was higher than the SMR of 1.21 reported for this same cohort with 3 fewer years of follow-up (Manz et al., 1991, 199061). In terms of site-specific cancer mortality, excesses were found for respiratory cancer (SMR = 1.71, 95% CI = 1.24−2.29) and rectal cancer (SMR = 2.30, 95% CI = 1.05−2.47). Increased risk for lymphatic and hematopoietic cancer (SMR = 2.16, 95% CI = 1.11−3.17) were also noted largely attributable (SMR = 3.73, 95% CI = 1.20−8.71) to lymphosarcoma (i.e., non-Hodgkin's lymphoma). A dose-response relationship was observed across quartiles of cumulative TCDD for all-cancer mortality ($p < 0.01$). The SMRs for these quartiles were 1.24, 1.34, 1.34, and 1.73. Dose-response relationships were not observed for lung cancer or hematopoietic cancers using this same metric. Dose-response relationships were not observed with cumulative TEQ for any of the cancer sites examined (i.e., all cancers, lung cancer, hematopoietic cancer).

2.4.1.1.1.3.3.2. *Study evaluation.*

The approach used in the Flesch-Janys et al. (1998, 197339) study offers a distinct advantage over earlier analyses involving the same cohort. Three more years of follow-up were available, and the characterization of exposure using the "area under the curve" better captures changes in cumulative exposure using a person-years approach rather than cumulative TCDD at the time of last exposure. As noted previously, other occupational exposures or cigarette smoking are unlikely to have biased the study findings. A sufficient length of follow-up had accrued, and dose-response associations were evident. Dioxin-like compounds were evaluated in this study. For TCDD, the mean concentration was 101.3 ng/kg at the time of measurement. For other higher chlorinated congeners, the corresponding mean (without TCDD) was 89.3 ng/kg.

2.4.1.1.1.3.3.3. *Suitability of data for TCDD dose-response modeling.*

The data used in this study satisfy most of the considerations developed for performing a quantitative dose-response analysis. However, latency was not examined in this study. Dose-response analyses were, therefore, limited to a subsequent study of this cohort (Becher

et al., 1998, 197173) which did examine latency and supersedes the Flesch-Janys et al. (1998, 197339) study.

2.4.1.1.1.3.4. Becher et al. (1998, 197173).

2.4.1.1.1.3.4.1. *Study summary.*

The Becher et al. (1998, 197173) quantitative cancer risk assessment for the Hamburg cohort was highlighted in the 2003 Reassessment as being appropriate for conducting dose-response analysis. The integrated TCDD concentration over time, as estimated in the Flesch-Janys et al. (1998, 197339) study, was used as the exposure variable. Estimates of the half-life of TCDD based on the sample of 48 individuals with repeated measures were incorporated into the model that back-calculated TCDD exposures to the end of the employment (Flesch-Janys et al., 1996, 197351). This method took into account the age and body fat percentage of the workers. In Becher et al. (1998, 197173), the analysis used the estimate of cumulative dose (integrated dose or area under the curve) as a time-dependent variable.

Poisson and Cox regression models were used to characterize dose-response relationships. Both models were applied to internal comparisons where a person-years offset was used and to an external comparison where an offset of expected number of deaths was used. The person-years offset was used to account for varying person-time accrued by workers across exposure categories. The use of the expected number of deaths as an offset allows risks to be described in relation to that expected in the general population. Within each classification cell of deaths and person-years, a continuous value TCDD and TEQ levels based on the geometric mean were entered into the Poisson model. For the Cox model, accumulated dose was estimated based on area under the curve for TCDD, TEQ, TEQ without TCDD, and β-hexachlorocyclohexane. These other coexposure metrics were adjusted for in the Cox regression analyses. Other covariates considered included in the models were year of entry, year of birth, and age at entry into the cohort. A background level of 3.4 ng/kg blood fat for the German population was used (Päpke et al., 1994, 198279). A variety of latencies was evaluated (0, 5, 10, 15, and 20 years), and attributable risk and absolute risk were estimated. The unexposed cohort of gas workers was used for most internal analyses.

Internal and external comparisons using the Poisson model found positive associations with TCDD exposure and mortality from all cancers combined. The slope associated with the

continuous measure of TCDD (μg/kg blood fat × years) for the internal comparison was 0.027 ($p < 0.001$), which decreased to 0.0156 ($p = 0.07$) after adjusting for age and calendar period. The slope for the external comparison was 0.0163 ($p = 0.055$); this estimate was not adjusted for other covariates. For TEQ, the slopes based on the internal comparisons were 0.0274 ($p < 0.001$) in the univariate model and 0.0107 ($p = 0.175$) in the multivariate model after adjusting for age and calendar period. The external estimate of slope for TEQ was 0.0109 ($p = 0.164$). Cox regression of TCDD across six exposure categories, with a lag of 0 years, found a statistically significant linear trend ($p = 0.03$) and those in the upper exposure group had a RR of 2.19 (95% CI = 0.76–6.29). These estimates were adjusted for year of entry, age at entry, and duration of employment. A similar pattern was observed with the Cox regression analysis of TEQ; the linear test for trend, however, was not statistically significant at the alpha level of 0.05 ($p = 0.06$).

Cox regression models that included both TCDD and TEQ (excluding TCDD) were applied. In this model, the slope (β) for TCDD was 0.0089 ($p = 0.058$), while the coefficient for TEQ (excluding TCDD) was -0.024 ($p = 0.70$). This suggests that confounding by other dioxin-like compounds was unlikely and the increased risk of cancer was due to TCDD exposure. For all TEQs combined, the slope was 0.0078 ($p = 0.066$).

The authors used multiple Cox models to evaluate the effect of latency. The slope estimates for both TCDD and TEQ increased dramatically with increasing latency. The slope estimates for TCDD increased from 0.0096 to 0.0160 ($p < 0.05$) when latency was increased from 0 to 20 years. Similar changes in the TEQ slopes were noted (0.0093 to 0.0157). Evaluations of dose-response curves found that the best-fitting curve was concave in shape, thereby yielding higher risk at low exposure. Differences between the fit of the class of models considered [i.e., $RR(x,\beta) = \exp(\beta \log(kx = 1))$], however, were small.

Attributable risks were generated only for TCDD, as the data suggested no effects with other TEQs. The additional lifetime risk of cancer assuming a daily intake of 1 pg TCDD/kg body weight/day was estimated to range between 0.001 and 0.01.

2.4.1.1.1.3.4.2. *Study evaluation.*

The Becher et al. (1998, 197173) study represent perhaps the most detailed analyses performed on any cohort to date. The findings were robust, as similar patterns were found with

and without using the gas supply worker cohort as the referent group. Exposures to other potential confounding coexposures, such as dioxin-like compounds, were taken into account, and workers with exposure to other carcinogens (e.g., lindane) were excluded. Furthermore, latency was examined in this study, unlike earlier studies of this cohort.

2.4.1.1.1.3.4.3. *Suitability of data for TCDD dose-response modeling.*

This study was included in the quantitative dose-response modeling for the 2003 Reassessment (U.S. EPA, 2003, 537122). The data in the Becher et al. (1998, 197173) study are suitable for conducting quantitative dose-response modeling. The exposure data capture cumulative exposure to TCDD as well as exposures to other dioxin-like compounds. The length of the follow-up is sufficient, and the study appears to not be subject to confounding or other types of biases. Therefore, this study is utilized in quantitative dose-response analysis.

2.4.1.1.1.4. ***The Seveso cohort.***

Several studies have evaluated the morbidity and mortality effects of residents exposed to TCDD following a July 10, 1976, accidental release through an exhaust pipe at a chemical plant in the town of Meda near Seveso, Italy. The released fluid mixture contained 2,4,5-T, sodium trichlorophenate, ethylene glycol, and sodium hydroxide. Vegetation in the area showed immediate signs of damage, and in the days following the accident, residents developed nausea, headaches, eye irritation, and dermal lesions, particularly children.

This accident transported TCDD up to 6 km from the plant. Soil samples taken near the plant revealed average levels of TCDD that ranged from 15.5 $\mu g/m^2$ to 580.4 $\mu g/m^2$ in the most contaminated area near the plant (referred to as Zone A) (Bertazzi et al., 2001, 197005). Zone A covered 87 hectares and extended 2,200 m south from the plant. Another, more distant contaminated zone (Zone B) covering 270 hectares also had contaminated soil levels, but the TCDD concentration range was much lower (1.7–4.3 $\mu g/m^3$). A reference zone (Zone R), which surrounded the two contaminated areas, had lower TCDD soil levels (range: 0.9–1.4 $\mu g/m^3$) and included approximately 30,000 residents. Following the accident, most residents in Zone A left the area. Although residents in Zone B remained, they were under strict regulations to avoid consuming homegrown products. In total, 736, 4,737, and 31,800 individuals lived in Zones A, B, and R, respectively. Within days of the accident, 3,300 animals (mostly poultry and rabbits)

were found dead. Emergency slaughtering was undertaken to prevent TCDD from entering the food chain, and within 2 years more than 80,000 animals had been slaughtered. Mechanisms were put into place for long-term follow-up of these residents. Unlike the other studies based on occupational cohorts, the follow-up of this population allows for risks to be characterized for females.

The mortality studies from Seveso published to date have not incorporated serum TCDD levels that were measured in individuals. Needham et al. (1997) describe the collection of serum samples from a sample of the exposed population and control subjects in 1976. In 1988, human exposure to TCDD was assessed by measuring small volumes of serum remaining from medical examinations done in 1976. An examination of these data revealed some of the highest serum TCDD levels ever reported, that the half-life of TCDD in this population was between 7 and 8 years, and that half-life varied between women and men. The half-life of TCDD in serum was longer in women (~9 years) than in men (~7 years) (Needham et al., 1994, 200030). In this report, the findings of studies that characterized cancer risks in relation to exposure to TCDD from the 1976 accident are highlighted. These studies include comparisons of cancer mortality rates to the general population based on zone of residence at the time of accident (Bertazzi et al., 2001, 197005; Consonni et al., 2008, 524825). More recent work done by Warner et al. (2002, 197489) investigated the relationship between serum-based measures of TCDD and breast cancer among participants in the Seveso Women's Health Study (SWHS).

2.4.1.1.1.4.1. Bertazzi et al. (2001, 197005).

2.4.1.1.1.4.1.1. *Study summary.*

Several studies have reported on the mortality experience of Seveso residents. The more recent publications having a longer follow-up of the cohort are evaluated here. In 2001, the findings from a 20-year mortality study of Seveso residents was published (Bertazzi et al., 2001, 197005). The Bertazzi et al. (2001, 197005) study was an extension of the 10- and 15-year follow-ups for mortality (Bertazzi et al., 1989, 197013; Bertazzi et al., 1997, 197097; Pesatori et al., 1998, 523076) and the 10-year follow-up for cancer incidence (Bertazzi et al., 1993, 192445).

In this cohort, TCDD exposures were assigned to the population using a three-level categorical variable representative of the individual's place of residence (Zones A, B, or R) at the

time of the accident or when the person first became a resident of the zone, if that was after 1976. An external comparison to the province of Lombardy was made by generating rate ratios (RR) using Poisson regression techniques. Person-years of follow-up were tabulated across strata defined by age, zone of residence, duration of residence, gender, calendar time, and number of years that had elapsed since the time of exposure. Mortality rates during the preaccident period also were compared to evaluate potential changes in rates due to the accident and to evaluate whether patterns were consistent before and after the accident.

No overall excess in mortality rates from all cancer sites combined was observed in Zones A or B (combined) when compared to the reference population of Lombardy (n = 9 million residents) (RR = 1.0, 95% CI = 0.9–1.2). Analyses of site-specific cancer mortality revealed statistically significant excesses among residents in Zones A or B (combined) for cancer of the rectum (RR = 1.8, 95% CI = 1.0–3.3) and lymphatic and hematopoietic malignancies (RR = 1.7, 95% CI = 1.2–2.5). Lymphatic and hematopoietic malignancies were elevated in women (RR = 1.8, 95% CI = 1.1–3.2) and in men (RR = 1.7, 95% CI = 1.0–2.8).

Analyses stratified by the number of years since first exposure (i.e., 1976) revealed higher risk among men with an increased number of years elapsed. Similar to other studies, the RR for all cancers (combined) was 1.3 (95% CI = 1.0-1.7) among men 15–20 years after first exposure. No such increase after 15 years postexposure, however, was noted in women (RR = 0.8, 95% CI = 0.6–1.2).

2.4.1.1.1.4.1.2. *Study evaluation.*

Ascertainment of mortality appears to be excellent. Vital status was established using similar methods for both the exposed and reference populations. No individual data were collected and, therefore, the possibility that confounding by individual characteristics such as cigarette smoking cannot be entirely dismissed. Bertazzi et al. (2001, 197005) do note that the sociodemographic characteristics of residents in the three zones were similar based on independently conducted surveys, and no differences in chronic respiratory disease were found across the different zones. If excess mortality was attributable to cigarette smoking, such excesses would be expected to be evident during the entire study period. Latency analyses revealed elevated risks 15–20 years postaccident. Finally, no excesses were observed for other smoking-related cancers of the larynx, esophagus, pancreas, and bladder. The observed excesses

in all cancer mortality do not appear to be attributed to differential smoking rates between the two populations.

To examine potential for bias due to noncomparability in the two study populations, a comparison of cancer mortality rates between the Seveso regions and the reference population of Lombardy was conducted. Elevated rates for brain cancer mortality were noted in Seveso relative to Lombardy, but the higher rates of leukemia mortality were found in Lombardy relative to Seveso. That no excess was reported for all cancer sites combined lends credence to the hypothesis that the exposure to TCDD from the accident increased rates of cancer after a sufficient period of latency.

Stratified analyses were performed across several categorical variables including gender and time since exposure. The numbers of cancer site-specific deaths are quite small in many of the 5-year increments since first exposure. The study, therefore, has limited statistical power to detect differences in mortality rates among the comparison groups for many cancer sites.

Bertazzi et al. (2001, 197005) assigned exposures based on zone of residence. Soil sampling within each zone revealed considerable variability in TCDD soil levels within each zone. Moreover, some individuals would have left the area shortly after the accident, and determining the extent to which individuals in Zone B who were subject to the recommendations near the time of the accident adhered to them is difficult. As a result, exposure misclassification is possible, and the use of individual measures of TCDD level in serum is preferred over zone of residence for determining exposure. As noted by the authors, the study is better suited to "hazard identification" than to quantitative dose-response analysis.

2.4.1.1.1.4.1.3. *Suitability of data for TCDD dose-response modeling.*

Given the variability in soil TCDD levels within each zone and the lack of individual level, no effective dose can be estimated for quantitative dose-response analyses. Uncertainty in identifying the critical exposure window for the Seveso cohort is a key limitation. The evaluation of this study indicates that this study is not suitable for quantitative dose-response analysis.

2.4.1.1.1.4.2. Warner et al. (2002, 197489).

2.4.1.1.1.4.2.1. *Study summary.*

To date, Warner et al. (2002, 197489) is the only published investigation of the relationship between serum-based measures of TCDD and cancer in Seveso. Eligible participants from the Seveso Women's Heath Study (SWHS; see Section 2.4.1.2.1.4 for details) were women who, at the time of the accident in 1976, were 40 years of age or younger, had lived in one of the most highly contaminated zones (A or B), and had adequate sera collected soon after the explosion. Enrollment in SWHS was begun in March 1996 and lasted until July 1998. Of the total 1,271 eligible women, 981 agreed to participate in the study. Cancer cases were identified during interview and confirmed through review of medical records. Information on other risk factors including reproductive history and cigarette smoking was obtained through interview.

Serum volumes greater than 0.5 mL collected between 1976 and 1981 volume were analyzed. Most sera were collected in 1976/77 (n = 899); samples were collected in 1978−1981 for 54 women, and in 1996/97 for 28 women. For most samples collected after 1977, serum TCDD levels were back-extrapolated using a first-order kinetic model with a 9-year half-life (Pirkle et al., 1989, 197861). For 96 women with undetectable values, a serum level that was equal to one-half the detection level was used.

Analyses were based only on women who provided serum samples; no extrapolation of values to a larger population was done. Risks were therefore generated using data collected at an individual level. Serum TCDD was analyzed as both a continuous variable and a categorical variable. The distribution of serum TCDD levels of the 15 cases of breast cancer was examined in relation to the distribution of all women in the SWHS. The median exposure was slightly higher among with the 15 cases of breast cancer (71.8 ppt) compared to those without (55.1 ppt), and the exposure distribution among breast cancer cases appeared to be shifted to the right (i.e., the exposures were higher but followed the same distribution); however, no formal test of significance was conducted.

Warner et al. (2002, 197489) used Cox proportional hazards modeling techniques to evaluate risk of breast cancer in relation to TCDD serum levels while controlling for a variety of potential risk factors. In all, 21 women had been diagnosed with cancer, and of these, 15 cases were cancer of the breast. The analysis revealed that for every 10-fold increase in TCDD

log-serum levels (e.g., from 10 to 100 ppt) the risk of breast cancer increased by 2.1 (95% CI = 1.0−4.6). Risk estimates also were generated across four categories (<20, 20.1−44, 44.1−100, >100 ppt), with the lowest category used as the reference. The RRs estimated in the third and fourth highest exposure categories were 4.5 (95% CI = 0.6−36.8) and 3.3 (95% CI = 0.4−28.0). Although statistical significance was not achieved for either category, likely because of the small number of cases, the greater than threefold risk evident in both categories is worth noting. Given that the reference category had only one incident case underscores the limited inferences that can be drawn from these analyses. The authors adjusted for numerous potential confounders, but observed no differences between the crude and adjusted results; the authors, therefore, presented unadjusted risks.

2.4.1.1.1.4.2.2. *Study evaluation.*

The findings from the Warner et al. (2002, 197489) study differ from reports in earlier studies in which mortality outcomes noted the absence of an SMR association. The design of this study is much stronger than earlier ones, given the improved characterization of exposure, the ability to compare incidence rates within the cohort, the ability to control for potential confounding variables at an individual level, and the availability of incident outcomes. The use of incident cases (versus mortality data) should also help minimize potential bias due to disease survival. Another important advantage was the ability to measure TCDD near the time of the accident, thereby reducing the potential for exposure measurement error.

A potentially important limitation of the Warner et al. (2002, 197489) study was that information was collected only from those who were alive as of March 1996. Therefore, TCDD and other relevant risk factor data could not be collected for those who had previously died of breast cancer. Thirty-three women could not participate because they were either too ill or had died. Of these, three died of breast cancer. Given that there were only 15 breast cancer cases, the exclusion of these 3 cases could have dramatically impacted the findings in either direction.

Another limitation was that, at the time of the follow-up, most women were still premenopausal and therefore, most of the cohort (average age = 40.8 years) had not yet attained the age of greater risk of breast cancer (average age at diagnosis among the cases in this cohort was 45.2 years). Although comparable data from Italy were not found, the median age of diagnosis for breast cancer among U.S. women from 2003−2007 was 61 years (Altekruse et al.,

2010). An ongoing follow-up of the cohort should be completed by 2010, which should allow for increased number of incident breast cancers to be identified. Given that the current analyses were based only on 15 incident cases, this will substantially improve the statistical power of the study. A secondary benefit is that the increased follow-up will allow for an investigation of possible differential effects according to the age the women were at the time of exposure.

2.4.1.1.1.4.2.3. *Suitability of data for TCDD dose-response modeling.*

Several aspects of the Warner et al. (2002, 197489) study are weaknesses in the consideration of this study for further dose-response modeling. Only 15 cases of breast cancer were available, and no increases in risk were found with serum TCDD exposures between 20.1 and 44 ppt ($n = 2$) when compared to those with <20 ppt ($n = 1$). The average age at the time of enrollment was 40.8 years while the average age at diagnosis among the cases was 45.2 years. As most women had not yet reached the age when breast cancer cases are typically diagnosed, additional follow-up of the cohort would improve the quantitative dose-response analysis and strengthen this study. A key strength of this study, however, is that Warner et al. (2002, 197489) includes an investigation of the relationship between individual serum-based measures of TCDD and cancer in Seveso. Despite the weaknesses, this study meets the evaluation considerations and criteria for inclusion and will be analyzed for quantitative dose-response modeling.

2.4.1.1.1.4.3. Pesatori et al. (2003, 197001).

2.4.1.1.1.4.3.1. *Study summary.*

Pesatori et al. (2003, 197001) published a review of the short- and long-term studies of morbidity and mortality outcomes in the Seveso cohort in 2003. This paper presented cancer incidence data from 1977 to 1991 for Seveso males and females residing in Zones A, B and R relative to an external population (i.e., uncontaminated areas). Mortality data are also presented for a 20-year follow-up (1976−1996) relative to the reference population. As in the original Bertazzi et al. (2001, 197005) study, RRs were estimated using Poisson regression. No associations were noted for zone of residence and all cancer mortality for either males or females. Although no cases were reported in Zones A and B, soft tissues sarcoma was associated with residence in males from Zone R (RR = 2.6, 95% CI = 1.1−6.3). Among males, residence in Zones A and B was associated with lymphatic and hematopoietic cancer (RR = 1.9,

95% CI = 1.1−3.1). This increased risk was due primarily to non-Hodgkin's lymphoma, which accounted for 8 of the 15 incidence cases (RR = 2.6, 95% CI = 1.3−5.3). Among females, increased incidence of multiple myeloma (RR = 4.9, 95% CI = 1.5−16.1), cancer of the vagina (RR = 5.5, 95% CI = 1.3−23.8), and cancer of the biliary tract (RR = 3.0, 95% CI = 1.1−8.2) was associated with residence in Zones A and B.

2.4.1.1.1.4.3.2. *Study evaluation.*

Study limitations of the Pesatori et al. (2003, 197001) study included exposure misclassification from the use of an ecological measure of exposure (region of residency at time of accident) and low statistical power for some health endpoints. For e.g., all of the RRs presented above for specific cancer mortality among females in the Pesatori et al. (2003, 197001) study were based on fewer than five incident cases.

2.4.1.1.1.4.3.3. *Suitability of data for TCDD dose-response modeling.*

As with the studies of mortality among Seveso residents, the Pesatori et al. (2003, 197001) study does not capture TCDD exposure on an individual basis, and soil TCDD levels considerably vary within each zone. Therefore, the quality of the exposure data is insufficient for estimating the effective dose needed for quantitative dose-response analysis.

2.4.1.1.1.4.4. Baccarelli et al. (2006, 197036).

2.4.1.1.1.4.4.1. *Study summary.*

Given previous findings from Seveso, Baccarelli et al. (2006, 197036) examined t(14;18) translocations in the DNA of circulating lymphocytes of healthy dioxin-exposed individuals. These translocations are associated with the development of cancer, namely follicular lymphomas. The study included 211 healthy subjects of the Seveso area, and 101 who had developed chloracne. The investigators analyzed data from 72 high-TCDD plasma level individuals (≥10 ppt) and 72 low-TCDD plasma levels (<10 ppt). A three-level categorical variable was used to evaluate dose-response. This variable was developed by dividing those with exposures ≥10 ppt into two groups: 10– <50 ppt, and 50−475.0 ppt. Trained interviewers administered a questionnaire that collected data on demographic characteristics, diet, and residential and occupational history.

The prevalence of t(14;18) was estimated as those individuals having a t(14;18) positive blood sample divided by the t(14;18) frequency (number of copies per million lymphocytes). Baccarelli et al. (2006, 197036) found that the frequency of t(14;18) was associated with plasma TCDD levels, but no association between TCDD and the prevalence of t(14;18) was detected.

2.4.1.1.1.4.4.2. *Study evaluation.*

Whether the frequency of t(14;18) associated with plasma TCDD levels translates into an increased risk of lymphoma is uncertain as prospective data of TCDD on those who developed non-Hodgkin's lymphoma are lacking. Moreover, the t(14;18) translocation could be an important event in the pre-B stage cell that contributes to tumorigenicity, however subsequent exposure to carcinogenic agents might be necessary for t(14;18) cells to develop into a malignancy (Höglund et al., 2004, 199130).

2.4.1.1.1.4.4.3. *Suitability of data for TCDD dose-response modeling.*

Given that current TCDD plasma levels were measured for this study, it is unclear if the effects of lymphocyte translocations may be due to initial high exposure or are a function of the cumulative exposure for a longer exposure window. Additionally, whether the frequency of t(14;18) associated with plasma TCDD levels translates into an increased risk of lymphoma is unknown. Dose-response analysis for this outcome, therefore, was not conducted.

2.4.1.1.1.4.5. Consonni et al. (2008, 524825).

2.4.1.1.1.4.5.1. *Study summary.*

Consonni et al. (2008, 524825) analyzed cancer mortality in the Seveso cohort with the addition of a 25-year follow up period. Similar analytic methods as Pesatori et al. (2003, 197001) were applied with 25 years of follow-up added to the analysis (Consonni et al., 2008, 524825). An important addition in this paper was the presentation of RRs for Zone R, which had the lowest TCDD levels. Poisson regression models were used to calculate RRs of mortality using Seregno as the reference population. Cancer deaths observed in Zones A and B were 42 and 244, respectively.

No statistically significant differences in all cancer mortality relative to the reference population were noted in any of the zones (Zone A: RR = 1.03, 95% CI = 0.76−1.39; Zone B:

RR = 0.92, 95% CI = 0.81−1.05; Zone R: RR = 0.97, 95% CI = 0.92−1.02). Statistically significant excesses in mortality from non-Hodgkin's lymphoma (RR = 3.35, 95% CI = 1.07−10.46) and multiple myeloma (RR = 4.34, 95% CI = 1.07−17.52) were observed in the area with the highest TCDD levels (Zone A). No other statistically significant increases in cancer mortality relative to the reference population were apparent. The absence of elevated breast cancer mortality among women in this study was noteworthy, as this finding differs from the results of a study of Seveso women for which TCDD exposures were estimated using serum samples (Warner et al., 2002, 197489).

2.4.1.1.1.4.5.2. *Study evaluation.*

Although no individual-level data on smoking were available, the potential for confounding is likely minimal. Independent smoking surveys found that the smoking prevalence rates in Desio, one of cities affected by the accident, were similar to those in districts just outside the study area (Cesana et al., 1995, 594366). As mentioned earlier, one would expect elevated RRs over the entire study period if smoking had biased the study results, and not just after 15−20 years since exposure to TCDD.

2.4.1.1.1.4.5.3. *Suitability of data for TCDD dose-response modeling.*

The lack of individual-level exposure data precludes quantitative dose-response modeling using these data.

2.4.1.1.1.5. *Chapaevsk study.*

Industrial contamination of dioxin in the Chapaevsk region of Russia has been the focus of research on the environmentally-induced cancer and other adverse health effects. The Chapaevsk region is located in the Samara region of Russia and has a population of 83,000. The region is home to a chemical plant that produced lindane and its derivatives between 1967 and 1987, which are believed to be responsible for local dioxin contamination. Soil sampling has demonstrated a strong gradient of increased TCDD concentrations with decreased proximity to the chemical plant (Revich et al., 2001, 199843).

2.4.1.1.1.5.1. Revich et al. (2001, 199843).

2.4.1.1.1.5.1.1. *Study summary.*

Revich et al. (2001, 199843) used a cross-sectional study to compare mortality rates of Chapaevsk residents to two external populations of Russia and the region of Samara. Mortality rates for all cancers combined among males in Chapaevsk were found to be 1.2 times higher when compared to the Samara region as a whole and 1.3 times higher than Russia. Similar to other studies, statistically significant excess was noted in men (SMR = 1.8, 95% CI = 1.6−1.9) but not in women (SMR = 0.9, 95% CI = 0.8−1.1). Among men, the excess was highest for the smoking-related cancers of the lung (SMR = 3.1, 95% CI = 2.6−3.5) and larynx (SMR = 2.3, 95% CI = 1.2−3.8) and urinary organs (SMR = 2.6, 95% CI = 1.7−3.6). Among females, there was no increased SMR for all cancer sites combined, but excesses for breast cancer (SMR = 2.1, 95% CI = 1.6−2.7) and cancer of the cervix (SMR = 1.5, 95% CI = 1.0−3.1) were statistically significant.

Revich et al. (2001, 199843) also compared age-standardized cancer incidence rates in Chapaevsk to those in Samara. Although statistical tests examining these differences were not reported, higher incidence rates were observed for all cancers combined, cancer of the lip, cancer of the oral cavity, and lung and bladder cancer among males in Chapaevsk. Considerably lower cancer incidence rates also were observed for prostate cancer, cancer of the esophagus, and leukemia/lymphoma among males from Chapaevsk. Among females, incidence rates were higher in 1998 for all cancers in Chapaevsk when compared to Russia and the Samara region, an observation that appears somewhat counter to the presented SMR of 0.9 for all cancer mortality from 1995−1998. Like mortality, rates of breast cancer incidence among women in Chapaevsk were higher than in Russia, as were rates of cervical cancer. Leukemia/lymphoma rates were higher among women in Chapaevsk than in those who lived in the reference populations of Samara and Russia. This finding is contrary to the finding for males who had lower rates of leukemia/lymphoma in Chapaevsk.

2.4.1.1.1.5.1.2. *Study evaluation.*

Although the Revich et al. (2001, 199843) findings suggest TCDD exposures in Chapaevsk are quite high relative to other parts of the world (Akhmedkhanov, 2002, 197140), evaluation of health outcomes to date have been based on ecological data only. This analysis did

not adjust for the influence of other risk factors (e.g., smoking, reproductive characteristics) that could contribute to increased cancer rates for lung cancer in men and breast cancer in women. Given that both the SMRs and SIRs for cancer outcomes vary considerably between men and women, this suggests the possibility that occupational exposures might be a contributing factor in these adverse health outcomes.

Future research in Chapaevsk includes plans to conduct a breast cancer case-control study. Women who were born from 1940 onward and who have been diagnosed with breast cancer before the age of 55 were included in the study, although the plan to characterize TCDD using serum is uncertain (Revich et al., 2005, 198777).

2.4.1.1.1.5.1.3. *Suitability of data for TCDD dose-response modeling.*

This study did not meet the considerations and criteria for inclusion in a quantitative dose-response assessment. Given the lack of exposure data on an individual basis, no effective dose can be estimated for this study population. As such, no dose-response modeling was conducted.

2.4.1.1.1.6. *The Air Force Health ("Ranch Hands" cohort) study.*

Between 1962 and 1971, the U.S. military sprayed herbicides over Vietnam to destroy crops that opposition forces depended upon, to clear vegetation from the perimeter of U.S. bases, and to reduce the ability of opposition forces to hide. These herbicides were predominantly a mixture of 2,4-D, 2,4,5-T, picloram, and cacodylic acid (Institute of Medicine, 2006, 594374). A main chemical sprayed was Agent Orange, which was a 50% mixture of 2,4-D and 2,4,5-T. TCDD was produced as a contaminant of 2,4,5-T and had levels ranging from 0.05 to 50 ppm (Institute of Medicine, 1994, 594376). A series of studies have investigated cancer outcomes among Vietnam veterans. A review of military records to characterize exposure to Agent Orange led Stellman and Stellman (1986, 594380) to conclude that assignment of herbicide levels should not be based solely on self-reports or a crude measure such as military branch or area of service within Vietnam. Investigations have been performed on the Ranch Hands cohort, which consisted of those who were involved in the aerial spraying of Agent Orange between 1962 and 1971. More elaborate methods were used to characterize exposures among these individuals, and these studies are summarized below.

2.4.1.1.1.6.1. Akhtar et al. (2004, 197141).

2.4.1.1.1.6.1.1. *Study summary.*

Akhtar et al. (2004, 197141) investigated the incidence of cancer in the Ranch Hand cohort, which was published after the release of the 2003 Reassessment document (U.S. EPA, 2003, 537122). The Ranch Hand Unit was responsible for aerial spraying of herbicides, including Agent Orange, in Vietnam from 1962 to 1971. Cancer incidence in the Ranch Hand cohort were compared to a cohort that included other Air Force personnel who served in Southeast Asia during the same period but were not involved in the spraying of pesticides. Health outcomes were identified during the postservice period that extended from the time each veteran left Southeast Asia until December 31, 1999. In contrast to previous analyses of this cohort, the Akhtar et al. (2004, 197141) study took into account concerns that both the comparison and spraying cohorts had increased risks of cancer, and addressed the possibility that workers with service in Vietnam or Southeast Asia might have increased cancer risk. The authors addressed the latter concern by adjusting risk estimates for the time spent in Southeast Asia and for the proportion of time spent in Vietnam.

The Ranch Hand cohort comprised 1,196 individuals, and the comparison cohort had 1,785 individuals. The comparison cohort was selected by matching date of birth, race, and occupation (i.e., officer pilot, officer navigator, nonflying officer, enlisted flyer, or enlisted ground personnel). TCDD levels were determined using serum levels collected from veterans who completed a medical examination in 1987. For those who did not have a serum measure taken in 1987, but provided one in subsequent years, TCDD levels were back-extrapolated to 1987 using a first-order kinetic model that assumed a half-life of 7.6 years. Those with nonquantifiable levels were assigned a value of the limit of detection divided by the square root of 2. A total of 1,009 and 1,429 individuals in the Ranch Hand and comparison cohorts, respectively, provided serum measures that were used in the risk assessment. Veterans also were categorized according to the time their tours ended. This date corresponded to changes in herbicide use. These categories were before 1962 or after 1972 (no herbicides were used), 1962−1965 (before Agent Orange was used), 1966−1970 (when Agent Orange use was greatest), and 1971−1972 (after Agent Orange was used). Information on incident cases of cancer in the cohort was determined from physical examinations and medical records. Some malignancies were discovered at death and coded from the underlying causes of death as detailed on the death

certificate. A total of 134 and 163 incident cases of cancer were identified in the Ranch Hand and comparison cohort, respectively. Akhtar et al. (2004, 197141) describe case ascertainment verified by record review as being complete.

External comparisons were made based on the expected cancer experience derived from U.S. national rates using SIRs and the corresponding 95% confidence interval. Person-years and events were tabulated by 5-year calendar and age intervals.

When compared to the general population, no statistically significant excesses in all cancer incidence were observed for either the Ranch Hand (SIR = 1.09, 95% CI = 0.91−1.28) or the comparison cohort (SIR = 0.94, 95% CI = 0.81−1.10). Statistically significant differences were found for three site-specific cancers in the Ranch Hands cohort relative to the general population. Excesses were noted for malignant melanoma (SIR = 2.33, 95% CI = 1.40−3.65) and prostate cancer (SIR = 1.46, 95% CI = 1.04−2.00). In contrast, a reduced SIR was found for cancers of the digestive system (SIR = 0.61, 95% CI = 0.36−0.96). The excess in prostate cancer was also noted in the comparison cohort (SIR = 1.62, 95% CI = 1.23−2.10) relative to the general population. External comparisons were repeated by restricting the cohorts to the period when Agent Orange was used (1966−1970). Again, no statistically significant excesses in all cancer incidence were noted in the Ranch Hand (SIR = 1.14, 95% CI = 0.95−1.37) or comparison cohort (SIR = 0.94, 95% CI = 0.80−1.11). Statistically significant excesses continued to be observed for malignant melanoma (SIR = 2.57, 95% CI = 1.52−4.09) and prostate cancer (SIR = 1.68, 95% CI = 1.19−2.33) in the Ranch Hand component of the cohort. No other statistically significant differences were found among Ranch Hands personnel.

For internal cohort analyses, veterans were assigned to one of four exposure categories. Those in the comparison cohort were assigned to the "comparison category." Ranch Hand veterans that had TCDD serum levels <10 ppt were assigned to the "background" category. Those with a TCDD levels >10 ppt had their TCDD level estimated at the end of their Vietnam service with a first-order kinetic model that used a half-life of 7.6 years. These back-extrapolated values that were less than 118.5 ppt were assigned to a "low" exposure group, while those with values above 118.5 ppt were classified as "high" exposure. Akhtar et al. (2004, 197141) used Cox regression models to describe risks across the exposure groups using the comparison category as the reference. Risks were adjusted for age at tour, military occupation, smoking history, skin reaction to sun exposure, and eye color. Internal cohort analyses were

restricted to those who spent no more than 2 years in Southeast Asia and Ranch Hand workers who served exclusively in Vietnam, and the comparison cohort who served exclusively outside of Vietnam.

Statistically significant excesses of cancer incidence (all sites combined) were observed in the highest two exposure groups. A statistically significant trend test ($p = 0.04$) was detected based on the RRs for the background-, low-, and high- exposure groups: 1.44 (95% CI = 0.82−2.53); 2.23 (95% CI = 1.24−4.00), and 2.02 (95% CI = 1.03−3.95). For malignant melanoma, the RRs across the three increasing exposure categories were 2.99, 7.42, and 7.51. The corresponding risk estimates for prostate cancer were 1.50, 2.17, and 6.04.

2.4.1.1.1.6.1.2. *Study evaluation.*

An important strength of this study is the manner in which TCDD exposure was estimated. Serum data were available for most veterans, and therefore, generalizing exposure from a small sample of cohort members is not a concern as was the case with the NIOSH and Hamburg cohorts. Back-extrapolating to derive past exposures was based on a methodology that has been applied in many of the cohorts, thereby facilitating risk comparisons. An additional strength of the study is the examination of incidence as a measure of disease occurrence rather than mortality.

In contrast to the previous analysis (Ketchum et al., 1999, 198120) the analysis by Akhtar et al. (2004, 197141) was restricted to individuals who spent no more than 2 years in Southeast Asia. Previous research had demonstrated that increased time spent in Southeast Asia was associated with an increased risk of cancer. Confounding might have been introduced given that the comparison cohort spent much more time in Southeast Asia than the Ranch Hands. To illustrate, the median number of days spent in Southeast Asia was 790 for comparison cohort members, and the median days for the Ranch Hand cohort in the background, low, and high exposure groups were 426, 457, and 397, respectively. After restricting to those who spent at most 2 years, statistically significant associations were observed for all cancer sites combined, prostate cancer, and malignant melanoma using the internal cohort comparisons.

An important issue in the study is the high correlation between 2,4,5-T and 2,4-D, given that both were used in equal concentrations in Agent Orange. As a result, distinguishing the effects of each is impossible. This point is relevant, given that 2,4-D has been associated with

prostate cancer in several studies. As a result, the dose-response association with prostate cancer might be due to 2,4-D exposure and not TCDD. This issue also has implications for the interpretation of the dose-response pattern for all cancer sites combined, given that incident prostate cancers accounted for 4 of the 12 incident cases in the high-exposure group.

2.4.1.1.1.6.1.3. *Suitability of data for TCDD dose-response modeling.*

The ascertainment of incident cases and characterization of exposure to TCDD based on serum measures are strong features of the cohort. Confounding by 2,4-D is a major concern. Since delineating the independent effects of other Agent Orange contaminants is not possible, quantitative dose-response analysis was not conducted on this study.

2.4.1.1.1.6.2. Michalek and Pavuk (2008, 199573).

2.4.1.1.1.6.2.1. *Study summary.*

Michalek and Pavuk (2008, 199573) recently published an updated analysis of the incidence of cancer and diabetes in the cohort of Ranch Hand veterans. As with the Akhtar et al. (2004, 197141) analysis, the study included a comparison cohort of other Air Force veterans who served in Southeast Asia at the same time but were not involved with the spraying of herbicides. This study extended previous analyses (Henriksen et al., 1997, 197645; Ketchum et al., 1999, 198120) by addressing the number of days of herbicide spraying, calendar period of service, and the time spent in Southeast Asia. Veterans who attended at least one of five examinations were eligible for inclusion. Incident cancer cases also were identified from medical records.

The methods used to determine TCDD exposures were as described above in the review of the Akhtar et al. (2004, 197141) study. Blood measures also were taken in 1992, 1997, and 2002 for subjects with no quantifiable TCDD levels in 1987, those who refused in 1987, and those new to the study. TCDD dose at the end of service in Vietnam was assigned to Ranch Hands that had TCDD levels above background using a a first-order kinetic model and constant half-life of 7.6 years. Each veteran was then assigned to one of four dose categories: comparison veteran, background (i.e., Ranch Hands with 1987 levels of TCDD ≤10 ppt), low (Ranch Hands with 1987 levels of TCDD 10.1−91 ppt), and high (Ranch Hands with 1987 levels of TCDD ≥118.5 ppt). Serum TCDD estimates are available for 1,597 veterans in the comparison cohort,

and 986 veterans in the Ranch Hand cohort. The comparison cohort was selected by matching on date of birth, race, and occupation of the Ranch Hands.

Michalek and Pavuk (2008, 199573) used Cox regression to characterize risks of cancer incidence across the three upper exposure categories using the comparison category as the referent group. Risk estimates were adjusted for year of birth, race, smoking, body mass index at the qualifying tour, military occupation, and skin reaction to sun exposure. Tests for trend for increased risk of cancer were conducted by testing the continuous covariate $\log_{10}$TCDD.

Overall, no association between the TCDD exposure categories and RR of all-site cancer was observed. Those in the highest exposure group had an RR of 0.9 (95% CI = 0.6−1.4). Stratified analyses by calendar period of service showed more pronounced risk for those who served before 1986 (when higher amounts of Agent Orange were used). A statistically significant dose-response trend ($p < 0.01$) was observed for cancer risk and $\log_{10}$TCDD exposure. The RRs for the background, low, and high groups used in these comparisons were 0.7 (95% CI = 0.4−1.3), 1.7 (95% CI = 1.0−2.9), and 1.5 (95% CI = 0.9−2.6). A statistically significant increase, however, was noted when analyses were restricted to those who had sprayed for at least 30 days before 1967 and spent time in Southeast Asia (RR = 2.2, 95% CI = 1.1−4.4).

2.4.1.1.1.6.2.2. *Study evaluation.*

Michalek and Pavuk (2008, 199573) used the same study population that Akhtar et al. (2004, 197141), and so it has the same strengths and limitations as noted above. The follow-up, however, extends an additional 5 years (until the end of 2004). The findings for the dose-response analyses were not as compelling as the earlier Akhtar et al. (2004, 197141) findings.

2.4.1.1.1.6.2.3. *Suitability of data for TCDD dose-response modeling.*

The key limitation precluding dose-response analysis for the Michalek and Pavuk (2008, 199573) study is the possible confounding from the inability to control for 2,4-D and other agents used in Agent Orange. As such, quantitative dose-response analysis was not conducted on this study.

2.4.1.1.1.7. *Other studies of potential relevance to dose-response modeling.*

2.4.1.1.1.7.1. Hooiveld et al. (1998, 197829)—Netherlands workers.

2.4.1.1.1.7.1.1. *Study summary.*

Hooiveld et al. (1998, 197829) re-analyzed the mortality experience of a cohort of workers employed in two chemical plants in the Netherlands using 6 additional years of follow-up from an earlier study (Bueno et al., 1993, 196993). The cohort consisted of those employed between 1955 and June 30, 1985, and vital status was ascertained until December 31, 1991 (i.e., 36 years of follow-up). These cohort members were involved in the synthesis and formulation of phenoxy herbicides, of which the main product was 2,4,5-trichlorophenoxyacetic acid and monochloroacetic acid. This cohort, with a shorter follow-up interval than the original study (t' Mannetje et al., 2005, 197593), was included in the IARC international cohort. The cohort consisted of 1,167 workers, of which 906 were known to be alive at the end of the follow-up. The average length of follow-up was 22.3 years, and only 10 individuals were lost to follow-up.

The authors used detailed occupational histories to assign exposures. Workers were classified as exposed to phenoxy herbicides or chlorophenols and contaminants if they worked in selected departments (i.e., synthesis, finishing, formulation, packing, maintenance/repair, laboratory, chemical effluent waste, cleaning, shipping-transport, or plant supervision); were exposed to the accident in 1963; or were exposed by proximity (i.e., if they entered an exposed department at least once a week). The 1963 accident was the result of an uncontrolled reaction in the autoclave in which 2,4,5-trichlorophenol was synthesized; an explosion resulted, with subsequent release of PCDDs that included TCDD. Based on these methods of exposure assignment, 562 workers were deemed to be exposed to phenoxy herbicides or chlorophenols, and 567 were unexposed. Due to limited information, 27 workers were classified as having unknown exposure.

TCDD exposures also were assigned using serum measured on a sample of workers who were employed for at least 1 year and first started working before 1975. Dioxin-like compounds including PCDDs were also measured in the serum samples but were not analyzed for this study. Of the 144 subjects who were invited to provide samples, 94 agreed. TCDD levels were back-extrapolated to the time of maximum exposure using a one-compartment, first-order kinetic model that used a half-life estimate of 7.1 years. The mathematical model used was

ln(TCDDmax) = ln(TCDD) + lag × ln(2)/7.1. The lag was defined as the number of years since last exposure for those exposed by virtue of their normal job duties. For those exposed as a result of the accident in 1963, the lag was defined as the number of years since the accident occurred.

The authors made external comparisons of cohort mortality to the Netherlands population using the SMR statistics. Poisson regression was used to perform internal cohort comparisons using unexposed workers as the referent. RRs (measured using rate ratios) generated from the Poisson model also were used to compare mortality based on low, medium, and high TCDD serum-derived categories. The Poisson model included the following covariates as adjustment factors: age, calendar period at end of follow-up, and time since first exposure.

When compared to the general population, workers had an excess mortality from cancer (SMR = 1.5, 95% CI = 1.1−1.9), based on 51 cancer deaths. Generally, no excesses were observed for site-specific cancers. The exception included eight deaths from cancers of the urinary organs (SMR = 3.9, 95% CI = 1.7−7.6). Although not statistically significant, SMRs comparable in magnitude to other studies were detected for non-Hodgkin's lymphoma (SMR = 3.8, 95% CI = 0.8−11.0) and Hodgkin's disease (SMR = 3.2, 95% CI = 0.1−17.6). A statistically significant excess of cancer mortality (n = 20 deaths among occupational workers) also was also observed relative to the general population when analyses were restricted to those exposed as a result of the 1963 accident (SMR = 1.7, 95% CI = 1.1−2.7). Three deaths from prostate cancer were also noted among these workers (SMR = 5.2, 95% CI = 1.1−15.3), but no excess was observed with any other cancer site.

Internal cohort comparison also demonstrated an increased risk of all cancer mortality among those exposed to phenoxy herbicides, chlorophenols, and contaminants relative to those unexposed (RR = 4.1, 95% CI = 1.8−9.0). A statistically significant increased risk was also noted for respiratory cancer mortality (RR = 7.5, 95% CI = 1.0−56.1). Analyses across categories of TCDD exposure revealed excesses in cancer mortality for all cancer sites combined; however, no dose-response trend was apparent.

2.4.1.1.1.7.1.2. *Study evaluation.*

Several other studies that have characterized cohorts by TCDD levels have used the area under the curve approach and thus have derived an exposure metric that is time dependent.

Hooiveld et al. (1998, 197829) instead created an exposure metric to capture the maximum exposure attained during the worker's employment. Characterizing risks using this metric assumes that other TCDD exposures accrued during a workers' lifetime are not relevant predictors of cancer risk.

2.4.1.1.1.7.1.3. *Suitability of data for TCDD dose-response modeling.*

One study limitation is that although dioxin-like compounds were measured in the serum samples, Hooiveld et al. (1998, 197829) reported associations with mortality for TCDD only. There is some utility to examining dose-response analyses using alternative exposure metrics as those constructed in this cohort. However, the small number of identified cancer deaths, limitations in terms of the exposure assignment (based on nonrepresentative sample, and maximum exposure level) and concern over potential confounding by co-exposures preclude using these data for a dose-response analysis.

2.4.1.1.1.7.2. t' Mannetje et al. (2005, 197593)—New Zealand herbicide sprayers.

2.4.1.1.1.7.2.1. *Study summary.*

t'Mannetje et al. (2005, 197593) described the mortality experience of a cohort of New Zealand workers who were employed in a plant located in New Plymouth. The plant produced phenoxy herbicides and pentachlorophenol between 1950 and the mid-1980s. This study population also was included in the international cohort of producers and sprayers of herbicides that was analyzed by IARC (Kogevinas et al., 1997, 198598; Saracci et al., 1991, 199190). In this 2005 study, analyses were restricted to those who had worked at least 1 month; clerical, kitchen, and field research staff were excluded. The authors followed up 1,025 herbicide producers and 703 sprayers from 1969 and 1973, respectively, until the end of 2000.

The cohort consisted of two components: those involved with the production of herbicides and those who were sprayers. For the herbicide producers, exposures were determined by consulting occupational history records; no direct measures of exposure were available. Each department of employment was assigned to one of 21 codes as in the IARC international cohort (Saracci et al., 1991, 199190). Industrial hygienists and factory personnel with knowledge of potential exposures in this workforce classified each job according to potential to be exposed to TCDD, other chlorinated dioxins, and phenoxy herbicides. Exposure

was defined as a dichotomous variable (i.e., exposed and unexposed). Among producers, 813 were classified as exposed, with the remaining 212 considered unexposed.

The "sprayer" component of the cohort includes those who were registered in the national registry of applicators at any time from January 1973 until the end of 1984. For the sprayers, detailed occupational information was lacking. Exposure was, therefore, based on an exposure history questionnaire completed in a previous study of congenital malformations (Smith et al., 1982, 198586). This questionnaire, administered to 548 applicators in 1980 and 232 applicators in 1982, achieved a high response rate (89%). Participants were asked to provide information about 2,4,5-T-containing product use on an annual basis from 1969 up to the year the survey was completed. As the use of 2,4,5-T ceased in the mid-1980s, data on occupational exposure to TCDD among these workers are fairly complete. Virtually all sprayers (699 of 703) were exposed to TCDD, higher chlorinated dioxins, and phenoxy herbicides.

Deaths among workers were identified through record linkage to death registrations in the New Zealand Health Information Service. Electoral rolls, drivers' licenses, and social security records also were consulted to confirm identified deaths. External comparisons of mortality were made to the New Zealand population using the SMR statistic. The mortality follow-up for the producers began on January 1, 1969 and extended until December 31, 2000. For the sprayers, the follow-up period extended from January 1, 1973 until December 31, 2000. A total of 43 cancer deaths occurred in the producer group and 35 cancer deaths occurred in the sprayer group in the cohort. Where possible, stratified analyses by duration of employment and department were conducted. The departments examined for producers included synthesis, formulation and lab, maintenance and waste, packing and transport, other, and unexposed. SMRs were generated using the New Zealand population as an external referent. A linear test for trend was applied to evaluate dose-response trends according to categories of duration of employment. Stratified analyses also were also done for sprayers who started working before 1973, as TCDD levels in 2,4,5-T produced at the New Zealand plant dropped dramatically after 1973. Although an SMR was presented for female producers, given that only one cancer death was observed, this study can provide no insight on differential risks between the sexes.

Among TCDD-exposed producers, for all cancers combined, no statistically significant excess mortality was found when compared to the general population (SMR = 1.24, 95% CI = 0.90−1.67). No dose-response trend in the SMRs for all cancers was observed with

duration of employment (p = 0.44). No statistically significant elevated SMR was observed in any of the duration of employment categories for any of the six specific departments examined. A statistically significant positive linear trend, however, was noted among synthesis workers (p = 0.04). There was some suggestion of reduced mortality in the upper exposure levels for workers in the formulation and lab departments. For sprayers, the SMR for all cancer sites combined was not elevated relative to the New Zealand general population (SMR = 0.82, 95% CI = 0.57−1.14), nor was a dose-response pattern observed with increasing duration of employment (p = 0.86). Additionally, no statistically significant excess in cancer mortality for all sites combined was evident in workers who were first employed either before 1973 (SMR = 0.75, 95% CI = 0.50−1.07) or from 1973 on (SMR = 1.81, 95% CI = 0.59−4.22). For site-specific analyses of cancer mortality, an excess of multiple myeloma was observed among production workers relative to the general population (SMR = 5.51, 95% CI = 1.14−16.1). This SMR was based on three deaths. No statistically significant excess (or deficit) of mortality was found for any other cancer site examined in either the sprayers or the producers.

2.4.1.1.1.7.2.2. *Study evaluation.*

The physical activity demands of spraying contribute to a healthy worker effect that manifests itself in a lower SMR based on both external comparisons to the general population as a referent, and the SMR generated for the producers in the cohort. The analyses conducted using a simple dichotomy of exposure and duration of employment are limited, as nearly all of the sprayers were unexposed.

The dose-response pattern with duration of employment coupled with the observation that higher levels of exposure to TCDD occurred among workers in the synthesis department is an important finding. These workers were also exposed to several other contaminants, however, that include processing chemicals, technical products, intermediates, and byproducts (Kauppinen et al., 1993, 594388). These included phenoxy herbicides and dioxin-like compounds such as chlorinated dioxins. Since the dichotomous exposure measure was based on exposure to TCDD, chlorinated dioxins and phenoxy herbicides, the associated dose-response analyses presented in this study should be interpreted cautiously in light of the inability to either characterize or control for these potential confounders. As such, these co-exposures might have contributed to the dose-response pattern observed with increased duration of employment in the synthesis workers.

2.4.1.1.1.7.2.3. *Suitability of data for TCDD dose-response modeling.*

Although the study authors completed a subsequent analysis of this cohort using serum-derived TCDD (McBride, 2009, 198490), the lack of individual-level TCDD exposures precludes dose-response modeling.

2.4.1.1.1.7.3. McBride et al. (2009, 198490)—New Zealand herbicide sprayers.

2.4.1.1.1.7.3.1. *Study summary.*

McBride et al. (2009, 198490) recently published the mortality experience of the New Zealand cohort in relation to serum estimates of TCDD levels. This study included 1,599 workers who were employed between 1969 and November 1, 1989, which was the date that 2,4,5-T was last used. As in their study published earlier in the same year (McBride et al., 2009, 197296), the follow-up period extended from the first day of employment until December 31, 2004. Vital status was ascertained through record linkage to the New Zealand Health Information Service Mortality Collection and the Registrar General's Index to Deaths for deaths up to 1990.

All current and former workers who lived within 75 km of the plant were invited to provide serum samples. A total of 346 of the eligible workers (68%) provided samples, which represented 22% of the overall study population (346/1599). Based on the serum measures, 70% (241/346) had been exposed to TCDD. This percentage is similar to the estimated 71% of workers who were deemed to have been exposed based on a review of occupational records. The mean serum TCDD value was 9.9 ppt. The highest exposures were observed for those employed in the trichlorophenol operation (23.4 ppt). Values among unexposed workers averaged 4.9 ppt, which is close to the background level of 3.9 ppt among individuals of similar age in the New Zealand general population (Bates et al., 2004, 197113). Details on smoking histories of individuals were also collected for the 346 individuals who provided serum, allowing for an examination of the potential confounding role that smoking might have on derived risk estimates for TCDD.

Cumulative exposure to TCDD as a time-dependent metric was estimated for each worker. A detailed description of the methods used to derive TCDD exposure was described in Aylward et al. (2009, 197187). The qualitative TCDD scores available for those with serum measures were used to estimate the cumulative exposures based on a half-life of approximately

7 years. A time-dependent estimate of TCDD exposure was derived and the area under the curve was used to obtain cumulative workplace TCDD exposure above background levels. Model performance appears modest as the model explained only 30% of the variance (adjusted R^2) when these TCDD exposure estimates were compared with actual serum levels (Aylward et al., 2009, 197187).

As with previous analyses of the cohort (McBride et al., 2009, 197296; t' Mannetje et al., 2005, 197593), external comparisons to the New Zealand general population were made using the SMR statistic. The SMR statistic also was used to compare mortality across four exposure groups relative to the general population, as defined by the serum TCDD estimates: 0–68.3, 68.4–475.0, 475.1–2085.7, and ≥2085.8 ppt-month. The proportional hazards model also was used to conduct internal cohort comparisons across these same four exposure groups. In these analyses, age was used as the time variable, and the covariates of date of hire, sex, and birth year were included in the proportional hazards model. The cut-points for these four exposure categories were chosen so that approximately equal numbers of deaths were included in each category.

Consistent with earlier SMR analyses of the same cohort, no increased cancer mortality was observed among "ever" exposed workers in this cohort when compared to the general population (SMR = 1.1, 95% CI = 0.9–1.4). No statistically significant excess was noted for any of the site-specific cancers, although there was some suggestion of increased risk of soft tissue sarcoma (SMR = 3.4, 95% CI = 0.1–19.5), multiple myeloma (SMR = 2.2, 95% CI = 0.2–8.1), non-Hodgkin's lymphoma (SMR = 1.6, 95% CI = 0.3–4.7), and cancer of the rectum (SMR = 2.0, 95% CI = 0.7–4.4). No statistically significant increases in cancer mortality (all sites combined) was found in any of the four exposure categories as measured by the SMR statistic, nor was a dose-response trend noted with increasing exposure categories. No dose-response trends (based on SMR analyses) were noted for five site-specific cancers examined (i.e., digestive organs, bronchus, trachea and lung, soft tissue sarcomas, lymphatic and hematopoietic tissue, and non-Hodgkin's lymphoma), although SMRs for three of the four exposure categories exceeded 2.0 for non-Hodgkin's lymphoma.

In contrast to the external cohort comparisons, the RRs generated with the proportional hazards model supported a dose-response trend, as rate ratios increased across increasing TCDD exposure categories. The RRs and their 95% confidence intervals relative to the lowest of the

four groups were 1.05 (95% CI = 0.48−2.26), 1.38 (95% CI = 0.64−2.97) and 1.58 (95% CI = 0.71−3.52). Neither the linear (p = 0.29) or quadratic (p = 0.82) test for trend, however, was statistically significant. An increased risk of lung cancer mortality was observed in the highest TCDD exposure category relative to the lowest (RR = 5.75, 95% CI = 0.76−42.24). The tests for trend for lung cancer, however, also were not statistically significant.

A smoking survey was administered to a sample of surviving workers of this cohort, and smoking prevalence was found to be slightly higher among those with higher cumulative exposure (61%) compared to lower exposures (51−56%). These minor differences in smoking prevalence unlikely was a strong enough confounder to explain the fivefold increase in risk of lung cancer found in the highest exposure category. Although the smoking data assessment was a strength of the study, it was limited to only sample of workers and was not available for those who died of lung cancer.

2.4.1.1.1.7.3.2. *Study evaluation.*

Given high rates of emigration, loss to follow-up (22%) was a potential concern in this study. If comparable emigration rates did occur among the general population then the SMRs would be underestimated. It is unclear to what extent emigration occurred among the general population and whether emigration in both the worker and general populations was dependent on health status. If emigration rates were comparable among these two populations, the associated bias from the under-ascertainment of mortality in the lost to follow-up group would likely attenuate a positive association between TCDD and cancer mortality. Among the worker population, there was not much evidence of differential loss to follow-up with respect to exposure as average exposures were lower (3.2 ppt) among those loss to follow up compared to those with complete follow-up (5.7 ppt). Previous studies among this population also found slightly higher loss to follow-up rates among the unexposed (23%) compared to the exposed (17%) workers (t' Mannetje et al., 2005, 197593).

McBride et al. (2009, 198490) did not present results using a continuous measure of TCDD exposure (lagged or unlagged) as was done in most other occupational cohorts. Additionally, the modeling did not consider the use of different periods of latency.

2.4.1.1.1.7.3.3. *Suitability of data for TCDD dose-response modeling.*

There is no evidence that the authors considered exposure metrics that are consistent with environmental cancer-causing agents such as exposure modeling that takes latency into account. Given that past occupational cohort studies of TCDD-exposed workers have consistently demonstrated stronger association with lag interval of 15 years, such an approach should be applied to this cohort. This precludes this study from consideration for quantitative dose-response modeling.

2.4.1.1.1.7.4. McBride et al. (2009, 197296)—New Zealand herbicide sprayers.

2.4.1.1.1.7.4.1. *Study summary.*

McBride et al. (2009, 197296) published an updated analysis of the mortality of the New Zealand cohort. The follow-up period was from January 1, 1969 to December 31, 2004 extending the previous study by an additional 4 years. In contrast to the previous study where the cohort comprised individuals employed for at least 1 month prior to 1982 (or 1984) (t' Mannetje et al., 2005, 197593), the cohort in this study consisted of all those who worked at least one day between January 1, 1969 and October 1, 2003. This resulted in a cohort of 1,754 workers, of which 247 died in the follow-up interval. Seventeen percent of the cohort members were lost to follow-up, which could be a source of selection bias if loss to follow-up was related to both the exposure metrics and the health outcome of interest. Previous data from this cohort (t' Mannetje et al., 2005, 197593), however, showed fairly comparable loss to follow-up rates among the unexposed (23%) and the exposed populations (17%).

Comparisons to the New Zealand general population were made using the SMR statistic. Stratified analyses were conducted by duration of employment (<3 months, ≥3 months), sex, latency (<15 years, ≥15 years), and period of hire (<1976, ≥1976). The authors defined latency as the period between the day last worked and the earliest of date of death, date of emigration or loss to follow-up, or December 31, 2004.

The overall SMR for mortality from all cancer sites combined relative to the New Zealand population was 1.01 (95% CI = 0.85−1.10). Although not statistically significant there was suggestion of an increased risk of rectal cancer (SMR = 2.03; 95%CI = 0.88–4.01) among the employees. SMRs for lymphatic and hematopoietic cancers (overall SMR = 1.21, 95% CI = 0.52–2.39) included 3.12 (95% CI = 0.08−17.37) for Hodgkin's disease,

1.59 (95% CI = 0.43–4.07) for non-Hodgkin's lymphoma and 3.73, 95% CI = 1.20−8.71), and 1.66 (95% CI = 0.20–5.99) for multiple myeloma. No statistically significant excess of cancer mortality was noted among workers employed for <3 months (SMR = 1.19, 95% CI = 0.65−2.00), or for ≥3 months (SMR = 0.98, 95% CI = 0.75−1.26). A statistically significant excess of digestive cancers was found for those who worked fewer than 3 months relative to the New Zealand population (SMR = 2.52, 95% CI = 1.15−4.78). No excesses were observed for any site-specific cancers when analyses were restricted to those who worked for 3 or more months. No statistically significant elevated SMRs were found for all cancers (combined) either for a latency period of fewer than 15 years (SMR = 1.14, 95% CI = 0.72−1.71) or a latency period of ≥15 years (SMR = 0.96, 95% CI = 0.72−1.26). Similarly, no statistically significant excess in cancer mortality was observed for all cancer sites combined, or any site-specific cancer when analyses were stratified by date of hire (<1976, ≥1976) or by sex. The SMR among women who were employed at the site was 0.68 (95% CI = 0.45−1.00).

2.4.1.1.1.7.4.2. *Study evaluation.*

High rates of emigration in New Zealand (9% among workers in the cohort) contributed to a fairly high loss to follow-up (22% among workers) during the study period. The loss to follow-up would reduce the overall mortality estimates among the workers, which could underestimate the SMRs if loss to follow-up (and health status) was not comparable in the general population. For example, it is unclear if workers and the general population who emigrated were sicker than those remaining in the cohort. Previous data from the cohort workers suggests that loss to follow-up rates were slightly higher among the low and unexposed populations (McBride, 2009, 198490; t' Mannetje et al., 2005, 197593) worker population, so presumably the highly exposed workers were not lost to follow-up more so than other workers.

2.4.1.1.1.7.4.3. *Suitability of data for TCDD dose-response modeling.*

This study extended the mortality follow-up and included stratified analyses to investigate effect modification by period of latency, sex, and date of hire. A key limitation was the lack of direct measures of exposure for study participants which precluded estimating effective dose needed for dose-response modeling. This study did not meet the considerations and criteria for inclusion in quantitative dose-response analysis.

2.4.1.1.2. *Key characteristics of epidemiologic cancer studies*

See Table 2-1 at the end of the chapter for a comparison of the length of follow-up, latency period used, the half-life for TCDD used, and the fraction of TEQs accounted for by TCDD (when applicable) for each study.

2.4.1.1.3. *Feasibility of TCDD cancer dose-response modeling—summary discussion by cohort.*

2.4.1.1.3.1. ***Using the NIOSH cohort in dose-response modeling.***

It is important to evaluate the NIOSH cohort in cancer dose-response modeling of TCDD. This cohort is the largest assembled to date, direct measures of TCDD based on sampling are available, and the lengthy follow-up interval allows for latent effects to be taken into account. Further, although this cohort consists mostly of male workers, these workers were occupationally exposed to TCDD daily, as compared to the acute accidental exposures of other occupational cohorts. Although the most recent analyses of a subset of the NIOSH cohort showed no association between serum TCDD levels and cancer mortality, the study authors did not examine latency effects (Collins et al., 2009, 197627). Incorporation of latency intervals is important in light of the stronger dose-response relationships that consistently have been observed with a 15−20 year latency interval in previous investigations of the NIOSH and other cohorts (Steenland et al., 2001, 197433).

Most published studies of the NIOSH cohort did not evaluate exposures to dioxin-like compounds. An exception is the analysis by Steenland et al. (2001, 197433). Although Steenland et al. (2001, 197433) did not incorporate individual-level data on dioxin-like compounds, based on their previous work (Piacitelli et al., 1992, 197275) they assumed that TEQ occupational exposures occurred as a result of TCDD alone in this population. TCDD exposures provided a better fit to the data than the TEQ-based metric, and 15-year latencies improved the fit for both metrics (relative to unlagged exposures). The lifetime risk estimates for an increase in 10 TEQs (pg/kg of body weight/day/sex) ranged from 0.05−0.18%. The value added for this measure is the incorporation of the contribution of other dioxin-like compounds to the background rates.

Blue collar workers, such as those in the NIOSH cohort, typically have higher rates of smoking than the general population (Bang and Kim, 2001, 197081; Lee et al., 2007, 594391).

This potential source of confounding would be expected to produce a higher SMR for lung cancer mortality, and could contribute to the excess noted in the cohort with longer lag intervals. This bias, however, likely is not large as no statistically significant excess of nonmalignant respiratory mortality was found in these workers. Any associated bias from smoking would be expected to be smaller for comparisons conducted within the cohort, as fellow workers would be expected to be more homogeneous with respect to their risk factor profile than with an external general population referent group. Stratified analyses using both internal and external comparison groups also did not identify important differences in associations with TCDD exposure between smoking and nonsmoking cancers. Thus, fatal cancer risk estimates reported for workers in the NIOSH cohort appear to provide a reasonable estimate of the carcinogenic potency of TCDD.

Although the Steenland et al. (2001, 197433) study did not directly account for the possible confounding effects of other occupational exposure, the authors did address this source of potential bias. No known occupational exposures to carcinogens occurred, with the exception of 4-aminobiphenyl, which occurred at one plant. Two deaths from mesothelioma also occurred in the cohort, so some exposure to asbestos might also have occurred in the cohort (Fingerhut et al., 1991, 197375). The statistical analyses suggested that the inability to control for other occupational exposures would not have unduly affected risk estimates generated from internal cohort comparisons. For instance, the removal of one plant at a time from the analysis did not materially change dose-response estimates generated from the Cox model (Cheng et al., 2006, 523122). Moreover, adding a variable to represent plant in the Cox regression had little impact on the risk estimates. Given that other occupational exposures varied by plant, a change in risk estimates would be expected if such exposures were strong confounders.

The Cheng et al. (2006, 523122) analysis provides important information about the impact of applying kinetic models to the data. The CADM TCDD kinetic model resulted in dramatic decreases in the TCDD cancer mortality risk estimates when compared to the one-stage compartmental model that had been applied. Although Cheng et al. (2006, 523122) suggested that the CADM model provides a better fit to the data than the typically used simple one-compartmental model, statistical comparisons of model fit were not reported. Therefore, there is value in presenting the range in risk estimates across different models when characterizing dose-response relationships.

Finally, the half-life of TCDD is generally recognized to vary according to body fat percentage, data that were not available for the NIOSH workers. The inability to account for between-worker variability in body fat would introduce exposure measurement error. That body fat percentage would not be expected to correlate with cumulative exposure to TCDD exposure, however, would limit the potential for misclassification bias. The effect of any nondifferential exposure measurement error likely would serve to attenuate the risk estimates of the study.

2.4.1.1.3.2. *Using the BASF cohort in dose-response modeling.*

The availability of blood lipid data for TCDD allows for characterization of cumulative TCDD exposures in the BASF cohort. TCDD blood lipid data were collected for 90% of the surviving members of the cohort (138 of 154) and these serum measures were used to generate TCDD exposure estimates for all 254 cohort members. Therefore, the potential for misclassification from extrapolating these exposures to the entire cohort may not be as likely as for the NIOSH cohort where sera data were available for only a small fraction of workers. These data were, however, collected long after the accident (36 years) and had to be back-extrapolated to derive the initial exposures.

The data on this cohort included several risk factors such as cigarette smoking and body mass index. One advantage is that cumulative TCDD levels by body mass index can be estimates on an individual-level basis. As expected, the derived cumulative measures appear to compare well with severity scores of chloracne. The finding that more pronounced risks are found 15−20 years after first exposure are also consistent with findings from several other cohorts (Bertazzi et al., 2001, 197005; Fingerhut et al., 1991, 197375; Manz et al., 1991, 199061).

One key limitation of the BASF cohort is its relatively small sample size (n = 243), which limits the ability to evaluate dose-response relationships for site-specific cancers. Also, the quality of the ascertainment of cancer incidence cannot be readily evaluated as the geographic area of the cohort is not covered by a tumor registry. Ott and Zober (1996, 198101) state that nonfatal cancers could have been more likely to be missed in early years, which could partially contribute to the larger standardized incidence ratio found for cancer with longer latencies. Commenting on risk differences derived from incident and decedent cancer outcomes is difficult. Among those comprising the cohort, the ascertainment of incident outcomes was recognized to

be less complete in early years. Although the ascertainment of mortality outcomes was generally regarded to be good among the 243 workers, some workers who died or moved likely were missed when the cohort was constructed. These deaths would have been more likely to have occurred several years before the second component of the cohort was assembled.

The use of the SMR statistic for this study population is associated with important sources of uncertainties. Deaths were surely missed, particularly for the third component of the cohort that accounts for approximately 38% (94/247) of the entire cohort; this factor would serve to underestimate the overall SMR. As mentioned before, this component of the cohort was assembled through the recruitment of workers known to be alive in 1986. Despite this limitation, the characterization of exposure data and availability of other risk factor data at an individual level allow the development of quantitative dose-response analyses.

2.4.1.1.3.3. *Using the Hamburg cohort in dose-response modeling.*

The Hamburg cohort lacked data on cigarette smoking, and, therefore, effect estimates could not be adjusted for this covariate. Additional analyses that excluded lung cancers resulted in an even stronger dose-response relationship between all cancer mortality and TCDD. Serum levels of TCDD also were also not associated with smoking status in a subgroup of these workers (Flesch-Janys et al., 1995, 197261) suggesting that smoking is not likely a confounder of the association between all cancer mortality and TCDD.

An important limitation of the cohort is the reliance on blood and tissue measurements of 190 workers that likely represent a highly selective component of the cohort. This subset of workers was identified at the end of the observation period, and therefore, excludes workers who died or could not be traced. There are uncertainties in deriving department- and period-specific estimates for a period that extends over three decades using this number of workers. Additionally, the criteria applied to the reference population could have introduced some bias. Workers were included only in the reference group if they had been employed for at least 10 years in a gas supply industry. The criteria were much different for the workers who were exposed to TCDD (only 3 months of employment). As a result, the reference group likely would be more susceptible to the healthy worker effect. Internal cohort comparisons, which should be void of such bias, however, generally produced results similar to those based on the external comparison population. Therefore, the Becher et al. (1998, 197173) study meets the criteria and

additional epidemiological considerations which allowed for development of quantitative dose-response analyses.

2.4.1.1.3.4. *Using the Seveso cohort in dose-response modeling.*

Unlike many of the occupational cohorts that were examined, data from the Seveso cohort are representative of a residential population whose primary exposure was from a single TCDD release. A notable exception is the BASF cohort where workers were exposed primarily through two accidents that occurred in the plant. The Seveso data, therefore, might permit cancer dose-response investigations in women and children.

Uncertainty in identifying the critical exposure window for most of the outcomes related to the Seveso cohort is a key limitation. An important feature of the Seveso cohort, however, is that TCDD levels were much lower among those in the highest exposure zones in Seveso (medians range from 56−136 ng/kg) (Eskenazi et al., 2004, 197160) than those in the occupational cohorts who had TCDD exposures that were sometimes more than 1,000 ng/kg. Given these dramatic differences in exposures, the standardized mortality ratios (after incorporating a 15−20 year latency period) for all cancer sites combined are remarkably similar between the Seveso and the occupational cohort analyses. Perhaps more importantly, the data from Seveso might be more relevant for extrapolating to lower levels, given that exposures to TCDD are two orders of magnitude higher than background levels (Smith and Lopipero, 2001, 198585).

The Warner et al. (2002, 197489) study found a positive association between serum levels of TCDD and breast cancer. As noted previously, ascertainment of incident cases for all cancers would allow for a dose-response relationship to be evaluated. Moreover, future breast cancer analyses in this cohort should strengthen the quantitative dose response analyses of this specific cancer site. The strengths of the Warner et al. (2002, 197489) study outlined earlier suggest that this study should be considered for cancer dose-response modeling.

Earlier Seveso studies likely are unsuitable for conducting quantitative risk assessment. These previous studies used an indirect measure of TCDD exposure, namely, zone of residence. Soil concentrations of TCDD varied widely in these three zones (Zone A: 15.5−580.4 ppt; Zone B: 1.7−4.3 ppt; and Zone R: 0.9−1.4 ppt), which could have resulted in considerable exposure misclassification. The Warner et al. (2002, 197489) study greatly improved the

characterization of TCDD exposure using serum measures, and also allowed for control of salient risk factors that may have resulted in bias due to confounding.

At this time it is unclear whether any study has examined the relationship between cancer and serum estimates of TCDD among Seveso males exposed from the 1976 accident.

2.4.1.1.3.5. ***Using the Chapaevsk related data in dose-response modeling.***

Currently, individual-level exposure data are lacking for residents of this area and there is no established cohort for which cancer outcomes can be ascertained. These limitations, therefore, preclude the inclusion of Chapaevsk data in a quantitative dose-response analysis.

2.4.1.1.3.6. ***Using the Ranch Hands cohort in dose-response modeling.***

An important limitation of the Ranch Hands cohort for TCDD and cancer dose-response modeling is an inability to isolate TCDD effects from the effects of other agents found in the associated herbicides. Exposure to other dioxin-like compounds was not estimated in this study and could confound the previously reported associations. As such, dose-response analyses on this population were not conducted.

2.4.1.1.4. *Discussion of general issues related to dose-response modeling*

2.4.1.1.4.1. ***Ascertainment of exposures.***

Several series of epidemiological data have used serum measures to estimate TCDD levels. Serum data offer a distinct advantage in that they provide an objective means to characterize TCDD exposure at the individual level. The serum measures in the occupational cohorts, however, are limited in two important ways. First, these samples are generally collected from small subsets of the larger cohorts; therefore, using these measures to extrapolate to the remainder of the cohort could introduce bias due to exposure misclassification. The second limitation is related to estimating the half-life of TCDD. As noted previously, exposures to TCDD were back-extrapolated several decades from serum samples collected among surviving members of several cohorts. This approach was used in the NIOSH, Ranch Hands, BASF, New Zealand, and Hamburg cohorts. The reported half-life of TCDD among these populations was reported between 7.1 to 9.0 years and shown to vary with several individual characteristics including age, body fat composition, and smoking. The derivation of half-lives

from a sample of workers, and application of these estimates to retrospectively characterize exposure can introduce uncertainty into the lifetime exposure estimates. It is important to note, however, that sensitivity analyses results in several studies have been fairly consistent when evaluating the impact of half-life of TCDD (Flesch-Janys et al., 1995, 197261; Steenland et al., 2001, 197433).

A unique advantage of the Seveso study is that serum measures were taken shortly after the accident, and therefore characterization of TCDD exposure in this population does not depend on assumptions needed to back-extrapolate exposures several decades.

2.4.1.1.4.2. *Latency intervals.*

Many of the epidemiological studies indicate stronger associations between TCDD and cancer outcomes once a latency period has been considered. Generally, risks are higher when a lag period of 15−20 years is included. As noted previously, this observation is consistent with many other environmental carcinogens such as radon, radiation, and cigarette smoking. That recent exposures do not contribute to increased cancer risk provides some support that the initiation and promotion phases might occur many years before death making recent exposures irrelevant for these analyses. The ability to discriminate between models of varying latency, however, was limited in many studies. The application of biologically based modeling could provide additional important insights on which phase(s) of carcinogenesis TCDD exerts an influence. Such modeling, however, would necessitate having data on an individual-level basis. Ideally, this modeling would use cancer incident data rather than mortality outcomes, given that for many cancers, the median survival time exceeds 5 years.

2.4.1.1.4.3. *Use of the SMR metric.*

The occupational cohorts and the studies in Seveso and Chapaevsk have made inferences regarding the effects of TCDD on mortality using the SMR. When compared to the general population, the healthy worker effect may result in a downward bias in the SMR. This often can manifest as SMRs less than 1 for several causes of mortality. The effect of this bias is, however, generally lower for cancer outcomes. Cancer outcomes, whether incidence or death, typically occur later in life and do not generally affect an individual's ability to work at earlier ages.

There are several approaches that can be taken to minimize potential biases introduced by the healthy worker effect, which would account for workers being healthier than the general population. Comparisons of mortality (or cancer incidence) can be made to other cohorts of similar workers. If done properly, this can allow for some control of characteristics such as sociodemographic characteristics and smoking as the two populations can be matched by these factors. However, it may be the case that other working populations are exposed to other harmful exposures, thereby making it difficult to estimate risk associated with a specific agent (such as TCDD) in the cohort of interest. A second and preferred approach to control for the healthy worker effect, should it prove feasible, is to conduct comparisons of health outcomes in relation to exposure within the cohort. These comparisons are less likely to be influenced by other potential confounding variables such as smoking, socioeconomic status, and other occupational exposures that are generally more homogeneous within the cohort relative to external populations. Moreover, the mechanisms used to identify health outcomes and follow individuals over time are generally applied in the same manner to all cohort members. Taken together, where different comparisons have been made to generate risk estimates, those that have been conducted using internal cohort comparisons are preferable.

In addition to potential bias from the health worker effect, the comparison of SMRs between studies is not always straightforward and is not recommended by some (Myers and Thompson, 1998, 594395; Rothman, 1986, 046091). The SMR is the ratio of the observed number of deaths to the expected number of deaths and is often referred to as the method of indirect standardization. The expected number of deaths is estimated by multiplying the number of person-years tabulated across individuals in the cohort, stratified by age, by rates from a reference population that are available for the same strata. Therefore, each population cohort will have an estimated number of cases derived using a different underlying age structure. As outlined by Rothman (1986, 046091), the mortality rates might not be directly comparable to each other, although the impact of such bias will be much less if the age-distribution of the cohorts is similar. While it might be reasoned that the TCDD exposed workers would have similar age distributions this is in fact not the case (Becher et al., 1998, 197173; Ott et al., 1993, 594322; Thiess et al., 1982, 064999). This may be due to exposure occurring both chronically, as well as from acute exposures due to accidental releases that happened at various times at different plants. This is evident with the Hamburg and the BASF cohorts, as most individuals

comprising the BASF cohort were employed at the time of the accident (1953/1954), while most of the Hamburg cohort (852/1048) was employed after 1954; the follow-up of these cohorts ended at approximately the same time.

The method of direct standardization allows for a more meaningful comparison of mortality rates to be made between cohorts. With this approach, weights (usually based on age and sex) are drawn from a standard population and are, in turn, applied to disease rates for the same strata observed in the cohort of interest. A comparison of weighted rates between different cohorts would then be based on the same population standard.

Despite these limitations in comparing SMRs between studies, Armstrong (1995, 594397) argues that the comparisons are valid if the underlying stratum specific rates in each exposure grouping are in constant proportion to external rates. Comparisons of the SMRs between studies will be biased only if there is an interaction between age and TCDD (i.e., the RR of disease due to exposure differs by age). For cancer outcomes, the finding that associations become stronger after a period of latency is incorporated into the analyses suggests that this assumption does not hold true. That is, risk estimates would be lower among young workers. Similarly, for noncancer outcomes, some of the data from the Seveso cohort suggests differential effects according to the age at exposure.

The use of the SMR might also be biased in that workers exposed to TCDD could be subject to more intensive follow-up than the general population, and as a result, differential coding biases with cause of death might occur. Moreover, some cohorts (e.g., the BASF cohort) have been assembled, in part, by actively seeking out survivors exposed to accidental releases of dioxins. As such, they would not include persons who have died or who were lost to follow-up. This would result in underascertainment of deaths and SMRs developed from these data. The use of an internal cohort comparison offers distinct advantages to overcome potential sources of selection bias. Given these uncertainty about comparability across the different studies, conducting a meta-analysis of cancer outcomes for TCDD using the SMR statistic is not warranted for this analysis.

2.4.1.1.4.4. ***All cancers versus site-specific.***

An important consideration for quantitative dose-response modeling is the application of models for all cancers combined, or for site-specific cancers. Consistency is often lacking for

site-specific cancers, which might be due in large part to the relatively small number of cases identified for site-specific cancers in the cohorts. Although the risk estimates produced for all cancer sites have important limitations and uncertainties, the data are far more consistent in terms of the magnitude of an association and latency intervals. The IARC evaluation has put forth the possibility of a pleuripotential mode of action between TCDD and the occurrence of cancer. Despite the criticism of this assertion by some (Cole et al., 2003, 197626), the general consistency of an increased risk for all-cancer mortality across the occupational cohorts when latency intervals have been incorporated, provides adequate justification for dose-response quantification of all cancer sites combined.

2.4.1.1.4.5. ***Summary of epidemiologic cancer study evaluations for dose-response modeling.***

All epidemiologic cancer studies summarized above were evaluated for suitability of quantitative dose-response assessment using the TCDD-specific considerations and study inclusion criteria. The results of this evaluation are summarized in a matrix style array (see Table 2-2) at the end of this section, and descriptively in Appendix B. Table 2-4 summarizes the key epidemiologic cancer studies suitable for further TCDD dose-response analyses.

2.4.1.2. *Noncancer*

In this section, the available epidemiological data that could be used in a dose-response analysis for noncancer endpoints are evaluated. Because many of the key studies also evaluated cancer outcomes, the noncancer studies are presented in the same order as presented in Section 2.4.1.1. Generally, the strengths and limitations of the cancer studies also apply to the noncancer outcomes. In this section, key features of these studies that have direct relevance to modeling of noncancer outcomes in particular are highlighted. To reduce redundancy, a detailed overview of many of these cohorts and studies are not provided here. Instead, the reader should refer to Section 2.4.1.1.1.

2.4.1.2.1. ***Noncancer cohorts.***

2.4.1.2.1.1. ***The NIOSH cohort.***

2.4.1.2.1.1.1. Steenland et al. (1999, 197437).

2.4.1.2.1.1.1.1. *Study summary.*

The 1999 published report of NIOSH workers exposed to TCDD also conducted external cohort comparisons to the U.S. general population using SMRs for mortality outcomes other than cancer (Steenland et al., 1999, 197437). Analyses are based on 3,538 workers employed at 8 plants from 1942 to 1984. SMRs were based on a mortality follow-up that was extended until the end of 1993. Cox regression analyses were used to compare mortality risk in relation to TCDD exposure within the cohort.

2.4.1.2.1.1.1.2. *Study evaluation.*

Overall, no statistically significant differences in all-cause mortality (SMR = 1.03, 95% CI = 0.97−1.08) were observed. Mortality from ischemic heart disease (SMR = 1.09, 95% CI = 1.00−1.20) and accidents (SMR = 1.25, 95% CI = 1.03−1.50) was slightly elevated. Based on the external comparison population, the dose-response relationship for ischemic heart disease observed with the SMRs calculated across TCDD exposure septiles was not statistically significant ($p = 0.14$). Overall, excess risk was not evident for diabetes, cerebrovascular disease, or nonmalignant respiratory disease using the external population comparisons. Internal cohort comparisons using the Cox regression model were performed using 0 and 15-year lag intervals. A dose-response trend was observed for the derived ratios across the unlagged cumulative TCDD exposure septiles for ischemic heart disease ($p = 0.05$) and diabetes ($p = 0.02$). For ischemic heart disease mortality, those in the upper two septiles had rate ratios of 1.57 (95% CI = 0.96−2.56) and 1.75 (95% CI = 1.07−2.87), respectively, relative to those in the lowest septile. In contrast, an inverse dose-response relationship was observed for diabetes mortality. The inverse association found for diabetes is inconsistent with the positive association reported in the Ranch Hands study (Michalek and Pavuk, 2008, 199573). However, previous reports have questioned the use of death certificates as the means to ascertain outcome as diabetes may be under-reported especially among descendents with diabetes who die from cancer (McEwen and TRIAD, 2006, 594400).

2.4.1.2.1.1.1.3. *Suitability of data for TCDD dose-response modeling.*

The inverse association with diabetes precludes dose-response analysis for this outcome. The dose-response relationship between TCDD exposure and ischemic heart disease mortality was not statistically significant at the alpha level of 0.05 and was not observed in other cohorts. Furthermore, fatal outcomes are not a suitable basis for development of an RfD. For these reasons, dose-response analysis for this outcome is precluded.

2.4.1.2.1.1.2. Collins et al. (2009, 197627).

2.4.1.2.1.1.2.1. *Study summary.*

Collins et al. (2009, 197627) recently described the mortality experience of Dow employees who worked in Midland, Michigan. This plant produced 2,4,5-trichlorophenol between 1942 and 1979, and 2,4,5-T between 1948 and 1982. The cohort consisted of 1,615 workers exposed to TCDD from as early as 1942; the follow-up of the cohort extended until 2003.

TCDD exposures were derived using serum samples obtained from 280 surviving individuals. A simple one-compartment, first-order pharmacokinetic model was used to estimate time-dependent TCDD measures. The area under the curve approach was then applied to estimate cumulative TCDD exposure above background. A half-life of 7.2 years for TCDD based on earlier work was incorporated into the exposure estimation (Flesch-Janys et al., 1996, 197351).

Collins et al. (2009, 197627) made an external comparison of the mortality rates of the cohort to the U.S. general population using the SMR statistic. Noncancer causes of death included all causes, diabetes, cerebrovascular disease, nonmalignant respiratory disease, cirrhosis of the liver, and accidents. Overall, no statistically significant difference in all-cause mortality of these workers was detected when compared to the general population (SMR = 0.9, 95% CI = 0.9−1.0). Except for cirrhosis of the liver (SMR = 0.4, 95% CI = 0.1−0.8), no differences were found for any of the noncancer causes of death relative to the general population.

Internal cohort analyses based on cumulative measures of TCDD were conducted for mortality from diabetes, ischemic heart disease, and nonmalignant respiratory disease using the Cox regression model. These models adjusted for possible confounders such as year of hire and

birth year. No statistically significant association was found between continuous measure of TCDD and these causes of death.

2.4.1.2.1.1.2.2. *Study evaluation.*

Given that the external comparisons may result in bias from the healthy worker effect, results from the internal cohort comparisons using the Cox regression model are preferred. These analyses were performed for diabetes, ischemic heart disease, and nonmalignant respiratory disease. TCDD levels for these workers were estimated using a simple one-compartment pharmacokinetic model (Aylward et al., 2007, 197175). The hazard ratios generated from the Cox regression model were not statistically significant for any of the three noncancer outcomes modeled.

2.4.1.2.1.1.2.3. *Suitability of data for TCDD dose-response modeling.*

No association of an increased risk for an adverse effect was observed with any of the noncancer outcomes. In addition, since noncancer mortality was the endpoint being examined, dose-response modeling based on this population was not conducted.

2.4.1.2.1.2. ***The BASF cohort.***

2.4.1.2.1.2.1. Ott and Zober (1996, 198101).

2.4.1.2.1.2.1.1. *Study summary.*

In 1996, Ott and Zober published a report on the mortality experience of the cohort of 243 BASF male workers who were accidentally exposed to 2,3,7,8-TCDD in 1954 or in the clean up that followed. The mortality follow-up of this cohort extended until the end of 1992. External comparisons of mortality were made to the German population using the SMR statistic. Internal cohort comparisons were also made by estimating cumulative TCDD for the cohort using serum measures that were obtained from 138 workers. Ott et al. (1993, 594322) provided a detailed account of the methodology to estimate TCDD. Briefly, a cumulative measure of TCDD expressed in µg/kg was derived, by first estimating the half-life of TCDD using individuals who had repeated serum measures; the half-life was estimated to be 5.8 years. Individual-level data on body fat were used to account for the influence of body fat on decay rates. Half-life estimates of TCDD varied (range: 5.1−8.9 years) and were dependent on body fat

composition (20% and 30%, respectively). This approach differed from previous analysis of this cohort that used a constant 7-year half-life (Ott et al., 1993, 594322). TCDD levels at the time of serum sampling were then estimated as the product of TCDD concentration in blood lipid and the total lipid weight for each worker. Nonlinear models then were applied to estimate the contribution of duration of exposure to TCDD dose extrapolated to the time of exposure.

External comparisons to the German population using the SMR statistic also were examined across dose categories. The noncancer causes of death examined by Ott and Zober (1996, 198101) included all-cause mortality, diseases of the circulatory system, ischemic heart disease, diseases of the digestive system, external causes, suicide, and residual causes of death. Overall, no statistically significant differences in the SMR with the general population for all-causes of death (SMR = 0.9, 95% CI = 0.7−1.1) were found. No statistically significant differences were noted for any of the other causes of death examined.

Ott and Zober (1996, 198101) performed internal cohort comparisons using the Cox regression model. These analyses found no dose-response patterns when cause-specific mortality was examined across increasing cumulative TCDD exposure categories. Although an inverse association for diseases of the respiratory system (SMR = 0.1, 95% CI = 0.0−0.8) was detected, it was based only on 1 reported case. Many of these comparisons are limited by small sample sizes as 92 deaths occurred in the cohort, and of these, 31 were from cancer. Also, the third component of the cohort was identified primarily from former employees who were alive in 1986. As a result, the SMR based on the general population might be underestimated by the exclusion of deceased workers.

2.4.1.2.1.2.1.2. *Study evaluation.*

As noted previously, caution should be exercised in the interpretation of SMR values of noncancer outcomes as they could be influenced by the healthy worker effect. Although the mechanism of identifying vital status appears to be excellent and unbiased, SMRs might be underestimated for the cohort due to the manner in which they were constructed. Specifically, a large component of the cohort was assembled by actively seeking out former workers who were known to be alive in 1986.

2.4.1.2.1.2.1.3. *Suitability of data for TCDD dose-response modeling.*

No dose-response patterns were observed between TCDD and the noncancer outcomes in the Ott and Zober (1996, 198101) study. Therefore, dose-response modeling was not conducted.

2.4.1.2.1.3. ***The Hamburg cohort.***

2.4.1.2.1.3.1. Flesch-Janys et al. (1995, 197261).

2.4.1.2.1.3.1.1. *Study summary.*

Flesch-Janys et al. (1995, 197261) reported on the mortality experience of a cohort of individuals employed by an herbicide-producing plant in Hamburg, Germany, covering the period 1952 to 1992. As described in more detail in Section 2.4.1.1.1.3, the authors developed a cumulative measure of TCDD using serum measures from 190 workers. This study also examined the relationship between total TEQ and mortality. In the study population, the mean TEQ without TCDD was 155 ng/kg, and for the mean TEQ including TCDD was 296.5 ng/kg.

Risks relative to the unexposed referent group of gas workers were estimated using Cox regression across six exposed TCDD groups (i.e., the first four quintiles, and the ninth and tenth deciles). A linear dose-response relationship was found with all causes of mortality and cardiovascular mortality ($p < 0.01$). The RR for all cardiovascular deaths in the upper exposure category was 1.96 (95% CI = 1.15−3.34), although there was no evidence of a linear dose-response trend ($p = 0.27$). The dose-response relationship was most marked for ischemic heart disease, with a RR of 2.48 (95% CI = 1.32−4.66) in the highest exposure group. A dose-response relationship was also observed across TEQ groupings for all cause mortality, cardiovascular disease mortality, and ischemic heart disease mortality. The authors did not perform joint modeling of TEQ (without TCDD) and TCDD, so determining the extent that dioxin-like compounds contributed to an increased risk of mortality is not possible.

2.4.1.2.1.3.1.2. *Study evaluation.*

The Flesch-Janys et al. (1995, 197261) study lacks information on other potential risk factors for cardiovascular disease, which could result in confounding if those risk factors are also related to TCDD exposure. Dose-response patterns were strong, however, and persisted across numerous TCDD (and TEQ) exposure categories based on the use of an external reference group (i.e., gas workers) or based on the internal comparison. The findings based on the internal

comparison are noteworthy in that these groups should be more homogenous with respect to confounding factors. As noted previously, the poor correlation between TCDD and smoking among workers and similar smoking prevalence between the workers and the external gas company workers suggest that smoking was not likely a confounder of the TCDD and cardiovascular disease relationship. No other evaluation of noncancer mortality outcomes has been undertaken in this cohort since 1995.

A strength of the Flesch-Janys et al. (1995, 197261) study was that it included the collection of blood serum measures, which provided an objective measure of TCDD exposure. Blood serum data, however, were obtained only for 16% of the cohort. The assumption of the first-order kinetic elimination model is critical, given that measures were taken at the end of follow-up. The model also assumed the half-life of TCDD was 6.9 years. If the kinetics are not first order, or if the half-life estimate is inaccurate, estimates of TCDD levels during exposure would be biased, particularly for workers having longer periods between exposure and PCDD and PCDF assays. Sensitivity analyses completed by the authors suggest that such bias is not likely to present because the results were unaffected when different model assumptions regarding kinetic and half-lives were examined. The lack of an impact on RR estimates with varying half-life estimates was similar to findings by Steenland et al. (2001, 197433).

2.4.1.2.1.3.1.3. *Suitability of data for TCDD dose-response modeling.*

Despite the aforementioned study strengths, the study focused on fatal outcomes such as all cause mortality, cardiovascular disease mortality, and ischemic heart disease mortality. As such, dose-response analysis was not conducted since these outcomes are not suitable for development of an RfD.

2.4.1.2.1.4. ***The Seveso Women's Health Study (SWHS).***

Eskenazi et al. (2000, 197162) presented an overview of the SWHS. The SWHS is the first comprehensive epidemiologic study of the reproductive health of a female population exposed to TCDD. The primary objective of the SWHS is to investigate the relationship of TCDD and several reproductive endpoints, including endometriosis, menstrual cycle characteristics, birth outcomes, infertility, and age at menopause. A second phase of follow-up

that focuses on osteoporosis, thyroid hormone, breast cancer, diabetes, and metabolic syndrome is expected to be completed in 2010.

Women were eligible for participation in the SWHS if they resided in Zones A and B (the most contaminated areas) at the time of the explosion, were 40 years of age or younger at the time of the explosion in 1976, and samples of their blood were collected and stored between 1976 and 1980. The enrollment of women in the SWHS began in March 1996 and continued until July 1998. Of the 1,271 eligible women, 17 could not be found, 21 had died, and 12 were too ill to participate. Of the 96% of the remaining women, 80% (n = 981) participated in the study. Participation in the SWHS included a blood draw and an interview by a trained nurse who was blind to subjects' TCDD level and zones of residence at the time of the accident. The interview included detailed information on potential confounders including occupational, medical, and reproductive, and pregnancy history. Also, women who were premenopausal were asked to undergo a vaginal ultrasound and pelvic exam and to complete a daily diary on menstruation.

Depending on the health outcome under study, TCDD exposures were characterized for the women at different times. For example, TCDD exposure levels were estimated at the time of the accident for some studies and at the time of conception for others. The SWHS study population has been used to investigate associations between maternal TCDD levels and the following health outcomes: menstrual cycle characteristics (Eskenazi et al., 2002, 197168); endometriosis (Eskenazi et al., 2002, 197164); birth outcomes (Eskenazi et al., 2003, 197158); age at menarche (Warner et al., 2004, 197490); age at menopause (Eskenazi et al., 2005, 197166); uterine leiomyomas (Eskenazi et al., 2007, 197170); and ovarian function (Warner et al., 2007, 197486). An evaluation of the studies in chronological order is presented in this section.

2.4.1.2.1.4.1. Eskenazi et al. (2002, 197168)—Menstrual cycle characteristics.

2.4.1.2.1.4.1.1. *Study summary.*

Eskenazi et al. (2002, 197168) evaluated serum TCDD exposures in relation to several menstrual cycle characteristics in the SWHS. A total of 981 women who were 40 years of age or younger at the time of the accident comprised the SWHS. The following exclusion criteria was applied 44 years of age or older, women with surgical or natural menopause, those with Turner's

syndrome, and those who in the past year had been pregnant, breastfed, or used an intrauterine device or oral contraceptives.

A trained interviewer collected data on menstrual cycle characteristics using a questionnaire. Women were asked to indicate how long their cycles were, whether the cycles were regular (e.g., irregular cycle defined as length varied by more than 4 days), how many days the menstrual flow lasted, and whether this flow was "scanty, moderate, or heavy." Information was also collected on obstetric and gynecological conditions. TCDD exposures were derived from serum samples collected in 1976−1985. The authors selected the earliest available serum sample, and back-extrapolated to 1976 values using either the Filser model (Kreuzer et al., 1997, 198088) for women aged 16 years or younger in 1976 (n = 20) or the first-order kinetic model (n = 6) (Pirkle et al., 1989, 197861).

Serum TCDD levels were transformed using the log10 scale, and the relationships between these levels and length of menstrual cycle and days of menstrual flow were examined using linear regression. The authors applied logistic regression to characterize the risk between $\log_{10}$TCDD and heaviness of flow or regularity of cycle. In these analyses, moderate or heavy flow and regular cycle were used as the reference categories. Stratified analysis was performed by menarcheal status at the time of the accident.

Overall, the association with TCDD exposure (per 10-fold increase) and length of menstrual cycle was not statistically significant for premenarcheal (β = 0.93, 95% CI = −0.01, 1.86) women or postmenarcheal women (β = −0.03, 95% CI = −0.61, 0.54). The corresponding estimates found for days of menstrual flow were β = 0.18 (95% CI = −0.15, 0.51) and β = 0.16 (95% CI = −0.18, 0.50), respectively. Reduced flow was not associated with TCDD when compared to moderate or heavy flow (odds ratio [OR] = 0.84, 95% CI = 0.44, 1.61); effect modification by menarcheal status, however, was evident (p = 0.03). Specifically, women exposed to TCDD who were premenarcheal had lower odds of reduced flow, while those exposed to TCDD who were postmenarcheal did not. These findings counter the hypothesis that TCDD exposure is related to ovarian dysfunction. Finally, statistically significant ORs were found between serum TCDD levels (per 10-fold increase) and having an irregular cycle (OR = 0.46, 95% CI = 0.23, 0.95). This inverse association was evident in both premenarcheal women (OR = 0.50, 95% CI = 0.18, 1.38) and postmenarcheal women (OR = 0.41, 95% CI = 0.15, 1.16).

2.4.1.2.1.4.1.2. *Study evaluation.*

Overall, the findings from the Eskenazi et al. (2002, 197168) study suggest that exposures to TCDD can affect menstrual cycle characteristics among women who were exposed before menarche. Exposures to TCDD were well characterized using serum samples available on an individual-level basis, and the design allowed for the influence of other risk factor data to be controlled for in regression analyses. Analysis of TCDD levels and the length of menstrual cycle in premenarcheal women produced associations that were largely not statistically significant at the alpha level of 0.05, but may have some biological significance. However, it is unclear whether the endpoints that were measured constitute adverse health outcomes as they are not definitive markers of ovarian dysfunction. Another source of uncertainty is measurement error due to the subjective nature of menstrual flow reporting. Any resulting misclassification of the outcome should be nondifferential, as the measurement error is unlikely to be dependent on TCDD exposure.

2.4.1.2.1.4.1.3. *Suitability of data for TCDD dose-response modeling.*

The lack of a clear adverse health outcome related to TCDD exposure is a weakness of this study. Although it is difficult to define the critical window of exposure for quantitative exposure calculations, it can be estimated for the women that were premenarcheal at the time of the accident as 13 years. Therefore, this study is suitable for further consideration for quantitative dose-response modeling.

2.4.1.2.1.4.2. Eskenazi et al. (2002, 197164)—Endometriosis.

2.4.1.2.1.4.2.1. *Study summary.*

The SWHS provided the opportunity to investigate the association between serum TCDD levels and endometriosis (Eskenazi et al., 2002, 197164). The rationale the authors provided for undertaking this study was the experimental animal studies that suggested an association, the high prevalence of endometriosis among infertile women where breast milk concentrations of dioxin are high, and the unknown etiology of endometriosis. The study consisted of 601 women who were younger than 30 years at the time of the Seveso accident. Stored sera that had been collected between 1976 and 1980 were also available for these women.

Given that laparoscopy could not be performed on women unless clinically indicated, no "gold" standard was available for endometriosis diagnosis. Based on the results of a validation study they conducted in a clinical population, the researchers classified women as having endometriosis based on symptom report, gynecologic exam results, and vaginal ultrasound.

TCDD was measured in sera in 1976 for 93% of the women. Values for women whose serum TCDD levels were collected after 1977 and had values exceeding 10 ppt were back-extrapolated to 1976 using either the Filser model (<16 years of age) (Kreuzer et al., 1997, 198088) or a first-order kinetic model (≥16 years) (Pirkle et al., 1989, 197861). These estimates of TCDD were then modeled as both continuous (on a log scale) and categorical (≤20, 20.1−100, and >100 ppt) exposures.

Polytomous logistic regression was applied within the cohort used to generate RRs. In relation to women in the lowest exposure category, the RR for endometriosis among women in the middle and upper categories was 1.2 (90% CI = 0.3−4.5) and 2.1 (90% CI = 0.5−8.0), respectively. The trend tests were not statistically significant for either the categorical (p = 0.25) and continuous measures of TCDD (p = 0.84).

2.4.1.2.1.4.2.2. *Study evaluation.*

It is important to note that disease misclassification could have led to an underestimate of the true risk of endometriosis if this misclassification was not differential with respect to TCDD exposure. Also, younger women were likely to be under-represented as those who had never been sexually active could not be examined due to cultural reasons. Other dioxin-like compounds (PCDD, PCDFs, or polychlorinated biphenyls [PCBs]) were not considered because of small serum volumes, but any potential TEQ exposures occurring in the population were thought to be mostly attributable to TCDD in the exposed women.

2.4.1.2.1.4.2.3. *Suitability of data for TCDD dose-response modeling.*

Given that no statistically significant dose-response patterns were observed with either log-transformed or across TCDD exposure categories, and that the elevated risks among those with higher exposures had very wide confidence intervals (that included unity) quantitative dose-response analyses were not recommended for this outcome.

2.4.1.2.1.4.3. Eskenazi et al. (2003, 197158)—Adverse birth outcomes.

2.4.1.2.1.4.3.1. *Study summary.*

Eskenazi et al. (2003, 197158) examined the relationship between serum TCDD levels and birth outcome measures. Analyses were based on 745 of the 981 women enrolled in the SWHS who reported having been pregnant (n = 1,822). Most of these pregnancies (888 pregnancies among 510 women) occurred after the accident. Analysis of spontaneous abortions was restricted to 769 pregnancies among 476 women that did not end in abortion or in ectopic or molar pregnancy. Congenital anomalies were evaluated for the 672 pregnancies that did not end in spontaneous abortion. For the birth outcomes of fetal growth and gestational age, analysis was performed using 608 singleton births from women without hypertensive pregnancy disorders.

TCDD exposures were based on serum measures, most of which were taken shortly after the accident. Serum was collected in 1976−1977 for 413 women, between 1978 and 1981 for 12 women, and in 1996 for 19 women. TCDD exposures based on serum samples collected from 1977 onward were back-extrapolated to 1976.

Statistical analyses were performed on pregnancies that ended between 1976 and the time of interview. A continuous measure of $\log_{10}$TCDD (base 10 scale) was used to investigate associations with adverse birth outcomes. Logistic regression was used to characterize the relationship between TCDD exposure spontaneous abortions, small for gestational age, and preterm birth (<37 weeks gestation). Linear regression was used to describe the relationship between TCDD and birth weight (in grams) and gestational age (in weeks).

The risk estimates were adjusted for a series of characteristics that included sex of infant, history of low birth weight child, maternal height, maternal body mass index, maternal education, maternal smoking during pregnancy, and parity. No association was evident between TCDD serum levels and spontaneous abortion for pregnancies between 1976 and 1998 (OR = 0.8, 95% CI = 0.6−1.2), or those between 1976 and 1984 (OR = 1.0, 95% CI = 0.6−1.6). No statistically significant associations (ORs ranged from 1.2−1.8) were found between $\log_{10}$ TCDD levels and preterm delivery, small for gestational age. Although the mean change in birth weight for pregnancies between 1976 and 1984 was fairly large (β = −92, 95% CI = −204 to 19), it also was not statistically significant at the alpha level of 0.05.

2.4.1.2.1.4.3.2. *Study evaluation.*

This study was well-designed with well characterized exposures. Statistically significant associations were not evident, although the birth-weight findings should be pursued with further follow-up of the cohort. As the authors point out, those who were most vulnerable at the time of the accident (the youngest) had not yet completed their childbearing years. While the study lacked exposure data for the fathers, the authors indicated that only a small proportion were believed to have high exposures to TCDD. The key limitation of the study was a reliance on self-reported measures of pregnancy history, which may lead to some misclassification of the birth outcomes. The observation that a large proportion of Seveso women had a voluntary abortion because of fears of possible birth defects due to exposures from the accident suggest an awareness bias is possible as a result of differential reporting of birth outcomes according to exposure status.

2.4.1.2.1.4.3.3. *Suitability of data for TCDD dose-response modeling.*

No statistically significant associations were found in the study; in addition, possible awareness bias could have influenced the self-reported measures of birth outcomes. Therefore, quantitative dose-response assessment was not considered for this study.

2.4.1.2.1.4.4. Warner et al. (2004, 197490)—Age at menarche.

2.4.1.2.1.4.4.1. *Study summary.*

Warner et al. (2004, 197490) examined the relationship between TCDD and age at menarche in the SWHS cohort. As described earlier in this report, the SWHS comprised 981 participants. This study was restricted only to those who were premenarcheal at the time of the accident (n = 282). The proportional hazards model was used to model TCDD exposures and age at menarche. Age at menarche was determined by questionnaire administered by a trained interviewer. Covariates examined as potential confounders included height, weight, body mass index, athletic training at the time of interview, smoking, and alcohol consumption.

TCDD exposures were determined using serum samples collected from 257 of these women between 1976 and 1977. For the remaining women, TCDD levels were quantified from measures collected between 1978 and 1981 (n = 23) and in 1996 (n = 2). TCDD levels were back-extrapolated to the time of the explosion in 1976. TCDD was modeled as both a

continuous variable (log_{10}TCDD) and a categorical variable based on quartile values (≤55.9, 56−140.2, 140.3–300, >300 ppt). The lowest group was further subdivided into those with levels ≤20, and >20 ppt; this cut-point represented background levels found in a sample of women living in an unexposed area.

No association was found between the continuous measure of TCDD and age at menarche (hazard ratio [HR] = 0.95, 95% CI = 0.83−1.09). Analyses restricted to those who were younger than 8 in 1976 produced similar results (HR = 1.08, 95% CI = 0.89−1.30). Additionally, no dose-response trend was observed with categorical measures of TCDD among all women, as well as those under the age of 8. Although not statistically significant at the alpha level of 0.05, TCDD exposures were later reported to be associated with age of menarche (HR = 1.20, 95% CI = 0.98−1.60) when analyses were restricted to 84 women under the age of 5 at the time of the accident (Warner and Eskenazi, 2005).

2.4.1.2.1.4.4.2. *Study evaluation.*

An important strength of the Warner et al. (2004, 197490) study is the ability to characterize TCDD exposures using serum samples that were collected shortly after the accident occurred. The outcome of interest, age at menarche, was determined by asking women "At what age did you get your first menstrual period?" Recent work suggests that self-reported measures of age at menarche decades later have modest agreement with responses provided during adolescence with recall varying by education and by history of an adverse birth outcome (Cooper et al., 2005, 594401). In the Seveso study, bias would be introduced if recall varied according to exposure levels.

2.4.1.2.1.4.4.3. *Suitability of data for TCDD dose-response modeling.*

Although the TCDD exposure characterization of study subjects was based on serum data, and no major biases were introduced from the study design, the analyses produced largely null associations. Therefore, quantitative dose-response assessment was not considered for this study.

2.4.1.2.1.4.5. Eskenazi et al. (2005, 197166)—Age at menopause.

2.4.1.2.1.4.5.1. *Study summary.*

Eskenazi et al. (2005, 197166) evaluated the relationship between age at onset of menopause and serum levels of TCDD among women in the SWHS. Of the 981 women who agreed to participate in SWHS, this analysis was restricted to those who had not reached natural menopause before the time of the accident and who were at least 35 years of age at the time of the interview. The recruitment and interview of women occurred approximately 20 to 22 years after the accident (March 1996–July 1998).

The population was divided into quintiles of serum TCDD levels for the categorical analysis. For most women (n = 564), TCDD levels were estimated from samples provided in 1976–1977. For the remaining women included in these analyses, TCDD levels were estimated from samples collected between 1978 and 1982 (n = 28) and between 1996 and 1997 (n = 24). As noted previously, exposure levels for women with post-1977 detectable levels of TCDD were back-extrapolated to 1976 using either the first-order kinetic model (Pirkle et al., 1989, 197861) (>16 years at time of accident) or the Filser model (<16 years at time of accident) (Kreuzer et al., 1997, 198088). Women were classified as premenopausal if they were still menstruating or if they had amenorrhea as a result of pregnancy or lactation (at the time of interview) with an indication of subsequent menstruation based on maintained diaries or further examination. Subjects for which amenorrhea had persisted for at least 1 year with no apparent medical explanation were classified into a natural menopause category. The category, surgical menopause, pertained to women with a medically confirmed hysterectomy or an oophorectomy. Finally, impending menopause was defined for subjects in which menstruation had been absent for 2 months, but who provided evidence of subsequent menstruation, or had a secretory endometrial lining, or indicated less predictable cycles in the previous 2–5 years. If participants' menopausal status could not be determined, they were grouped into the "other" category. This category included those for whom status could not be determined due to current use of oral contraceptives, hormone replacement therapy, or previous cancer chemotherapy.

Statistical analysis was based on both a continuous measure of log-transformed TCDD exposures and categories based on quintiles (<20.4 ppt; 20.4–34.2 ppt; 34.3–54.1 ppt; 54.2–118.0 ppt; >118.0 ppt). The Cox model was used to generate hazard ratios as estimates of relative risks and their 95% confidence intervals examining natural menopause as the outcome.

Several covariates previously identified as associated with menopausal status in the literature were considered as potential confounders. These covariates included body mass index, physical activity, premenopausal smoking, education, marital status, history of heart disease and other medical conditions, and other reproductive characteristics.

The RRs were found to increase across the second through fourth quintiles (RRs = 1.1, 1.4, and 1.6, respectively) of serum TCDD categories in relation to those in the lowest category, but not in the upper quintile (RR = 1.0, 95% CI = 0.6−1.8). A statistically significant test of trend was detected across the first four quartiles ($p = 0.04$) but not across all five quintiles ($p = 0.44$). A statistically significant association with onset of menopause was not detected (RR = 1.02, 95% CI = 0.8−1.3) based on the logTCDD continuous measure.

2.4.1.2.1.4.5.2. *Study evaluation.*

The categorical exposure results from this study support a nonmonotonic dose-related-association for earlier menopause with increased serum TCDD levels up to approximately 100-ppt TCDD serum, but not above. Eskenazi et al. (2005, 197166) speculated that the inverse "U" shape of the dose-response relationship is explained by the mimicking of hormones at lower doses of a chemical, while at higher levels the toxic effect of a chemical does not have the capacity to either inhibit or stimulate hormonal effects.

A study limitation is the potential for residual confounding due to adjustment based on current smoking status and not at the time of onset of menopause. It is unclear to what extent smoking status may differ between these two time periods and whether smoking is related to TCDD exposures in this cohort. Exposures to other dioxin-like compounds were not considered in this study because of small serum volumes, but any potential TEQ exposures occurring in the exposed population were thought to be mostly attributable to TCDD in the exposed women.

2.4.1.2.1.4.5.3. *Suitability of data for TCDD dose-response modeling.*

To date, this study is the only one that has examined the relationship between TCDD levels and onset of menopause. Although the findings suggest the possibility of a nonlinear dose-response function, the $\log_{10}$TCDD exposure metric was not statistically significant, nor were any category-specific hazard ratios statistically significant relative to the lowest category. Therefore, a quantitative dose-response analysis was not undertaken.

2.4.1.2.1.4.6. Warner et al. (2007, 197486)—Ovarian function.

2.4.1.2.1.4.6.1. *Study summary.*

Warner et al. (2007, 197486) investigated the association between serum TCDD levels and ovarian function in subjects in the SWHS who were younger than 40 in 1976 and for whom sera collected after the accident had been stored. These women were recruited from March 1996 until July 1998. Ovarian function analysis was limited to 363 women between 20 and 40 years of age and who were not using oral contraceptives. Of these, 310 underwent transvaginal ultrasound and were included in the functional ovarian cyst analysis. Ninety-six women were in the preovulatory stage of their menstrual cycles and were included in the follicle analysis. For the hormone analysis, 126 women who were in the last 2 weeks of their cycle were included.

The authors used logistic regression to examine the relationship between TCDD and the prevalence of ovarian follicles greater than 10 mm. Linear regression models examined the continuous outcome variables: number of ovarian follicles >10 mm and diameter of dominant ovarian follicle. Covariates considered for inclusion in the model were age at ultrasound, age at accident, age at menarche, marital status, parity, gravidity, lactation history, current body mass index, age at last birth, and smoking history. For the serum hormone analyses, estradiol and progesterone were measured in blood at the time of interview. Ovulation status was defined as a dichotomous variable (yes/no) based on a serum progesterone cut-point value of 3 ng/mL.

The adjusted ORs across categories of TCDD exhibited no dose-response trend for the presence of follicles in relation to TCDD in the follicular phase; also, no statistically significant differences were noted in any of the upper exposure categories relative to those in the lowest. The adjusted OR for the continuous measure of $\log_{10}$TCDD was 0.99 (95% CI = 0.4−2.2). A similar nonstatistically significant finding was found for $\log_{10}$TCDD in relation to ovulation in both the luteal (OR = 0.99, 95% CI = 0.5−1.9) and mid-luteal phases (OR = 1.03, 95% CI = 0.4−2.7). Analyses of progesterone and estradiol also were not related to serum TCDD levels for either the luteal or mid-luteal phases (p = 0.51 and p = 0.47).

2.4.1.2.1.4.6.2. *Study evaluation.*

The investigators found no relationship between serum TCDD levels and serum progesterone and estradiol levels among women who were in the luteal phase at the time of blood draw. No association with number of ovarian follicles detected from ultrasound.

Although no association was found, the authors suggested that the lack of significant results could be because the women in SWHS were all exposed postnatally and the relevant and critical time period for an effect might be in utero (animal studies support relevance of in utero exposures).

2.4.1.2.1.4.6.3. *Suitability of data for TCDD dose-response modeling.*

One limitation of the study was the lack of examination of confounding by dioxin-like compounds. The absence of associations between TCDD and adverse health effects in this study precludes conducting quantitative dose-response analyses.

2.4.1.2.1.4.7. Eskenazi et al. (2007, 197170)—Uterine leiomyoma.

2.4.1.2.1.4.7.1. *Study summary.*

Associations between TCDD exposures and uterine leiomyoma (i.e., fibroids) were also examined among 956 women in the SWHS (Eskenazi et al., 2007, 197170). The sample population was based on the on the original 981 SWHS participants excluding 25 women diagnosed with fibroids before the date of the accident (July 10, 1976). Women who previously had fibroids were identified both through the administered questionnaire and the review of medical records. Transvaginal ultrasounds were performed for 634 women to determine if they had fibroids at the time of follow-up. Similar to other SWHS studies, exposure to TCDD was estimated using serum collected from women shortly after the time of the accident, between 1978 and 1981 and in 1996. TCDD levels were back-extrapolated to 1976 levels.

The study authors performed statistical analyses using two definitions of fibroids as outcome measures. The first was fibroids detected before the study, and the second was fibroids detected via ultrasound. A proportional odds method Dunson and Baird (2001, 197248) developed was used to model the cumulative odds of onset of fibroids. This method combines historical and current information of diagnoses of fibroids. Continuous and categorical measures of TCDD were modeled. Regression models were adjusted for known or suspected risk factors of fibroids including parity, family history of fibroids, age at menarche, body mass index, smoking, alcohol use, and education.

2.4.1.2.1.4.7.2. *Study evaluation.*

Categorical measures of TCDD suggested an inverse dose-response relationship with the onset of fibroids. Relative to those with TCDD levels less than 20 ppt, those having TCDD exposures between 20.1 and 75.0 ppt and greater than 75.0 ppt had RRs of 0.58 (95% CI = 0.41−0.81), and 0.62 (95% CI = 0.44−0.89), respectively. The continuous measure of $\log_{10}$TCDD produced a hazard ratio of 0.83 (95% CI = 0.65−1.07).

2.4.1.2.1.4.7.3. *Suitability of data for TCDD dose-response modeling.*

The inverse association between TCDD and uterine fibroids supports the possibility of an anti-estrogenic effect of TCDD. The observed direction of the reported associations precludes quantitative dose-response modeling.

2.4.1.2.1.5. ***Other Seveso noncancer studies.***

2.4.1.2.1.5.1. Bertazzi et al. (1989, 197013); Consonni et al. (2008, 524825)—Mortality outcomes.

2.4.1.2.1.5.1.1. *Study summary.*

Several studies have evaluated the mortality of Seveso residents exposed to TCDD following the 1976 accident. The earlier section of this report described the designs of these studies and discussed their findings as they relate to cancer mortality. In this section, some of the findings for other causes of death are described. A key feature of these studies is that patterns of mortality among Seveso residents were investigated according to their zone of residence at the time of explosion relative to general population rates.

A 10-year mortality follow-up of residents of Seveso was published in 1989 (Bertazzi et al., 1989, 197013). Poisson regression was used to derive RRs for those who had lived in Zone A at the time of explosion using a referent group consisting of inhabitants who had lived in the uncontaminated study area. Between 1976 and 1986, no statistically significant difference was observed in all-cause mortality relative to the general population among those who lived in the most highly exposed area (Zone A) at the time of the accident. This finding was evident in both males (RR = 0.86, 95% CI = 0.5−1.4) and females (RR = 1.14, 95% CI = 0.6−2.1). A statistically significant excess in circulatory disease mortality was found among males relative to those in the referent population (RR = 1.75, 95% CI = 1.0−3.2); this increased risk was more

pronounced when the follow-up period was restricted to the first 5 years after the accident (1976−1981) (RR = 2.04, 95% CI = 1.04−4.2). Between 1982 and 1986, the RR decreased substantially and was not statistically significant (RR = 1.19, 95% CI = 0.4−3.5). Among females, a risk similar in magnitude was detected for circulatory disease mortality although it was not statistically significant (RR = 1.89, 95% CI = 0.8−4.2). Contrary to the calendar period-specific findings for males, the excess of circulatory mortality among females occurred between 1982 and 1986 (RR = 2.91, 95% CI = 1.1−7.8) and not between 1976 and 1981 (RR = 1.12, 95% CI = 0.3−4.5). The number of deaths in this cohort with the 10 years of follow-up was relatively small; in Zone A, 16 deaths were observed among males and 11 among females.

The most recently published account of the mortality experience of Seveso residents provides further information on follow-up of these residents until the end of 2001 (25 years after the accident) (Consonni et al., 2008, 524825). Three exposure groups were considered: Zone A (very high contamination), Zone B (high contamination), and Zone R (low contamination). The reference population consisted of those residents who lived in unaffected surrounding areas, as well as residents of five nearby towns. The authors used Poisson regression to compare mortality rates for each zone relative to the reference population.

For all causes of death, no excess was found in Zone A, B, or R relative to the reference population. Statistically significant excesses were noted for those who lived in Zone A relative to the reference population for chronic rheumatic heart disease (RR = 5.74, 95% CI = 1.83−17.99) and chronic obstructive pulmonary disease (RR = 2.53, 95% CI = 1.20−5.32). These risks, however, were based on only 3 and 7 deaths, respectively. For those in Zone A, no statistically significant excesses in mortality were noted for diabetes, accidents, digestive diseases, ischemic heart disease, or stroke. Among Zone A residents, stratified analysis by time since accident showed increased rates of circulatory disease 5−9 years since the accident (RR = 1.84, 95% CI = 1.09−3.12). Increased mortality from diabetes relative to the reference population was noted among females who lived in Zone B (RR = 1.78, 95% CI = 1.14−2.77).

2.4.1.2.1.5.1.2. *Study evaluation.*

The ascertainment of mortality in this cohort is nearly complete. Misclassification of some health outcomes, such as diabetes, may occur due to use of death certificate data.

The characterization of exposure is based on zone of residence. Soil sampling indicated considerable variability in TCDD soil levels, and therefore, the generation of risks based on zone of residence likely does not accurately reflect individual exposure. Exposure misclassification might also occur because residency in the areas does not necessarily reflect whether the individual would have been present in the area at the time the accident occurred. Any exposure misclassification would likely be nondifferential which would tend to bias the risk estimates towards the null.

Although some excess of circulatory disease mortality was found, the finding was not consistent between men and women. Moreover, excess circulatory disease mortality was more pronounced among men within the first 5 years of exposure, while, for women, the excess was more pronounced in years 5−10. Numerous other risk factors for circulatory disease were not controlled for in these analyses and may be confounders if related to TCDD exposure. Taken together, the possibility that TCDD increased circulatory disease mortality based on these data is tenuous at best.

2.4.1.2.1.5.1.3. *Suitability of data for TCDD dose-response modeling.*

There is considerable uncertainty in these data due to the potential for outcome and exposure misclassification. The lack of the individual-level TCDD levels and the examination of fatal outcomes reported in this study are not a suitable basis for development of an RfD. For these reasons, dose-response analysis for this outcome is not conducted.

2.4.1.2.1.5.2. Mocarelli et al. (1996, 197637; 2000, 197448)—Sex ratio.

2.4.1.2.1.5.2.1. *Study summary.*

A letter to the editor was the first report of a possible change in the sex ratio from dioxin among Seveso residents following the July 10, 1976 accident (Mocarelli et al., 1996, 197637). The authors reported that 65% (n = 48) of the 74 total births that had occurred from April 1977 to December 1984 were females. This male to female ratio of 26:48 (35%) is significantly different from the worldwide birth ratio of 106 males to 100 females (51%) (James, 1995,

197722). Between 1985 and 1994, the Seveso male to female ratio leveled out at 60:64 (48%). The authors suggested that the finding supported the hypothesis that dioxin might alter the sex ratio through several possible mechanistic pathways.

Mocarelli et al. (2000, 197448) later reported on an investigation between serum-based TCDD measures in parents and the sex ratio of offspring. In this study, serum samples were collected from mothers and fathers who lived in the areas at the time of the explosion, were between the ages of 3 and 45 at the time of the explosion, and produced offspring between April 1, 1977 and December 31, 1996. The study population included 452 families and 674 offspring, and serum measures were available for 296 mothers and 239 fathers. An estimate of TCDD at the time of conception was also examined in relation to male to female birth ratios. TCDD exposure estimates between the years of 1976 and 1996 were estimated using Filser's model (Kreuzer et al., 1997, 198088).

Mocarelli et al. (2000, 197448) used chi-square test statistics to compare observed sex ratio to an expected value of 0.51 in this Seveso population. Concentrations of TCDD were modeled as categorical variables in several ways. First, a dichotomous variable was used whereby unexposed parents were defined as those who lived outside Zones A, B, and R or had a serum TCDD concentration of less than 15 ppt; parents with exposures of 15 ppt or higher were considered exposed. Second, a trichotomous exposure variable was created that consisted of parents who (1) lived outside Zones A, B, and R or had serum concentrations of less than 15 ppt, (2) had serum concentrations of 15−80 ppt, and (3) had serum concentrations that exceeded 80 ppt. These cut-points were chosen as they represented tertiles based on the distribution of TCDD among parents. Analyses were conducted separately for paternal and maternal TCDD levels.

The overall proportion of 0.49 male births (based on male to female ratio of 328:346) was not significantly different from the expected proportion of 0.51 ($p > 0.05$). Statistically significant differences were found, however, if both parents had TCDD levels >15 ppt (sex ratio = 0.44) or just the father had serum TCDD levels >15 ppt (sex ratio = 0.44). No statistically significant differences were found when the fathers had TCDD levels less than 15 ppt, irrespective of the maternal levels. A dose-response pattern in the sex ratio was found across the paternal exposure categories. That is, the sex ratio decreased with increased paternal TCDD levels (linear test for trend, $p = 0.008$). In the unexposed group, the sex ratio (male to

female) was 0.56 (95% CI = 0.49−0.61), while in the highest exposure group (281.0−26,400.0 ppt) the corresponding sex ratio was 0.38 (95% CI = 0.28−0.49).

Stratified analyses by age at paternal exposure revealed that the sex ratio was altered to a greater degree among fathers who were younger than 19 at the time of the explosion. The male to female ratio among the unexposed fathers was 0.56 (95% CI = 0.50−0.62), while it was 0.38 (95% CI = 0.30−0.47) for those younger than 19 when exposed and 0.47 (95% CI = 0.41−0.53) for those exposed after 19. Regardless of the age at the time of exposure, however, fathers who were exposed had a statistically significantly different birth ratio (they were more likely to father girls) than those who were unexposed ($p < 0.05$).

Separate analysis of birth ratios based on paternal TCDD exposure estimated at the time of conception did not show the same dose-response pattern but did show strong evidence of consistently decreased male births relative to females. More specifically, the male to female birth ratios among the four successive quartiles (first through fourth) were 0.41, 0.33, 0.33, and 0.46.

2.4.1.2.1.5.2.2. *Study evaluation.*

Mocarelli et al. (2000, 197448) based the characterization of TCDD exposure on serum samples, which is an objective method for characterizing dose. Unlike for the occupational cohorts, serum measures for this study were taken close to the time of the accident, and therefore, back-extrapolation of TCDD exposures is unnecessary. Exposure received before the age of 19 at the time of the explosion were more strongly associated with a reduced male to female ratio than those received after the age of 19. The cut off age of 19 seems to be somewhat arbitrary, resulting in a highly uncertain critical exposure window. TCDD levels at the time of conception did not demonstrate a dose-response relationship, but paternal exposures resulted in consistently reduced male to female birth ratios (range: 0.33−0.46).

The study findings are unlikely to be influenced by age at conception as these values were found, on average, to be similar across calendar years. This suggests that age at conception was not an important confounder and that the birth ratio findings may be related to paternal exposures.

The methods used to identify births appear to be appropriate. Even if some under-ascertainment of births occurred, there is no reason to believe that ascertainment would be

related to TCDD exposure and the sex of the baby. Therefore, no bias is suspected due to incomplete birth ascertainment.

2.4.1.2.1.5.2.3. *Suitability of data for TCDD dose-response modeling.*

TCDD exposures were well-characterized, and internal cohort analyses demonstrate association between paternal TCDD levels at the time of the accident and birth ratio. However, the change in sex ratio was only statistically significant when exposure occurred before 19 years of age. It is impossible to identify the relevant time interval over which TCDD dose should be considered for dose-response analysis; specifically, it is difficult to discern whether the different sex ratio is a consequence of the initial peak exposure before 19 years of age or a function of the average cumulative exposure over this entire exposure window. Assuming the initial high exposure is the correct exposure window, using the initial exposures in a dose-response model would yield LOAELs that are too high to be relevant to factor into the RfD calculation. The differences between the two dose estimates are quite large. Dose-response analysis for this outcome, therefore, was not conducted.

2.4.1.2.1.5.3. Baccarelli et al. (2002, 197062; 2004, 197045)—Immunologic effects.

2.4.1.2.1.5.3.1. *Study summary.*

The relationship between TCDD and immunological effects was evaluated in a sample of Seveso residents (Baccarelli et al., 2002, 197062; Baccarelli et al., 2004, 197045). Both studies were based on findings from 62 individuals who were randomly selected from Zones A and B. An additional 59 subjects were chosen from the surrounding noncontaminated areas. Residency was based on where subjects lived at the time of the accident (July 10, 1976) (Landi, 1998, 594409). Frequency matching ensured that the two groups of subjects were similar with respect to age, sex, and cigarette smoking status.

TCDD levels were determined by mass spectrometric analysis of plasma samples. TCDD levels at the time of sampling were obtained, and estimates of levels at the time of the accident also were estimated by assuming an 8.2-year half-life (Landi, 1998, 594409). The plasma was also used to characterize levels of the immunoglobulins (Ig) IgG and IgM and the complement components C3 and C4. One subject was excluded due to lack of an immunological

evaluation. Analyses are, therefore, based on 58 subjects in the noncontaminated areas and 62 individuals from the contaminated areas.

Nonparametric tests were applied to test for differences between the two groups. Multiple regression also was used to describe the relationship between the variables. Adjustment was made for several potentially confounding variables that were collected via a questionnaire.

An inverse association was noted with increasing TCDD levels and plasma IgG levels; this result remained statistically significant after adjusting for other potential confounding variables in the regression models. Specifically, the slope coefficient and p-value for the unadjusted model were −0.35 ($p = 0.0002$) and for the adjusted model the p-value was 0.0004. The authors did not present the slope coefficient for the adjusted model in either paper but noted minimal differences between the adjusted and unadjusted results. In the 2004 analysis, the authors present IgG, IgM, IgA, C3, and C4 median and interquartile values across TCDD exposure quintiles. Decreased levels of IgG were observed in the highest exposure groups. Specifically, the median values across the five quintiles (for lowest to highest) were 1,526; 1,422; 1,363; 1,302; and 1,163. The Kruskal-Wallis test for differences across the TCDD categories was statistically significant ($p = 0.002$), which is consistent with the findings for the continuous measures of TCDD. This finding persisted after excluding those subjects with inflammatory diseases and those who used antibiotics or nonsteroidal anti-inflammatory drugs. For the other plasma measures, no dose-response relationship was apparent based on median values for IgM, IgA, C3, or C4 across TCDD quintiles. The authors highlight the need for additional research, particularly given the excess of lymphatic tumors noted in the area.

Exposure to other dioxin-like compounds for both the TCDD and nonexposed areas were reported to be at background levels.

2.4.1.2.1.5.3.2. *Study evaluation.*

Both TCDD exposure and health outcome measures are well characterized. TCDD exposures, in particular, are based on current serum measures and, therefore, are not dependent on assumptions needed to back-extrapolate to earlier time periods of exposure.

A dose-response relationship between TCDD and IgG is well documented for the unadjusted model, but no details are provided on the change in the slope coefficient when other covariates were added to the model.

Interpreting the inverse association between TCDD exposure and IgG in terms of clinical significance is not possible. The IgG values reported are much higher than those subjects with antibody immunodeficiency disorders.

2.4.1.2.1.5.3.3. *Suitability of data for TCDD dose-response modeling.*

Although the data support an inverse dose-response association between IgG and TCDD, because the relationship cannot be described in terms of clinical relevance with respect to a specific adverse health outcome, these data were not suitable for quantitative dose-response modeling.

2.4.1.2.1.5.4. Landi et al. (2003, 198362)—Gene expression.

2.4.1.2.1.5.4.1. *Study summary*

The impact of TCDD on the aryl hydrocarbon receptor (AhR) was evaluated by Landi et al. (2003, 198362) in a population-based study of Seveso residents. AhR, a mechanistically based biomarker of dioxin response, must be present for manifestation of most of the toxic effects of TCDD, including tumor promotion and immunological and reproductive system effects (Safe, 1986; Puga et al., 2000). AhR activates the transcription of several metabolizing enzymes in addition to certain genes (Whitlock, 1999). The primary objective of the study was to determine whether plasma levels of TCDD and TEQ are associated with the AhR-dependent pathway in lymphocytes among Seveso residents. The genes involved in the pathway that were examined included: AhR, aryl hydrocarbon receptor nuclear translocator, CYPA1A1 and CYP1B1 transcripts, and CYP1A1-associated 7-ethoxyresorufin O-deethylase (EROD).

Study recruitment occurred from December 1992 to March 1994. A total of 62 subjects were randomly chosen from the highest exposed zones in Seveso (Zones A and B), while 59 were chosen from the noncontaminated area (non-ABR). Those chosen from the noncontaminated zone were matched by age, sex, and smoking. Assignment of zones was based on place of residence where subjects lived at the time of the accident in 1976. Subjects provided data via questionnaire on a variety of sociodemographic and behavioral risk factors, including cigarette smoking. Multivariate models were adjusted for a variety of confounders including; adjustment for age, gender, date of assay, actin expression, postculture viability, experimental group, and cell growth.

TCDD levels were determined using high-resolution gas chromatography, and 21 other dioxins, or dioxin-like compounds, were measured to examine TEQ. Eleven measurements taken on the 121 subjects were deemed inadequate and excluded, but no further information was provided on these exclusions. Nine subjects from Zone B and fourteen subjects from Zone ABR had TCDD levels below that of detection, and were assigned a value equal to the lipid-adjusted detection limit divided by the square root of 2. The toxic equivalent for the mixture of dioxin-like compounds (i.e., TEQ) was calculated by summing the products of the concentration of each congener by its specific toxic equivalency factor.

The subjects provided between 5 and 50 mL of whole blood, which was centrifuged to separate mononuclear cells. The cells were frozen and later thawed. Cells were cultured, removed from the culture medium, and resuspended in a stimulation medium, 14 mL of which was used for RNA analysis. Reverse transcription-PCR was conducted and EROD was assayed. Differences in gene expression and EROD activity observed for various cell culture conditions were compared using paired t-tests. The unpaired Student's t-test was applied to test for differences between groups, while a Bonferroni factor was used to account for multiple comparisons. Data for continuous variables were log-transformed.

TCDD accounted for 26% of the TEQ among the study subjects, but varied by zone (35% in zone A and 18% in zone non-ABR). After adjusting for potential confounding, AhR was inversely related to plasma TCDD levels in uncultured cells ($p < 0.03$) and in mitogen-stimulated cells ($p < 0.05$). EROD was lower in cells cultured from subjects with higher plasma TCDD and TEQ levels, and the corresponding continuous measure of EROD was statistically significant ($p < 0.05$). No statistically significant associations with TCDD or TEQ were found with ARNT or CYP1B1 in uncultured cell medium, nor with CYP1A1 or CYP1B1 in mitogen-stimulated cells. In general, females had lower AhR transcripts and higher levels of dioxin.

Collectively, the findings suggest that TCDD exposure might reduce AhR expression in unstimulated cells. Therefore, TCDD could exert an influence on the AhR pathway regulation.

2.4.1.2.1.5.4.2. *Study evaluation.*

The study used biologically based measures of both TCDD exposures and biomarkers or AhR. Subject recruitment was based on randomly sampling of the cohort study population; some individuals with severe medical illnesses were excluded (Landi, 1998, 594409). Although

few details are provided on the number of subjects excluded for these reasons, given the objective nature of the biomarker outcomes that were evaluated, such exclusions are unlikely to be an important source of bias. The exclusion rates were also reported to be low and comparable across the zones (five subjects from the noncontaminated zone non-ABR and four subjects from zone B).

A strength of the study was the examination of other dioxin-like compounds via the TEQ analysis. A limitation of the study included the relatively small number of subjects which resulted in the grouping of several covariates, including TCDD exposures, into a small number of categories. As such, slope coefficients derived from modeling continuous measures were emphasized in the data presentation. Another key limitation of the study is the uncertainty of how effects on AhR translate into subsequent development of cancer and other chronic health effects.

2.4.1.2.1.5.4.3. *Suitability of data for TCDD dose-response modeling.*

It is unclear how associations between AhR biomarkers and TCDD levels translate into an increased risk of cancer. Dose-response analysis for this outcome, therefore, was not conducted.

2.4.1.2.1.5.5. Alaluusua et al. (2004, 197142)—Developmental dental effects.

2.4.1.2.1.5.5.1. *Study summary.*

Alaluusua et al. (2004, 197142) examined the relationship between TCDD and dental defects, dental caries, and periodontal disease among Seveso residents who were children at the time of the accident. Subjects were randomly selected from those individuals who had previously provided serum samples in 1976, which was shortly after the accident. A total of 65 subjects who were less than 9.5 years of age at the time of the accident, and who lived in Zones A, B, or R were invited to participate. Recruitment was initiated 25 years after the time of the Seveso accident. An additional 130 subjects from the surrounding area (outside Zones A, B, or R or "non-ABR zone") having the same age restriction were recruited. Subjects were frequency matched for age, sex, and education. Questionnaires were administered to these individuals to collect detailed information on dental and medical histories, education, and smoking behaviors. Ten subjects who had completed at least high school were randomly

excluded from the non-ABR zone to create groups with similar educational profiles. Participation rates for the ABR and non-ABR zones were 74 and 58%, respectively.

One dentist who was blind to the patients' TCDD exposure levels assessed dental aberrations. Dental caries was assessed using recommendations of the World Health Organization. Periodontal status was described following a detailed evaluation of the surfaces of the teeth. A radiographic examination was done to identify missing teeth, alveolar bone loss, deformities in the roots, and jaw cysts.

Comparisons of the presence of dental enamel defects according to exposure status were performed using logistic regression. Chi-square test statistics were applied to compare the distributions in the prevalence of dental defects across several categorical covariates (i.e., education, age, and serum TCDD level). For those who were younger than 5 at the time of the accident, dental defects were more prevalent among patients in zone ABR (42%) than those in the non-ABR zone (26%) ($p = 0.14$). Zone ABR is characterized by higher levels of soil TCDD levels relative to non-ABR. Serum levels permitted an improved characterization of risk as they were available at an individual level, rather than using a zone of residence. Defect prevalence was highest among those in the upper serum TCDD category (700−26,000 ng/kg) with 60% of subjects having dental defects. The continuous measure of serum TCDD was associated with developmental dental defects ($p = 0.007$) and hypodontia ($p = 0.05$).

2.4.1.2.1.5.5.2. *Study evaluation.*

Although the subjects with serum measures were selected randomly, no direct measures of TCDD were made in subjects from the unexposed area (i.e., non-ABR zones). That those who resided in the non-ABR areas had lower TCDD exposures would be a reasonable assumption. Alaluusua et al. (2004, 197142), however, provide few details about the sampling frame used to identify these participants. Despite this fact, it is important to note that a dose-response pattern was observed between TCDD exposure and presence of developmental defects in the ABR population alone ($p = 0.016$). This finding is based on 27 subjects with developmental dental defects. This positive association provides support for a quantitative dose-response modeling of dental aberrations. The numbers of such subjects are small, however, with one, five, and nine subjects having defects in the exposure groups of 31−226, 238−592, and 700−26,000 ng/kg TCDD, respectively.

TCDD exposures were characterized using serum measures for those who resided in zone ABR in 1976 (near the time of the accident). The authors could not account for additional exposure to TCDD across subjects that might have occurred since the time of the accident, so there is considerable uncertainty in delineating the critical exposure window for the reported effects. In addition, the lack of exposure data for those in the non-ABR zone, however, makes interpretation of the findings difficult. This difficulty is particularly evident, given that the prevalence of dental defects was less among those in the low exposure category of zone ABR (31−226 ng/kg TCDD) (10%) when compared to those in the non-ABR zone (26%).

2.4.1.2.1.5.5.3. *Suitability of data for TCDD dose-response modeling.*

Most of the considerations for conducting a dose-response analysis have been satisfied with the study population, although, exposure assessment uncertainties are a limitation of this study. For example, it is difficult to discern whether these health effects are a consequence of the initial high exposure during childhood or a function of the cumulative exposure for this entire exposure window beginning at the early age. If the latter is true, averaging exposure over the critical window would add considerable uncertainty to effective dose estimates given the large difference between initial TCDD body burden and body burden at the end of the critical exposure window. Despite the uncertainty in defining the critical window of exposure, dose-response analysis was conducted for this outcome.

2.4.1.2.1.5.6. Baccarelli et al. (2005, 197053)—Chloracne.

2.4.1.2.1.5.6.1. *Study summary.*

Baccarelli et al. (2005, 197053) published findings from a case-control study of 110 chloracne cases and 211 controls. The authors collected information on pigment characteristics and an extensive list of diseases. This study was performed to yield information about the health status of chloracne cases, TCDD-chloracne exposure response, and factors that could modify TCDD toxicity. TCDD was measured from plasma. Following adjustment for confounding, TCDD was associated with chloracne (OR = 3.7, 95% CI = 1.5−8.8), and the risk of chloracne was considerably higher in subjects younger than 8 at the time of the accidents (OR = 7.4, 95% CI = 1.8−30.3). Among individuals with lighter hair, the association between TCDD and chloracne was stronger than among those with darker hair.

2.4.1.2.1.5.6.2. *Study evaluation.*

Although a dose-response association was observed, chloracne is a rare health outcome likely only to occur among those highly exposed.

2.4.1.2.1.5.6.3. *Suitability of data for TCDD dose-response modeling.*

Given the very high TCDD levels needed to cause chloracne (e.g., Ott et al., 1993, 594322)), quantitative dose-response modeling to characterize risks for the general population with much lower TCDD exposures would be of little value. Therefore, quantitative dose-response assessment for the Baccarelli et al. (2005, 197053) study was not conducted.

2.4.1.2.1.5.7. Baccarelli et al. (2008, 197059)—Neonatal thyroid hormone levels.

2.4.1.2.1.5.7.1. *Study summary.*

Baccarelli et al. (2008, 197059) investigated the relationship between thyroid function and TCDD among offspring of women of reproductive age who were exposed in the 1976 accident. This health endpoint is relevant because thyroid function is important for energy metabolism and nutrients and for stimulating growth and development of tissues. Neonatal thyroid function at birth is evaluated through blood thyroid-stimulating hormone (b-TSH).

The study population was drawn from 1,772 women who were identified as having lived in the highly contaminated areas (Zones A or B) at the time of the accident or between July 10, 1976 and December 31, 1947; were of fertile age (born after 1947); and were alive as of January 1, 1994. A random sample of 1,772 unexposed women who lived in the reference area was selected using frequency matching by year of birth to the exposed women, and residency in the reference area at the time of the accident. The reference area represents the noncontaminated areas that surround the three zones of decreasing exposure (Zones A, B and R). In total, 55,576 women had lived in the reference area. Population registry offices (n = 472) were contacted to detect children born to these women. Records could be traced for virtually all subjects (1761/1772 exposed; 1762/1772 unexposed). Children born outside the Lombardy area were excluded as b-TSH could not be obtained for them. This accounted for 156 of the 1,170 children identified. The analyses were based on the remaining 56, 425, and 533 singletons born between January 1, 1994, and June 30, 2005 in Zone A, B, and from the reference area, respectively.

Thyroid function is tested in all newborns by b-TSH measures in the region of Lombardy where Seveso is located. These measures are obtained from blood samples taken 72 hours after birth using a standardized protocol. The b-TSH levels were log transformed to approximate a normal distribution. Linear regression analysis was used to conduct test for trends in mean b-TSH levels across different covariates. Logistic regression was used to assess associations between elevated b-TSH levels defined by the cutpoint of 5 μU/mL and residence in particular zones of contamination. The 5 μU/mL cutpoint for TSH measurements in neonates was recommended by WHO (1994) for use in neonatal population surveillance programs. Although WHO established the standard for increased neonatal TSH in the context of iodine deficiency disease, the toxicological implications are the same for TCDD exposure and include increased metabolism and clearance of T4. Generalized estimating equations were used to adjust the standard errors of the ORs for correlation between siblings.

The mean levels of b-TSH were positively associated with average soil TCDD concentrations in the three areas (Zone A: 1.66 μU/mL; Zone B: 1.35 μU/mL; and Zone R: 0.98 μU/mL) ($p < 0.001$). Plasma TCDD levels also were shown to be much higher in a group of 51 newborns that had b-TSH levels >5 μU/mL. Compared to the reference population, adjusted ORs were elevated for Zone B (OR = 1.90, 95% CI = 0.94−3.86) and Zone A (OR = 6.63, 95% CI = 2.36−18.6). These ORs were adjusted for gender, birth weight, birth order, maternal age at delivery, hospital, and type of delivery. The adjusted ORs however differed only slightly from those that were unadjusted (Zone B, OR = 1.79, 95% CI = 0.92−3.50; Zone A OR = 6.60, 95% CI = 2.45−17.8). Of the risk factors considered, both gender and birth weights were associated with neonatal b-TSH.

The paper also included an analysis of children born to 109 women who were part of the Seveso Chloracne Study (Baccarelli et al., 2005, 197053). A total of 51 children were born to 38 of these women, of these 12 lived in Zone A, 10 in Zone B, 20 in Zone R, and 9 from the reference population. Several congeners including TCDD were measured in maternal plasma. TCDD levels were extrapolated to the date of delivery using a first-order pharmacokinetic model (Michalek et al., 1996, 198893). The elimination rate used was 9.8 years based on the mean half-life estimate from a previous study of women in the Seveso region (Michalek et al., 2002, 199579). TEQs were calculated for a mixture of dioxin-like compounds by multiplying the concentration of each congener by its toxicity equivalence factor. The maternal average TEQ

was 44.8 ppt (range: 11.6–330.4) among 51 mothers. The measurement of noncoplanar PCBs occurred only later in the study (1996) and, therefore, total mean TEQs (i.e., including the sum of PCDDs, PCDFs, coplanar PCBs, and noncoplanar PCBs) are available only on a subset (n = 37) of the population. Dioxin-like congeners were examined in this study as several studies suggest associations between the sum of PCBs, or individual congeners having decreased thyroxine (T4; Longnecker et al., 2000, 201463; Sandau et al., 2002, 594406), and increased TSH (Alvarez-Pedrerol et al., 2008, 594407; Chevrier et al., 2007, 594408). The following confounders were examined by the authors in the plasma dioxin models: maternal body mass index, smoking habits, alcohol consumption, and neonatal age in hours at b-TSH measurement.

The authors used a linear model to examine the association between maternal TCDD levels and b-TSH. The standardized regression coefficient obtained from this model was 0.47 ($p < 0.001$). For the evaluation of TEQs, a similar association was noted for PCDDs, PCDFs, and coplanar PCBs (n = 51, β = 0.45, p = 0.005) but not with noncoplanar PCBs (n = 37, β = 0.16, p = 0.45). Multivariate regression models that were adjusted for several covariates (i.e., gender, birth weight, birth order, maternal age at delivery, hospital, and type of delivery) found statistically significant associations with plasma TCDD, PCDDs, PCDFs, and coplanar PCBs, but not with noncoplanar PCBs. The sum of all total TEQs from the measured compounds was not statistically significant (n = 37, β = 0.31, p = 0.14).

2.4.1.2.1.5.7.2. *Study evaluation.*

The Baccarelli et al. (2008, 197059) study satisfies the epidemiological considerations and criteria for determining whether dose-response modeling should be pursued. The outcome is well defined, and a dose-response pattern was observed. The study also contained a substudy that characterized TCDD and exposures to other dioxin-like congeners and used serum measures for a sample of mothers. Results were consistent among the zone of residence analysis and the substudy based on serum measures.

2.4.1.2.1.5.7.3. *Suitability of data for TCDD dose-response modeling.*

Given the potential for exposure misclassification due to variability in TCDD soil levels within each zone, modeling should rely on individual-level TCDD exposures derived from the serum sampling substudy. The study data provide an opportunity for quantitative dose-response

analyses as the critical exposure window of 9 months can be used for exposure assessment purposes.

2.4.1.2.1.5.8. Mocarelli et al. (2008, 199595)—Sperm effects.

2.4.1.2.1.5.8.1. *Study summary.*

Mocarelli et al. (2008, 199595) examined the relationship between TCDD and endocrine disruption and semen quality in a cohort of Seveso men. A total of 397 subjects of the eligible 417 males (<26 years old in 1976) from Zone A and nearby contaminated areas were invited to participate. Frozen serum samples were used to derive TCCD exposures. Also, 372 healthy blood donors not living in the TCCD-contaminated area were invited to participate. The researchers collected a health questionnaire and semen samples from participants. Analyses were based on 257 individuals in the exposed group and 372 in the comparison group.

Semen samples were collected postmasturbatory at home. Ejaculate volume, sperm motility, and sperm concentration were measured on these samples. Fasting blood samples also were collected from the subjects for reproductive hormone analyses, including 17β-estradiol (E_2), follicle stimulating hormone (FSH), inhibin B, luteinizing hormone (LH), and testosterone.

The researchers estimated serum concentrations of TCDD from samples provided in 1976−1977, and also in 1997−1998 for individuals whose earlier samples had TCDD values that exceeded 15 ppt. Serum concentrations for the comparison group were assumed to be less than 15 ppt in 1976 and 1977 and <6 ppt in 1998/2002 on the basis of serum results for residents in uncontaminated areas. The exposed and comparison groups were divided into three groups based on their age in 1976: 1−9, 10−17, and 18−26 years. Mocarelli et al. (2008, 199595) applied a general linear model to the sperm and hormone data and included exposure status, age, smoking status, body mass index, and occupational exposures as covariates. The study authors thoroughly addressed the potential for confounding.

Men exposed between the ages of 1 and 9 had reduced semen quality 22 years later. Reduced sperm quality included decreases in sperm count ($p = 0.025$), progressive sperm motility ($p = 0.001$), and total number of motile sperm ($p = 0.01$) relative to the comparison group. The opposite pattern was observed for several indices of semen quality among those aged 10−17 at the time of the accident; this included a statistically significant increase in sperm count ($p = 0.042$). The clinical significance of this increase is unknown. For the hormone analyses,

those in the exposed group had lower serum E_2 levels, and higher follicle stimulating hormone concentrations. Neither testosterone levels nor inhibin B concentrations were associated with TCDD exposure.

2.4.1.2.1.5.8.2. *Study evaluation.*

The findings of the Mocarelli et al. (2008, 199595) study support the hypothesis that exposure to TCDD in infancy/prepuberty reduces sperm quality. The changes in serum E2 and FSH concentrations are of unknown clinical significance, and cannot be considered adverse. Although most semen analysis studies have low compliance rates in general population samples (20−40%) (Jørgensen et al., 2001, 594402; Muller et al., 2004, 594403), the compliance rate in this study was much higher (60%). Given that the compliance rates were similar between the exposed and comparison groups and the strong differences detected across the two age groups, selection bias appears unlikely in this study.

2.4.1.2.1.5.8.3. *Suitability of data for TCDD dose-response modeling.*

Health outcomes are well defined in the Mocarelli et al. (2008, 199595) study, and exposures are well characterized using serum data. Because the men exposed to elevated TCDD levels between the ages of 1 and 9 had reduced semen quality 22 years later, it is difficult to identify the relevant time interval over which TCDD dose should be considered. Specifically, it is difficult to discern whether this effect is a consequence of the initial high exposure between 1 and 9 years of age or a function of the cumulative exposure for this entire exposure window beginning at the early age. However, the differences between these two dose estimates (the initial high exposure versus the cumulative exposure for the 9 year window) are minimal (i.e., within an order of magnitude). Despite the uncertainty in estimating the critical window of exposure, dose-response analysis for this outcome was conducted.

2.4.1.2.1.6. *The Chapaevsk study.*

2.4.1.2.1.6.1. Revich et al. (2001, 199843)—Mortality and reproductive health.

2.4.1.2.1.6.1.1. *Study summary.*

Revich et al. (2001, 199843) describe a series of investigations that have evaluated adverse health outcomes among residents of Chapaevsk where ecological measures of TCDD

have been noted to be higher than expected. In the earlier cancer section of this report, the cross-sectional comparisons of mortality that the authors carried out between Chapaevsk residents and a general population reference were described. Although the general focus of this paper is on cancer, the authors examined other adverse health outcomes.

For all-cause mortality, rates were found to be higher in Chapaevsk relative to the Samara region and other nearby towns. The magnitude of this increase, however, was not quantified in the review by Revich. Cardiovascular mortality accounted for nearly two-thirds of women's deaths and almost half of those among men. The rates of cardiovascular mortality among Chapaevsk men have been reported to be 1.14 times higher than those in Russia.

Revich et al. (2001, 199843) also reported on the occurrence of adverse reproductive events. Although the authors indicated that official medical information was used to make comparisons between regions, no details were provided about data quality, completeness, or surveillance differences across areas. The presented rates for reproductive health outcomes should be interpreted cautiously. A higher rate of spontaneous abortions (24.4 per 100 pregnancies finished by delivery) was found in Chapaevsk women relative to rates that ranged between 10.6 and 15.2 found in five other areas. The frequency of preeclampsia also was found to be higher in Chapaevsk women (44.1/100) relative to other towns, as was the proportion of low birth-weight babies and preterm births. The percentage of newborns with low birth weight was slightly larger in Chapaevsk (7.1%) when compared to other towns in Samara (5.1−6.2%); observed differences, however, were not statistically significant. The authors also reported on the sex ratio of newborns born between 1983 and 1997. These ratios (boys:girls) were highly variable and ranged between 0.79 and 1.29. Given the annual variability of this ratio on a year-to-year basis, it is unclear if this is largely due to natural fluctuations and to what extent this may result from prior TCDD (or other contaminants) exposure TCDD and other contaminants.

2.4.1.2.1.6.1.2. *Study evaluation.*

The review by Revich et al. (2001, 199843) highlights analyses that have been undertaken using largely cross-sectional data. Although soil sampling measures appear to demonstrate decreasing levels of TCDD in the soil with increasing distance from the plant, at this time, no individual-level TCDD exposure data are available. Increased rates of mortality relative

to the Samara region in Russia were observed among Chapaevsk men for all cancer sites combined; this excess risk however, was not observed among women. Although the authors provide compelling evidence of increased adverse events among residents of Chapaevsk, the study lacks a discussion about the validity of comparing health data across regions, and suffers from inherent limitations from ecological studies such as exposure misclassification.

2.4.1.2.1.6.1.3. *Suitability of data for TCDD dose-response modeling.*

As with the cancer outcomes presented in this study, the data for noncancer outcomes are limited by the absence of TCDD levels on an individual-level basis and information on other potential confounding variables that could have biased the comparisons. Additional studies are being undertaken to evaluate the relationship between TCDD and the sexual and physical development of boys. The cross-sectional nature of the data that were presented does not provide the necessary level of detail needed to estimate effective dose given the lack of individual-level exposure data. Therefore, a quantitative dose-response analysis was not conducted.

2.4.1.2.1.7. *The Air Force Health ("Ranch Hands" cohort) study.*

2.4.1.2.1.7.1. Michalek and Pavuk (2008, 199573)—Diabetes.

2.4.1.2.1.7.1.1. *Study summary.*

Michalek and Pavuk (2008, 199573) examined both the incidence of cancer and the prevalence of diabetes in the cohort of Ranch Hand workers exposed to TCDD. As noted previously, these veterans were responsible for aerial spraying of Agent Orange in Vietnam between 1962 and 1971. Exposure to TCDD was estimated using serum collected from participants in 1987 and assayed for TCDD. Exposure to TCDD was estimated using a first-order pharmacokinetic model with a half-life of 7.6 years and provided an estimate of TCDD at the end of the tour of duty in Vietnam. Veterans were grouped into four categories: comparison, background, low, and high. Diabetes was identified from diagnoses during the post-Vietnam era from medical records. Overall, no differences were shown in the RR of diabetes between the Ranch Hand unit and the reference group (RR = 1.21, $p = 0.16$). Stratified analyses by days of spraying (<90 days, ≥90 days), however, revealed a significant increase in risk of diabetes (RR = 1.32, $p = 0.04$) among those who sprayed for at least 90 days. A dose-

response relationship was also evident when log10TCDD was modeled in the combined cohort. Also, stratification by calendar period showed a dose-response relationship for those whose last year of service was during or before 1969.

2.4.1.2.1.7.1.2. *Study evaluation.*

The Michalek and Pavuk (2008, 199573) study provides an opportunity to characterize risks of diabetes as the study is not subject to some of the potential bias of case ascertainment based on death certificates (D'Amico et al., 1999, 197389). The quality of the TCDD exposure estimates is high, given that serum data were available at an individual-level basis for all Ranch Hand and comparison veterans used in the cohort. Although disentangling the effects of 2,4-D and TCDD is not possible because their concentrations in Agent Orange are equivalent, 2,4-D has not been associated with diabetes.

2.4.1.2.1.7.1.3. *Suitability of data for TCDD dose-response modeling.*

The reported dose-response relationship between TCDD and diabetes is supported by study strengths including the use of the individual-level level TCDD serum measures and the identification of diabetes through medical records are important strengths of the Michalek and Pavuk (2008, 199573) study. Nonetheless, the possible confounding from the inability to control for 2,4-D and other agents used in Agent Orange precludes a quantitative dose-response analysis.

2.4.1.2.1.8. *Other noncancer studies of TCDD.*

2.4.1.2.1.8.1. Ryan et al. (2002, 198508)—Sex ratio.

2.4.1.2.1.8.1.1. *Study summary.*

Ryan et al. (2002, 198508) conducted an investigation on the sex ratio in offspring of children of pesticide workers who were involved with the production of trichlorophenol and the herbicide 2,4,5-T in Ufa, Bashkortostan, Russia. Ufa was the site of a state agrochemical plant that has been in operation since the 1940s. Between 1961 and 1988, the plant employed more than 600 workers, most in their early 20s. Females, however, accounted for about 15% of the workforce that produced 2,4,5-T and 30% for 2,4,5-trichlorophenol.

Serum samples previously taken in 1992 among 60 men, women, and children from the factory and city of Ufa showed TCDD exposures that were approximately 30 times higher than

background levels (Ryan and Schecter, 2000, 594412). Blood data were subsequently measured on a sample of 20 workers between 1997 and 2000, and on 23 2,4,5-trichlorophenol workers between 1997 and 2001. In all, 84 individuals who provided blood samples formed the basis of the analysis in this study. Of these, 55 were exposed to 2,4,5-T and 29 were exposed to 2,4,5-trichlorophenol.

Ryan et al. (2002, 198508) reviewed company records for these workers to determine the number, sex, and date of birth of any children; birth data were available for 198 workers. Awareness of the study led other workers who had not provided serum to provide information on births that occurred 9 months after the time of first employment in the factory.

The authors calculated descriptive statistics for the 198 workers and compared them to values for the city of Ufa between 1959 and 1996. Tests of statistical significance were made using the z-test, and the chi-square test. The observed proportion of male births (0.40) among the factory workers was much lower than that for the city of Ufa (0.51) ($p < 0.001$). Stratified analyses revealed that this lower ratio was observed only among those paternally exposed to TCDD. Specifically, the proportion of male births among exposed fathers was 0.38 and among exposed mothers was 0.51. This pattern was observed in both the workers exposed to 2,4,5-T (proportion of male births = 0.40) and 2,4,5-trichlorophenol (proportion of male births = 0.35).

2.4.1.2.1.8.1.2. *Study evaluation.*

The Ryan et al. (2002, 198508) findings are consistent with earlier work completed for Seveso residents (Mocarelli et al., 2000, 197448). Although serum measures were available for 84 individuals, no dose-response of birth ratios was performed using exposure quantified at an individual-level basis. This approach would have been preferred and consistent with that which Mocarelli et al. (2000, 197448) used. All comparisons were made using an external comparison group, namely the sex ratio observed in Ufa between 1959 and 1996.

Although serum measures were used to describe TCDD exposure for a sample of the workers, individual-level dose estimates were not calculated for the study population. Specifically, exposures were characterized many years after exposure, and no attempt was made to back-extrapolate to the time of conception. The two groups of workers in the study also reportedly had high exposure levels of 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin. So, the group level exposure classification (by plant) did not allow consideration of confounding due to other

dioxin-like compounds. Another limitation of the study is that the study population is likely nonrepresentative of all workers employed at the plant. Participants included only those willing to provide serum samples and those who volunteered to participate in the study after learning about it in a public forum. If participation was dependent on TCDD exposures and the reproductive health of these subjects, then bias may have occurred.

2.4.1.2.1.8.1.3. *Suitability of data for TCDD dose-response modeling.*

The findings are notable in their consistency with those found in Seveso residents by Mocarelli et al. (2000, 197448). For the Ryan et al. (2002, 198508) study, serum data were quantified at an individual-level basis. Risk estimates, however, were not derived in relation to these exposures but instead in two separate subgroups (2,4,5-T and 2,4,5-trichlorophenol workers). This important limitation precludes the use of these data for quantitative dose-response modeling.

2.4.1.2.1.8.2. Kang et al. (2006, 199133)—Long-term health effects.

2.4.1.2.1.8.2.1. *Study summary.*

Kang et al. (2006, 199133) investigated the relationship between self-reported health measures and serum-based measures of TCDD in a group of 1,499 Vietnam veterans and a control group of 1,428 non-Vietnam veterans. The study subjects were identified from (1) reports of Army Chemical Corps detachments in Vietnam between 1966 and 1971, (2) personnel records of individuals involved in chemical operations who were on active duty between 1971 and 1974, and (3) class rosters of personnel who were trained at Fort McClellan in Alabama between 1965 and 1973. The comparison group was selected so that branch of service, time period, and military occupation were similar to those of the subjects with the exception that they did not serve in Vietnam. Although 2,872 Vietnam veterans and 2,732 non-Vietnam veterans were identified as potential subjects, those who were deceased as of December 1998 and those who had previously participated in a pilot study were excluded. The study targeted 2,247 Vietnam and 2,242 non-Vietnam veterans.

Exposure to TCDD was characterized for subsets of the study population that provided blood samples, specifically 795 of 1,085 (73%) Vietnam veterans and 102 of 157 (65%) non-Vietnam veterans. Details on these individuals selected for participation in the serum dioxin

study were not presented. The authors did state, however, that due to economic constraints, only 897` serum samples could be analyzed. Blood specimens were collected in 1999–2000 at individuals' homes. TCDD concentrations were analyzed by laboratory staff blind to the group status (i.e., Vietnam or non-Vietnam) of the study subjects.

Prevalent health outcomes were ascertained by self-reported information on selected conditions diagnosed by a medical doctor. The following conditions were included: diabetes, hepatitis (all types combined), heart disease, all cancer, nonmalignant chronic respiratory diseases, and hypertension. Health-related quality of life was evaluated using the SF-36 survey instrument (Ware et al., 1993, 004687).

Eligible veterans whose current residences (4,119 total) could be identified were contacted for study participation. Survey participation rates were 72.9% for Vietnam veterans, yielding data for 1,499 individuals, and 69.2% for non-Vietnam veterans, yielding data for 1,428 non-Vietnam veterans. The survey data showed that, relative to non-Vietnam veterans, Vietnam veterans were more likely to be regular smokers and to be obese. They also were more likely to be enlisted personnel, and a much higher proportion was 51 years of age or older (83.4% vs. 58.4%). After adjusting for age, race, smoking status, rank, and body mass index, the prevalence of self-reported health conditions was found to be statistically significantly higher in the Vietnam group. The adjusted odds ratios (OR) were as follows: diabetes, OR = 1.16 (95% CI = 0.91, 1.49); hepatitis, OR = 1.85 (95% CI = 1.30, 2.64); heart condition, OR = 1.09 (95% CI = 0.87, 1.38); all cancer, OR = 1.46 (95% CI = 1.02, 2.10); nonmalignant respiratory condition, OR = 1.41 (95% CI = 1.13, 1.76); and hypertension, OR = 1.06 (95% CI = 0.89, 1.27).

For those with Vietnam service, the mean serum TCDD concentrations were higher among those who reported spraying herbicides (4.3 parts per thousand [ppt]) than those who did not (2.7 ppt) ($p < 0.001$). The investigators did not back-extrapolate serum levels to the time when individuals last sprayed. The adjusted ORs (adjusted for age, cigarette smoking, body mass index, rank, and race) for most chronic health conditions examined revealed increased prevalence among Vietnam sprayers relative to non-Vietnam sprayers. These ORs were: diabetes, OR = 1.49 (95% CI = 1.10, 2.02); hepatitis, OR = 1.40 (95% CI = 0.92, 2.12); heart condition, OR = 1.41 (95% CI = 1.06, 1.89); all cancer, OR = 1.36 (95% CI = 0.91, 2.04); nonmalignant respiratory condition, OR = 1.57 (95% CI = 1.20, 2.07); and hypertension, OR = 1.26 (95% CI = 1.00, 1.58).

The investigators also examine the possibility of over-reporting of chronic health conditions by comparing the prevalence of self-reported conditions among 357 Vietnam sprayers who mean serum TCDD levels of 2.5 ppt compared to those who had levels less than 2.5 ppt. Prevalence of diabetes, heart condition, and hypertension, was higher among those with mean serum TCDD levels of 2.5 ppt, although no levels of statistical significance were reported. Data for cancer were not presented.

2.4.1.2.1.8.2.2. *Study evaluation.*

Because data were collected from only half of the individuals in the study target population, there is some potential for selection bias in this study. First, the study excluded those who had died before 1999, excluding potentially important TCDD-related adverse health effects that could result in death more than two decades after veterans had been actively spraying. Second, survey participation rates were modest: 72.9% for Vietnam veterans and 69.2% for non-Vietnam veterans. If those in poorer health were less inclined to participate, the prevalence of the selected chronic health conditions would be understated. Selection bias due to study participation could also be possible if, for example, those in poorer health also had high (or lower) exposures than those not participating in the study. The lack of direct evidence of differential participation and reports of comparable prevalence rates of hypertension and diabetes to other general populations suggests that selection bias may be minimal.

Because the data collected are cross-sectional, they are ill-suited for evaluating the relationship between the timing of exposure and the onset of disease. Whether any of the data could help identify when the chronic health conditions were diagnosed is unclear. Given the long period covered by the study, many of the self-reported health conditions likely were diagnosed some time ago, perhaps closer to the time of potential TCDD exposure. Such detail is needed to characterize health risks associated with specific TCDD levels, particularly given that TCDD levels have been demonstrated to decrease from time of last exposure.

An important strength of the study is the availability of blood sera for a subset of the study population, which allows for an objective determination of TCDD exposure. That serum TCDD levels were available for only 897 subjects, however, limits the ability to examine the relationship between measures of TCDD and prevalence of health outcomes without restricting the sample size or extrapolating exposure levels to the whole study population. For example,

among sprayers with available TCDD exposure data only 60 cases of diabetes and 69 cases of heart disease were examined relative to exposure. Also, the small number of cancers precluded a cancer site-specific analysis. Moreover, whether these TCDD levels are representative of the larger eligible population is difficult to gauge, given that deceased veterans and those whose current residences could not be determined were excluded.

The study relied on self-reported measures of disease prevalence. The ascertainment of chronic health conditions using self-reported data can be fraught with difficulties. For example, the sensitivity of self-reported data when compared to medical diagnosis has been shown to be poor for conditions such as diabetes and hypertension (Okura et al., 2004). As Kang et al. (2006, 199133) state, prevalence studies are not be well suited to examine rare diseases with short survival times such as cancer. In addition, self-reports of physician-diagnosed cancers by study subjects often lacks the sensitivity needed in most epidemiological studies as they can be influenced by a variety of factors including age and education (Navarro et al., 2006).

The potential for biases in the reporting of health outcomes between the sprayers and the non-Vietnam veterans (i.e., differential by TCDD exposure status) also is plausible, given the public attention that spraying of Agent Orange has received. Although the authors examined whether over-reporting was related to outcome prevalence among herbicide sprayers (prior to collection and determination of actual TCDD serum levels), the possibility exists that these subjects reporting could be influenced by their perceived level of exposure from herbicide spraying. The authors also examined the potential for misreported diabetes by conducting a medical records review of 362 veterans. Seventy-nine percent of the self-reported diabetes cases were confirmed with medical records. The documentation rate was also comparable between the Vietnam veterans and the non-Vietnam veterans suggesting that differential reporting was not an issue for this health outcome.

Because the Vietnam veterans group comprised professional sprayers, it is not unreasonable to assume that they would have been exposed to other potentially harmful agents either during their service in Vietnam, or from the end of their service to when they provided data in 1999–2000. This study did not control for other, potentially relevant occupational exposures.

2.4.1.2.1.8.2.3. *Suitability of data for TCDD dose-response modeling.*

Although the study demonstrates increased prevalence of several chronic health conditions, these findings should be interpreted with caution due to potential for selection and recall biases. The lack of demonstrated dose-response relationships with cancer or other outcomes precluded the use of these data for characterizing the dose response from TCDD.

2.4.1.2.1.8.3. McBride et al. (2009, 198490; 2009, 197296)—Noncancer mortality.

2.4.1.2.1.8.3.1. *Study summary.*

The McBride et al. (2009, 198490) mortality study of New Zealand workers employed as producer or sprayers with potential exposure to TCDD was described earlier in this report. These individuals were employed at a plant that manufactured 2,4,-dichlorophenoxyacetic acid, and later 2,4,5-T and 4-chloro-2-methyphenoxyacetic acid. In 1987, the plant closed and 2,4,5-T production ceased in 1988.

The cohort consisted of 1,754 individuals who were employed for at least one day at the New Plymouth site between January 1, 1969, and October 1, 2003. Vital status was determined until the end of 2004. Comparisons of mortality were made to the New Zealand general population using the SMR statistic. Exposure was characterized by duration of employment. Person-years of follow-up were tabulated across strata defined by age, calendar period, duration of employment, sex, latency, and period of hire. Analyses were stratified to compare risks by duration of employment (<3 or ≥3 months), latency (<15 or ≥15 years), and period of hire (<1976, ≥1976).

Overall, no statistically significant differences in all-cause mortality relative to the general population were found among those who worked for at least 3 months (SMR = 0.92, 95% CI = 0.80−1.06) or for less than 3 months (SMR = 1.23, 95% CI = 0.91−1.62). No statistically significant excesses were found for mortality from diabetes, cerebrovascular disease, heart diseases, or accidents. The incorporation of a latency period of 15 years revealed no statistically significant excesses for these same causes of death. Similarly, no excesses for any cause of death were noted among those who were hired either before or after 1976.

In subsequent analyses of the same cohort that used estimated TCDD levels from serum samples, McBride et al. (2009, 197296) found no excesses for all-cause mortality or mortality from diabetes or heart disease.

2.4.1.2.1.8.3.2. *Study evaluation.*

For the McBride et al. (2009, 198490) study, the size of the cohort is large enough to characterize mortality risks relative to the general population for most common causes of deaths. An important limitation of this study is the loss to follow-up of a substantial percentage of workers (22%). This would have impacted statistical power by reducing the number of deaths among the workers. If this incomplete ascertainment of mortality outcomes did not occur in a similar fashion with the general population then the SMR may also be biased.

For noncancer causes of death, the use of the SMR statistic is more likely to be influenced by the healthy-worker effect. Therefore, the findings obtained for these outcomes should be interpreted with caution. Subsequent analyses published by the same authors (McBride et al., 2009, 197296) provide improved characterization of TCDD exposure using serum samples.

2.4.1.2.1.8.3.3. *Suitability of data for dose-response analysis.*

Overall, no associations were evident between surrogate measures of TCDD (duration of employment, year of hire) and noncancer mortality outcomes. Further, the use of mortality endpoints is inconsistent with EPA RfD methodology. As such, these data do not support further use in a quantitative dose-response analysis.

2.4.1.2.1.8.4. McBride et al. (2009, 197296)—Noncancer mortality.

2.4.1.2.1.8.4.1. *Study summary.*

McBride et al. (2009, 197296) further analyzed the cohort of New Zealand workers to include estimates of TCDD exposure based on serum samples. Current and former employees who were still alive and living within 75 km of the site were asked to provide serum samples. Samples were collected from 346 workers representing 22% (346/1599) of the entire study population. These serum measures were used to estimate cumulative TCDD levels for all workers. The exposure assessment approach by Flesch-Janys et al. (1996, 197351) was used to estimate time-dependent exposures based on area under the curve models. This was based on a one-compartment first-order kinetic model with a half-life of 7.2 years.

Comparisons of mortality were made to the general population using the SMR statistic. The Cox proportional hazards model was used to conduct an internal cohort analysis across

four categories of cumulative TCDD levels for diabetes and ischemic heart disease mortality. The RRs generated from these models were adjusted for sex, hire year, and birth year. No diabetes deaths were observed among women, and therefore, analysis of this outcome was limited to men.

Relative to the general population, no difference in the all-cause mortality experience was observed in exposed cohort members (SMR = 1.0, 95% CI = 0.9−1.2). Similarly, no excess in these workers was observed for heart disease (SMR = 1.1, 95% CI = 0.9−1.5); cerebrovascular disease (SMR = 1.1, 95% CI = 0.6−1.9); diabetes (SMR = 0.7, 95% CI = 0.2−2.2); or nonmalignant respiratory disease (SMR = 0.8, 95% CI = 0.4−1.4). For the internal cohort analysis, the RR associated with cumulative categorical TCDD measure was 1.0 for both diabetes and ischemic heart disease.

2.4.1.2.1.8.4.2. *Study evaluation.*

The McBride et al. (2009, 197296) study extends the earlier work the same authors completed in two ways. First, serum measures were used to estimate cumulative TCDD with methodology that has been applied to several other cohorts of workers exposed to TCDD. Second, the authors used regression analyses that examined individual-level TCDD exposures in relation to various outcomes as part of the internal cohort comparisons. For noncancer outcomes, no dose-response associations with TCDD were observed with the internal comparisons. Also, as found with earlier analyses of this same cohort, no excess noncancer mortality relative to the New Zealand general population was observed.

Associations between TCDD and diabetes have been found previously in TCDD-exposed populations, most notably in the Ranch Hands cohort (Michalek and Pavuk, 2008, 199573). In this cohort, only five deaths from diabetes were identified, and of these, only three occurred among those who were exposed to TCDD. The study, therefore, has limited statistical power to characterize associations between TCDD and mortality from diabetes. Further, the identification of diabetes deaths is subject to misclassification errors due to under-reporting (McEwen and TRIAD, 2006, 594400).

2.4.1.2.1.8.4.3. *Suitability of data for TCDD dose-response modeling.*

McBride et al. (2009, 197296)) found no statistically significant associations in any of the noncancer causes of death. Furthermore, the use of mortality endpoints is inconsistent with EPA RfD methodology. Therefore, the data were not suitable for quantitative dose-response analysis for these outcomes.

2.4.1.2.2. ***Feasibility of dose-response modeling for noncancer.***

Relatively few study populations permit quantitative dose-response modeling to be performed for noncancer outcomes. The serum collected among Seveso men and women provide an opportunity to characterize risks for several health conditions in relation to TCDD exposure. The collection of these serum samples, shortly after the accident does not require the back-extrapolation of TCDD levels as in the occupational cohorts, which should reduce the exposure assessment uncertainty and minimize the potential for exposure misclassification.

An added feature of the SWHS is the detailed collection of other risk factor data from trained interviewers. These data allow for risk estimates to be adjusted for potential confounding variables. For the evaluations of reproductive health outcomes, this adjustment is critical given there are various documented risk factors for the different outcomes that were examined. For some health outcomes, continued follow-up of the cohort is needed, given that several of the Seveso studies suggest that those exposed at a very young age might be more susceptible to subsequent adverse health effects.

The findings of positive associations and dose-response relationships with serum-based measures of TCDD suggest several noncancer health outcomes could be associated with TCDD exposure. These health outcomes include neonatal thyroid function, sex ratio, diabetes, and semen quality. Although findings have suggested an association between TCDD and age at menopause, they were not statistically significant and no dose-response trend was observed. Weak or nonstatistically significant associations have been noted for endometriosis and menstrual cycle characteristics and do not support quantitative dose-response analyses.

Associations between TCDD exposure and cardiovascular disease have been noted in some, but not all, of the occupational cohorts, and also shortly after the accident among Seveso residents. Findings from the cohort studies based on external comparisons using the SMR statistic should be interpreted cautiously due to potential bias from the healthy worker effect.

Because the magnitude of the healthy worker bias is recognized to be larger for cardiovascular diseases than for cancer outcomes, risk estimates in some occupational cohorts might be underestimated for cardiovascular outcomes. Information on cardiovascular risk factors generally was not captured in these studies, and sensitivity analyses were generally designed to examine risk estimates generated for cancer outcomes.

2.4.1.2.3. *Summary of epidemiologic noncancer study evaluations for dose-response modeling.*

All epidemiologic noncancer studies summarized above were evaluated for suitability of quantitative dose-response assessment using the TCDD-specific considerations and study inclusion criteria. The results of this evaluation are summarized in a matrix style array (see Table 2-3) at the end of the chapter, and descriptively in Appendix B. The key epidemiologic noncancer studies suitable for further TCDD dose-response assessment are presented in Table 2-5.

2.4.2. Summary of Animal Bioassay Studies Included for TCDD Dose-Response Modeling

This section summarizes studies that have already met the in vivo animal bioassay TCDD study inclusion criteria (see Section 2.3.2). These studies are listed later in this section in Tables 2-6 and 2-7, for cancer and noncancer, respectively, and are considered in the dose-response modeling conducted later in this document (see Sections 4 and 5). The following sections are organized by reproductive studies, developmental studies, and general toxicity studies (subdivided by duration). They summarize the experimental protocol, the results, and the NOAELs and LOAELs EPA has identified for each study.

To evaluate and discuss studies consistently, doses were converted to nanograms per kilogram body weight per day (ng/kg-day) and were also adjusted for continuous exposure. Some doses were adjusted based on daily dietary intake and body weight. For these studies, EPA uses 10% of an animal's body weight as the daily feed rate. More commonly, doses were adjusted from 5 days/week to a 7 days/week standard adjustment, in which case administered doses were multiplied by 5 and divided by 7 to obtain continuous doses. To adjust for weekly dosing, the weekly administered doses were multiplied by the administration frequency per week (in days) and divided by 7 to give continuous doses.

Other exposure protocols used a single loading dose followed by weekly maintenance doses. To adjust these doses, the loading dose was added to the maintenance doses multiplied by the administration frequency, and this sum was divided by the exposure duration to give a continuous dosing rate. The doses administered in single dose studies were not averaged over the observation period.

2.4.2.1. *Reproductive Studies*

2.4.2.1.1. *Bowman et al. (1989, 543744; 1989, 543745) (and related Schantz and Bowman (1989, 198104); Schantz et al. (1986, 088206)).*

Female rhesus monkeys (6 to 10 years old; 8 per treatment) were exposed to 0 or 5 ppt (for 3.5 years), or 25 ppt (for 4 years) TCDD (purity not specified) (Bowman et al., 1989, 543744; Bowman et al., 1989, 543745; Schantz and Bowman, 1989, 198104; Schantz et al., 1986, 088206). Female monkeys were mated to unexposed males after 7 months (Cohort I) and 27 months (Cohort II) of exposure, then again 10 months postexposure (Cohort III). The average daily doses to mothers were equivalent to 0, 0.15, and 0.67 ng/kg-day. The 0.67 ng/kg-day dose group had reduced reproductive rates in both Cohorts I ($p < 0.001$) and II ($p < 0.025$; Bowman et al., 1989, 543744). The mean number of days of offspring survival ($p < 0.023$) also decreased. No effects on birth weight or growth, or physical evidence of toxicity (Bowman et al., 1989, 543745) were observed. Behavioral effects were observed in the offspring (Cohort I: 7, 6, and 0 offspring, respectively; Cohort II: 3, 5, and 0 offspring, respectively; Cohort III: 6, 7, and 3, respectively). In the 0.67 ng/kg-day dose group, the number of offspring was insufficient to form a group in either Cohorts I or II. Offspring in the 0.15 ng/kg-day dose group had alterations in social behavior of the mother-infant pairs (mothers had increased care giving, which appeared to be an effect of the infants and not due to the treatment of the mother) and peer group of the offspring after weaning (Cohort I offspring were more dominant or aggressive and exhibited more self-directed behavior; Bowman et al., 1989, 543745). The performance of learning tasks was inversely related to the level of TCDD in the body fat. Schantz and Bowman (1989, 198104) examined effects using discrimination-reversal learning (RL) and delayed spatial alteration (DSA). RL detected effects in the 0.15 ng/kg-day group as measured by retarded learning of the shape reversal ($p < 0.05$), but DSA did not. Schantz et al. (1986, 088206) combined the cohorts and looked at 5, 5, and 3 mother-infant pairs in the 0, 0.15, and

0.67 ng/kg-day groups, respectively. They found that TCDD-exposed mother-infant pairs spent more time in close, social contact compared to the controls (mutual ventral contact, $p < 0.025$; nipple contact, $p < 0.01$) and infants had reduced locomotor activity ($p < 0.05$), but the dose-effect was complex. Of note is that the control groups contained fewer males than did the TCDD-exposed groups.

In a follow-up study, Rier et al. (2001, 199843) examined the DLC levels of sera collected from some monkeys in this study. They reported that animals in this study had elevated serum PCB77 and PCB126 levels and an increased serum TEQ. In fact, the fractional contribution of serum TCDD levels to total serum TEQ was 30% in treated animals. In this study, it is not possible to determine the contribution of TCDD alone to the developmental effect due to the background contamination; thus, EPA has not developed a TCDD LOAEL from the study.

2.4.2.1.2. *Franc et al. (2001, 197353).*

To study the effects of subchronic, low-dose exposure to TCDD on the regulation and expression of the aryl hydrocarbon receptor (AhR), Franc et al. (2001, 197353) used rodent models with varying sensitivities to TCDD. Female Sprague-Dawley rats, inbred Long-Evans rats, and outbred Han/Wistar rats (8 per dose group) were dosed via oral gavage with 0, 140, 420, or 1,400 ng/kg TCDD (>99% purity) dissolved in corn oil once every 2 weeks for 22 weeks (0, 10, 30, and 100 ng/kg-day average daily doses). Animals were sacrificed 10 days after the final dosing. Body weights were recorded biweekly and just before sacrifice. After sacrifice, liver and thymus weights were determined. Liver tissue samples were removed and either frozen for RNA isolation followed by semiquantitative RT-PCR or homogenized and prepared for subcellular fraction analysis. Radioligand binding and immunoblotting techniques were used to measure AhR levels, and RT-PCR analysis was used to assess mRNA levels of AhR, aryl hydrocarbon nuclear receptor (ARNT), and CYP1A1.

Long-Evans rats exhibited significant ($p < 0.001$) decreased weight gain over time as compared to Sprague-Dawley and Han/Wistar rats as determined by repeated measures analysis of variance (ANOVA). Because body weight gain varied indirectly with TCDD exposure, liver and thymus tissue weights were normalized to body weight for data analysis. TCDD exposure led to a significant ($p < 0.05$) increase in relative liver weights at all three TCDD doses and in all

three rat strains, compared to the control groups. At the upper end of the TCDD dose range, Sprague-Dawley rats dosed with 100 ng/kg-day showed the greatest increase in relative liver weights (160% of the control values), while relative liver weights in Long-Evans and Han/Wistar rats were similar to each other, and also were elevated above control values by 10–20%. At the 30 and 100 ng/kg-day doses, the relative thymus weights were significantly lower ($p < 0.05$) in all rat strains compared to their corresponding controls, but the 10 ng/kg-day dose did not produce a statistically significant effect in any strain. However, absolute thymus weight was higher at all doses in Han/Wistar rats, which also had a higher control thymus weight.

Supporting observed differences in baseline TCDD sensitivity among the rat strains, liver AhR levels in the control groups as measured by radioligand binding were similar for Sprague Dawley and Han/Wistar rats, but were approximately two-fold higher for Long-Evans rats. A significant ($p < 0.05$) two-fold, dose-dependent increase in radioligand binding of liver AhR was observed at all TCDD doses relative to the control in Sprague-Dawley rats. At the 30 ng/kg-day dose, the AhR level for Long-Evans rats was significantly ($p < 0.05$) increased to approximately 250% of the control level.

AhR protein levels measured in the liver cytosol by immunoblotting were highest in the 10 and 30 ng/kg-day TCDD dose groups for all three rat strains. Significant ($p < 0.05$) increases in AhR levels were observed in the Sprague-Dawley rats that received 30 ng/kg-day, and in Long-Evans rats that received either 10 or 30 ng/kg-day. A significant ($p < 0.05$) decrease in AhR protein level was observed only at the 100 ng/kg-day dose in Han/Wistar rats. Liver AhR protein was not detectable by immunoblotting in nuclear extracts for any strain or dose. The study authors assert that AhR levels measured in cytosol correspond to measures in whole-tissue lysates as demonstrated in their previous work.

Based on RT-PCR analysis, all three rat strains showed similar responses in liver AhR mRNA following TCDD exposure. Liver AhR mRNA levels increased significantly ($p < 0.05$) as compared to control levels in all rat strains at 10 and 30 ng/kg-day and in Long-Evans rats at 100 ng/kg-day. The study authors observed that statistically significant increases in AhR mRNA levels in the liver were not always associated with statistically significant increases in AhR levels for a given strain and dose, but that the opposite (increases in AhR levels associated with increases in AhR mRNA levels) was always true. Changes in liver ARNT mRNA levels tended to increase with increasing TCDD dose, and the increases were significant ($p < 0.05$) in the

30 ng/kg-day dose groups of Long-Evans and Han/Wistar rats. At the 100 ng/kg-day TCDD dose, all rat strains showed a decrease in ARNT mRNA in the liver relative to controls with significant ($p < 0.05$) differences for the 100 ng/kg-day TCDD dose groups of Sprague-Dawley and Han/Wistar rats. Liver CYP1A1 mRNA induction was not detectable in control animals. A significant ($p < 0.05$) increase in liver CYP1A1 mRNA was observed in all rat strains administered 10 or 30 ng/kg-day TCDD. Liver CYP1A1 mRNA levels also were significantly ($p < 0.05$) elevated above controls in the 100 ng/kg-day groups although not to the same extent as in the 30 ng/kg-day groups. For all rat strains, the largest up-regulation for AhR and ARNT mRNA levels occurred in the 30 ng/kg-day TCDD dose groups.

The NOAEL for TCDD identified in this study is 10 ng/kg-day TCDD. At 10 ng/kg-day TCDD, the change in relative liver weight, while significantly ($p < 0.05$) increased in Sprague-Dawley rats, was determined (from Figure 5 in Franc et al., 2001, 197353) to be less than 10% and judged by EPA not to be biologically relevant. Also, at 10 ng/kg-day TCDD, the change in relative thymus weight, was not statistically significantly decreased in Sprague-Dawley, Han-Wistar or Long-Evans rats. The study LOAEL is 30 ng/kg-day, based on statistically and biologically significant increases in relative liver weight in Sprague-Dawley and Long-Evans rats and statistically and biologically significant decreases in relative thymus weight in Sprague-Dawley, Han-Wistar and Long-Evans rats.

2.4.2.1.3. *Hochstein et al. (2001, 197544).*

Adult female mink (12/treatment group) were administered dietary concentrations of 0.0006 (control), 0.016, 0.053, 0.180, or 1.40 ppb TCDD (purity >99.8%) for 132 days (Hochstein et al., 2001, 197544). This dose is estimated to be equivalent to 0.03 (control), 0.8, 2.65, 9, and 70 ng/kg-day assuming a food consumption of 5% of body weight per day. Females were mated with unexposed males beginning on treatment day 35. Females were allowed to mate every fourth day during a 29-day mating period or until a confirmed mating. Mated females were presented with a second male either the day after initial mating or 8 days later. In the 70 ng/kg-day group, the treated animals were lethargic after 4 to 5 weeks, with several having bloody (tarry) stools near the end of the trial. Two animals in the 70 ng/kg-day dose group died prior to study termination. These animals had lost a large percentage of their body weight (24−43%), and had pale yellow livers and intestinal hemorrhages. Histopathology from

both mink indicated marked diffuse hepatocellular vacuolation. The mean body weight decreased in all treatment groups including the control (losing an average of 3.29% of initial body weight), compared to a dose-dependent loss of up to 26% in the 70 ng/kg-day group. Mating and reproduction were considered subnormal in all groups. The number of females that gave birth in the 0.03 (control), 0.8, 2.65, 9, and 70 ng/kg-day dose groups were 5/12, 0/12, 3/12, 8/12, and 0/11, respectively. The study authors speculated that the subnormal breeding and reproductive performances in the control females likely were due to the indoor environment in which the mink were housed. In the three groups that gave birth, there was a dose-dependent decrease in kit body weight at birth, which was significant ($p < 0.05$) in the 9 mg/kg-day group compared to the controls. The body weight in the kits was not significantly different at 3 or 6 weeks after birth. Three-week survival rates of 71, 47, and 11% were recorded for kits in the 0.03 (control), 2.65, and 9 ng/kg-day dose groups, respectively. Six-week kit survival rates were 62, 29, and 11% in the 0.03 (control), 2.65, and 9 ng/kg-day dose groups, respectively.

In the adult females, clinical signs of toxicity were noted in the 70 ng/kg-day group near the end of the study and included alopecia and notably thickened, deformed, and elongated toenails. There was a dose-dependent decrease in plasma total solids, total protein, and osmolality that reached statistical significance ($p < 0.05$) in the two highest exposure groups. Anion gap was significantly decreased ($p < 0.05$) and alanine aminotranferase was significantly increased in the 70 ng/kg-day group compared to the controls. At terminal sacrifice, there was a dose-related decrease in body weight. There was a dose-related increase in liver weight that reached statistical significance ($p < 0.05$) in the 70 ng/kg-day dose group. The brains of 42% of the animals in the 70 ng/kg-day dose group had localized accumulation of lymphatic cells within the meninges with mild extension into the adjacent neuropil and mild gliosis. Of the 10 mink surviving to study termination in the 70 ng/kg-day group, 3 had periportal hepatocellular vacuolation. These same brain and liver lesions were not observed in the control mink.

As there were no litters produced in the low-dose group and pregnancy outcomes were not dose related, the 0.8 ng/kg-day exposure level does not inform the choice of NOAEL or LOAEL. Thus, the LOAEL for this study is 2.65 ng/kg-day (132-day maternal exposure duration) based on reduced kit survival (47% of control at 6 weeks). A NOAEL cannot be determined for this study.

2.4.2.1.4. *Hutt et al. (2008, 198268).*

Hutt et al. (2008, 198268) conducted a 3-month study investigating changes in morphology and morphogenesis of pre-implantation embryos as a result of chronic exposure to TCDD in female rats. The study authors administered 0 or 50 ng/kg TCDD (>99% purity) in corn oil via oral gavage to groups of 3 pregnant Sprague-Dawley rats on gestation days 14 and 21 and on postnatal days 7 and 14. The resulting female pups were divided into groups of 3 and administered 0 or 50 ng/kg TCDD (>99% purity) in corn oil (equivalent TCDD doses of 0 and 7.14 ng/kg-day) on postnatal day 21 and weekly thereafter until they reached 3 months of age. Pups were then mated, fertilization was verified, and pre-implantation embryos were harvested 4.5 days later. Pre-implantation embryos were examined using immunofluorescence microscopy to determine blastomere abnormalities.

No significant difference as compared to the control in pre-implantation embryotoxicity was observed following exposure to TCDD. Morphologically normal pre-implantation embryos were significantly ($p < 0.05$) reduced in 50 ng/kg TCDD exposed rats (15 of 41, 36.6%) compared to the control group (31 of 39, 79.5%). Pre-implantation embryos of TCDD-exposed rats included irregularities in mitotic spindles (13 of 18 were monopolar), chromosome patterns in metaphase, blastomere size and shape, blastomere nuclei shape in interphase, f-actin, and cytokinesis. The study authors concluded that the compaction stage of pre-implantation embryogenesis is the most sensitive following exposure to TCDD.

A LOAEL for this study is 50 ng/kg (7.14 ng/kg-day adjusted dose) for a significantly ($p < 0.05$) lower proportion of morphologically normal pre-implantation embryos during compaction stage in female Sprague-Dawley pups weekly for 3 months. A NOAEL cannot be determined for this study.

2.4.2.1.5. *Ikeda et al. (2005, 197834).*

Ikeda et al. (2005, 197834) studied the effect of repeated TCDD exposure to F0 dams on the male gonads of F1 generation and sex ratio in the F2 generation. Twelve female Holtzman rats were treated with a single dose of 400 ng/kg TCDD (≥98% purity) orally, via gavage, followed by weekly treatment doses of 80 ng/kg TCDD (16.5 ng/kg-day adjusted for continuous exposure of 10 weeks; specified 2 weeks premating, assumed 1 week for successful mating, 3 weeks of gestation, and specified 4 weeks to weaning) during mating, pregnancy, and

lactational periods (total exposure duration approximately 10 weeks). Corn oil served as the control in another group of 12 dams. Four dams were sacrificed on gestation day (GD) 20 to evaluate the in utero toxicity of TCDD. Litter sizes from the remaining eight dams were examined on postnatal day (PND) 2, and some of the F1 offspring were sacrificed to estimate TCDD tissue concentrations. The remaining offspring were weaned on PND 28. Some of the F1 (number not specified) offspring were mated with untreated females on PND 98, following which, litter size, sex ratio, weight, and anogenital distance of F2 pups were examined on PND 2. Mated and unmated F1 males were sacrificed and the testes, epididymis, seminal vesicle, and the ventral prostate were weighed; the cauda epididymis was weighed and examined for sperm count.

All fetuses in the control and TCDD group as a result of in utero exposure in the F0 generation survived. Litter size, sex ratio, and anogenital distance in the F1 generation on PND 2 were not altered as a result of in utero TCDD exposure. Pup weight was significantly ($p < 0.05$) lower in the TCDD-treated group than in controls. TCDD concentration in the adipose tissue of the F0 dams on GD 20 was significantly ($p < 0.05$) higher than in the liver. Adipose TCDD was significantly ($p < 0.01$) reduced at weaning, however, compared to concentrations on GD 20. F1 pup liver TCDD concentration increased significantly ($p < 0.01$) and was higher on PND 28 than PND2. The liver weight in F1 males increased by 14-fold at PND 28 compared to PND 2, implying a transfer of approximately 850 pg of TCDD from the dam to the F1 pup livers during lactation. TCDD also was detected in pup adipose tissue on PND 28. Body weight of TCDD-exposed F1 males was significantly ($p < 0.001$) lower than control males at weaning (PND 28). No significant differences in testis and cauda epididymis weights were observed between the control and treated groups. Ventral prostate weight in the F1 males exposed to TCDD, however, was approximately 60% lower than controls. No change in weight of the body, brain, testes, cauda epididymis, or seminal vesicle was observed at PND 120. Ventral prostate weight, however, was 16% lower than that of the control group ($p < 0.001$). Sperm count in the cauda epididymis of the F1 males was not affected by TCDD exposure.

Examination of F2 generation litters indicated no significant differences in litter size, pup body weight, and anogenital distance between TCDD-treated or vehicle control groups. The percentage of male F2 pups born to maternally and lactationally TCDD-exposed males was

significantly ($p < 0.05$) lower (38%) than those sired by control group males (52%). Every female mated with maternally TCDD-exposed F1 males delivered more female than male pups.

A LOAEL for TCDD of 16.5 ng/kg-day for an estimated 10 week exposure duration in F0 rat dams is identified in this study for decreased development of the ventral prostate in the F1 generation (60% lower than controls) and for significantly ($p < 0.05$) altered sex ratio (decreased percentage of males) in the F2 generation. A NOAEL cannot be determined for this study.

2.4.2.1.6. *Ishihara et al. (2007, 197677).*

Ishihara et al. (2007, 197677) examined the effect of repeated TCDD exposure of F0 males on the sex ratio of F1 offspring. Seven-week-old male ICR mice ($n = 127$) were divided into three groups and treated via gastric intubation with an initial loading dose of either 2 or 2,000 ng TCDD/kg BW or an equivalent volume of sesame oil (vehicle) as control, followed by a weekly maintenance doses of 0, 0.4, or 400 ng/kg until the animals were 12 weeks old. One week after the last exposure, the animals were mated with untreated female mice. On the day a vaginal plug was identified, F0 male mice were sacrificed and major organs including testes, epididymis, and liver were removed and weighed. Organ tissues also were examined for histopathological and immunohistochemical changes. Treatment levels, averaged over the 6 week period from start of treatment to mating (five maintenance doses), were 0, 0.095, and 950 ng/kg-day for the control, low dose and high dose groups, respectively.

All TCDD-treated males successfully impregnated untreated females and yielded viable offspring. Mortality, pup weights, and mating and fertility indices were not affected by TCDD exposure. There were no significant differences in body weights or in relative weights of testes, epididymis, or livers in the TCDD-treated F0 males compared to the control group. The livers of some animals (number not specified) in the high-dose group, however, were larger and heavier than in the controls or the low-dose group. Hence, tissues from the high-dose animals were selected for detailed immunohistochemical examination.

General histopathological findings in the TCDD-treated groups showed no changes in cell morphology in germ, Sertoli, and Leydig cells of the testes. Arrangement of the germ cells was normal and there was no difference in the epididymis spermatozoon number in either of the TCDD-treated groups compared to controls. Livers of some of the animals in the high-dose

group however, showed enlarged and vacuolated areas in the centrilobular area when compared to the low-dose group and the control group. Immunohistochemical and quantitative immunohistological findings showed a marked increase in staining intensity for cytochrome P450 (CYP)1A1 in the cytoplasm of the hepatocytes in the centrilobular area of the high-dose TCDD group compared to the cells in the low-dose and the control groups. In addition, proportions of immunoreactive CYP1A1 areas in the liver sections of the high-dose group were higher than in the low-dose and control groups. The proportions of immunoreactive CYP1A1 also varied across animals (n = 33) in the high-dose group.

In addition to the above findings, there was a dose-related decrease in the male/female sex ratio. The proportion of male offspring of the high-dose group was significantly lower ($p < 0.05$) than that observed in controls (46.2% versus 53.1%, respectively). Hepatic immunoreactive CYP1A1 staining levels in individual F0 males were strongly correlated with the sex ratio of their offspring.

A LOAEL for TCDD of 950 ng/kg-day for a 6 week exposure duration of F0 male mice is identified for significantly ($p < 0.05$) decreased male/female sex ratio (i.e., higher proportion of female offspring) in the F1 generation. The NOAEL is 0.095 ng/kg-day.

2.4.2.1.7. *Latchoumycandane and Mathur (2002, 197498) (and related: Latchoumycandane et al. (2002, 198365; 2002, 197839; 2003, 543746)).*

Latchoumycandane and Mathur (2002, 197498) conducted a study to determine whether treatment with vitamin E protected rat testes from TCDD-induced oxidative stress. Groups of albino male Wistar rats (n = 6) were administered an oral dose of 0 (vehicle alone) 1, 10, or 100 ng TCDD/kg-day for 45 days, while another group of animals (n = 6) was co-administered TCDD at the same doses, along with vitamin E at a therapeutic dose of 20 mg/kg-day for 45 days. At study termination, animals were fasted overnight, weighed, and sacrificed. Testis, epididymis, seminal vesicles, and ventral prostate were removed, weighed, and preserved for further examination. The left testis was used to determine daily sperm production, while the right testis was used for biochemical studies. Superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase activity were measured in the testes, along with production of hydrogen peroxide and lipid peroxidation.

Body weights of TCDD-treated rats did not differ significantly from the control group. Testis, epididymis, seminal vesicle, and ventral prostate weights in the TCDD-treated groups, however, decreased significantly ($p < 0.05$) when compared to controls. None of these changes were observed in the TCDD-exposed groups receiving vitamin E. There was a dose-related decrease in daily sperm production ($p < 0.05$) in all three TCDD-treated groups when compared to the control group. In contrast, the TCDD treatment groups that also received vitamin E did not show any significant changes in daily sperm production compared to the controls. The TCDD-treated groups also showed significantly ($p < 0.05$) lower activities of the antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase) than the control group. Levels of hydrogen peroxide and lipid peroxidation increased significantly ($p < 0.05$) in the testes of the rats treated with TCDD compared to the corresponding controls. The TCDD-treated groups that had been co-administered vitamin E show no difference in antioxidant enzyme activities or in reactive oxygen species production when compared with controls.

A LOAEL for TCDD of 1.0 ng/kg-day for a 45-day exposure duration in rats is identified in this study for significantly ($p < 0.05$) reduced sperm production and significantly ($p < 0.05$) decreased reproductive organ weights. A NOAEL cannot be determined for this study.

2.4.2.1.8. ***Murray et al. (1979, 197983).***

Male (10−16 per treatment) and female (20−32 per treatment) Sprague-Dawley rats were administered diets containing TCDD (purity >99%) to achieve daily concentrations of 1, 10, or 100 ng/kg-day through three generations. After 90 days of treatment, F0 rats were mated to produce F1a offspring. Thirty-three days after weaning of the last F1a litter, the F0 rats were mated again to produce F1b offspring. Some F0 rats were mated a third time for a cross-mating study. The F1b and F2 rats were mated at about 130 days of age to produce the F2 and F3 generations. No clinical signs of toxicity or changes in body weight and food consumption were observed in F0 rats during the 90 days of treatment before mating. The 100 ng/kg-day group was discontinued due to the lack of offspring. In the three surviving offspring (all males), no changes in appearance, body weight, or food consumption occurred. A dose of 10 ng/kg-day caused a consistent decreased body weight in both sexes of F1 and F2 rats, which was associated with decreased food consumption. A significant ($p < 0.05$) decrease in fertility in F1 and F2 rats

occurred, but not in F0 rats, administered 10 ng/kg-day. The number of live pups and gestational survival index were significantly ($p < 0.05$) decreased in the 100 ng/kg-day F0 rats and in the 10 ng/kg-day F1 and F2 rats. The gestational survival index also was significantly ($p < 0.05$) decreased in F2 rats administered 1 ng/kg-day. Postnatal survival was significantly ($p < 0.05$) reduced only in F2 rats administered 10 ng/kg-day. Growth (as measured by body weight) was affected at 10 ng/kg-day only in the third generation. In the 10 ng/kg-day group, a significant ($p < 0.05$) decrease in relative thymus weight and increase in liver weight also occurred in F_3 rats (weights were not measured in F2 rats). Additionally, mating 100 ng/kg-day TCDD-treated females with untreated males increased the percent of implants resorbed as assessed by uterine histopathology.

The reproductive LOAEL is 10 ng/kg-day, based on a significant ($p < 0.05$) decrease in fertility (33−37% lower than controls); decrease in the number of live pups (18−27% lower than controls); decrease in gestational survival (10−11% lower than controls); decrease in postnatal survival (32% lower than controls); and decreased postnatal body weight (14−19% lower than controls at weaning) in one or more generations. The reproductive NOAEL is 1 ng/kg-day.

2.4.2.1.9. ***Rier et al. (1993, 199987; 1995, 198566).***

Reir et al. (1993, 199987; 1995, 198566) examined the impact of chronic TCDD exposure on endometriosis in monkeys. Female rhesus monkeys (eight animals per treatment group) were exposed to 0, 5, or 25 ppt TCDD (purity not specified) in feed for 4 years. Previously, Bowman et al. (1989, 543745) determined that these dietary concentrations were equivalent to 0, 0.15, and 0.67 ng/kg-day, respectively. Ten years after termination of TCDD treatment, the presence of endometriosis was determined via laparoscopic surgical procedure, and the severity of the disease was assessed. The study authors reported that three monkeys in the 0.67 ng/kg-day exposure group died at 7, 9, and 10 years after termination of TCDD treatment. Autopsy results attributed the deaths to widespread and severe peritoneal endometriosis (all three monkeys) along with obstruction of the colon (one monkey) and blockage of the jejunum (one monkey). Other deaths also occurred in the control group (1 death from birthing complications and another from an unknown cause); in the 0.15 ng/kg-day dose group (1 death due to natural causes with no endometriosis), and in the 0.67 ng/kg-day dose group (1 death due to a breeding fight with no incidence of endometriosis). At study

termination, 17 live animals plus the 3 that had previously died of endometriosis were evaluated (total $n = 20$).

Incidence of endometriosis was significantly ($p < 0.05$) higher than in the control group with 71 and 86 % incidence rates in the 0.15 and 0.67 ng/kg-day dose groups, respectively, compared to 33% in the control group. Severity of endometriosis was also significantly ($p < 0.001$) correlated with TCDD dose. Staging by rAFS indicated that untreated control animals had either minimal or no incidence of endometriosis. In comparison, endometriosis was absent in 2 of the 7 monkeys in the 0.15 ng/kg-day dose group, while only 1 of the 7 animals in the high dose group was disease free. Moderate-to-severe disease was observed in 3 of the 7 animals in the 0.15 ng/kg-day dose group and 5 of the 7 animals in the 0.67 ng/kg-day dose group. Moderate-to-severe disease was not observed in the control group. The authors also compared the incidence and severity of endometriosis in TCDD-exposed animals with 304 normal, non-neutered females with no dioxin exposure and reported that the disease was not present in monkeys that were less than 13 years of age, while the disease rate was 30% among animals 13 years of age or older. The study authors report that these findings are in agreement with human and rhesus studies demonstrating that the prevalence of detectable endometriosis can increase with advanced age.

As noted previously, in a follow-up study, Rier et al. (2001, 198776) examined the DLC levels of sera collected from some monkeys in this study. They reported that animals in this study had elevated serum PCB77 and PCB126 levels and an increased serum TEQ; the fractional contribution of serum TCDD levels to total serum TEQ was 30% in treated animals. They also reported that the severity of the endometriosis corresponded to the serum PCB77 concentrations rather than total TCDD. In this study, it is not possible to determine the contribution of TCDD alone to the endometriosis due to the background contamination; thus, EPA has not developed a TCDD LOAEL from the study.

2.4.2.1.10. *Shi et al. (2007, 198147).*

Pregnant Sprague-Dawley rat dams (3 per treatment group) were administered 0, 1, 5, 50, or 200 ng/kg TCDD (purity >99%) in corn oil by gavage on GD 14 and GD 21 and on PND 7 and PND 14 for lactational exposure to pups (Shi et al., 2007, 198147). Ten female pups per treatment were selected and administered TCDD weekly at the same dose levels through their

reproductive lifespan (approximately 11 months). The corresponding equivalent daily TCDD doses are 0, 0.14, 0.71, 7.14, and 28.6 ng/kg-day. Vaginal opening was slightly but significantly ($p < 0.05$) delayed in 28.6 ng/kg-day females. Vaginal opening was also delayed, but not significantly, in the 0.14 and 7.14 ng/kg-day groups. Reproductive senescence with normal cyclicity was significantly ($p < 0.05$) accelerated beginning at 9 months in 7.14 and 28.6 ng/kg-day females. Serum estradiol concentrations were decreased at all time points across the estrous cycle in a dose-dependent manner with a statistically significant decrease ($p < 0.05$) in all but the lowest dose group. TCDD exposure, however, did not affect the number or size distribution of ovarian follicles; responsiveness of the pituitary gland to gonadotropin-releasing hormone, or serum profiles of FSH, LH, or progesterone.

A LOAEL for TCDD of 0.71 ng/kg-day for an 11-month exposure duration was identified in this study based on significantly ($p < 0.05$) decreased estradiol levels in offspring. The NOAEL for this study is 0.14 ng/kg-day.

2.4.2.1.11. ***Yang et al. (2000, 198590).***

Yang et al. (2000, 198590) studied the impact of TCDD exposure on the incidence and severity of endometriosis in female rhesus monkeys. Groups of 7- to 10-year old nulliparous cynomolgus monkeys were treated with 0 ($n = 5$), 1, 5, or 25 ($n = 6$ per group) ng/kg BW TCDD 5 days per week via gelatin capsules for 12 months. Because the monkeys received one capsule 5 days per week, the doses adjusted for continuous exposure were 0, 0.71, 3.57, and 17.86 ng/kg-day. Prior to TCDD administration, all animals had endometriosis induced during days 12–14 of the menstrual cycle by auto-transplantation of endometrial-strips in multiple abdominal sites. All TCDD-treated and control groups were laparoscopically examined during months 1, 3, and 6 to monitor the survival of endometrial implantations and to obtain peritoneal fluid to determine the concentration and immunotype of endometrial growth regulator cytokines interleukin-6 (IL-6) and interleukin-6 soluble receptor (IL-6sR). Because insufficient peritoneal fluids were present in the treated and control monkeys, however, the study authors collected blood samples at 6 and 12 months during laparoscopy for routine hematology and to assess the circulating levels of IL-6 and IL-6sR. All animals were sacrificed at 12 months, and circulating levels of gonadal steroids also were measured at the time of necropsy.

No changes were observed among treatment levels in general toxicological endpoints such as body weight changes, food consumption, hematological endpoints, general activity levels, and caretaker interaction. In addition, TCDD did not impact circulating levels of gonadal steroids measured during necropsy. Similarly, there were no differences in the number of menstrual cycles, the length of the menstrual cycle, and bleeding intervals. Endometrial implants were found in at least one site in all TCDD-treated and control monkeys during the first laparoscopic examination. Follow-up laparoscopies revealed that there was a continuous loss of endometrial implants over time in each dose group. At the 1-, 3-, and 6-month examination, the number of endometrial losses was not significantly different among different dose groups. At the 12-month examination, however, a significantly ($p < 0.05$) higher rate of survival of endometrial implants was observed in the 3.57 and 17.86 ng/kg-day dose groups compared to the control group. The highest rate of endometrial implant survival was observed in the ovaries regardless of the dose group. In contrast, all lesions disappeared from the left broad ligament, whereas two on the right broad ligament and one on the uterine fundus survived. There was a dose-dependent divergence in the growth response of endometrial implants following TCDD exposure. Both the maximum and minimum implant diameters in the 17.86 ng/kg-day dose group were significantly ($p < 0.05$) larger compared to controls. In contrast, the maximum and minimum implant diameters in the 0.71 ng/kg-day dose group were significantly ($p < 0.05$) smaller compared to controls. TCDD did not impact implant diameters in the 3.57 ng/kg-day dose group when compared to controls. Histological examinations revealed that endometrial glands and stromal cells were present in all surviving implants. Sections examined in the 17.86 ng/kg-day of TCDD possessed cystic endometrial glands that were more frequently observed in this dose group compared to other groups including controls. In addition, circulating levels of IL-6 were significantly ($p < 0.05$) lower in monkeys exposed to 17.86 ng/kg-day TCDD both at 6 and 12 months compared to the control group. In contrast, circulating levels of IL-6sR were significantly ($p < 0.05$) higher in animals treated with 3.57 and 17.86 ng/kg-day TCDD at 6 months, while the levels were higher only in the 17.86 ng/kg-day TCDD group at 12 months.

A LOAEL for TCDD of 17.86 ng/kg-day for a 1 year exposure duration was identified in this study for significantly ($p < 0.05$) increased endometriosis induced by endometrial implant survival, significantly ($p < 0.05$) increased maximum and minimum implant diameters, and

growth regulatory cytokine dysregulation (as assessed by significantly decreased IL-6 levels, $p < 0.05$). A NOAEL of 3.57 ng/kg-day is identified in this study.

2.4.2.2. *Developmental Studies*

2.4.2.2.1. *Amin et al. (2000, 197169).*

Amin et al. (2000, 197169) studied the impact of in-utero TCDD exposure on the reproductive behavior in male pups. Groups of pregnant Harlan Sprague-Dawley rats ($n = 108$ divided into 4 cohorts; number of animals in the TCDD treatment group is ~3 per dose group) were dosed via gavage with 0, 25, or 100 ng/kg-day TCDD (purity >98%) in corn oil on GDs 10−16. On the day of birth (PND 0), pups were examined for gross abnormalities and the number of live pups, their weights, and sex were recorded from each litter. Litters consisting of more than eight pups were reduced to eight, comprised of four males and four females when possible. Litters consisting of fewer than five pups were excluded from the study to minimize between-litter differences in growth rate, maternal behavior, and lactational exposure. After this exclusion, approximately 10 to 11 litters per exposure group remained. All pups were weaned on day 21 and one male and one female were retained to assess reproductive development, play behavior, reproductive behavior, and saccharin preference behavior. Both male and female pups were tested for saccharin preference between 189 and 234 days of age. A saccharin preference test was conducted for 8 days. For the first 4 days, rats were provided bottles containing tap water, and on days 5 and 6 the animals were provided a bottle containing water and a bottle containing 0.25% saccharin solution. On days 7 and 8, the animals were provided water and a bottle containing 0.50% of saccharin solution. A 0.50% saccharin solution was used because previous studies have reported that male rats exhibited a greater reduction in preference for this saccharin concentration compared to females, hence the sex difference in preference is more marked at this saccharine dose.

None of the treated dams exhibited any signs of toxicity as a result of exposure to TCDD. Gestational body weight, liver weight, litter size and percent live births were all comparable to the corresponding control group. Birth rate and weaning weight of the pups also were not affected by TCDD exposure. Sex-related water consumption, however, was significantly ($p < 0.001$) affected during the first 4 days with female pups drinking more water per 100 g of body weight compared to the respective male counterparts. Saccharin consumption was

significantly ($p < 0.001$) affected, with females consuming greater amounts of saccharin solution per 100 g body weight compared to the corresponding males. Additionally, both male and female pups drank significantly ($p < 0.001$) more of the 0.25% saccharin solution compared to the 0.50% saccharin solution. Females of all exposure groups consumed less of both the 0.25 and 0.50% saccharin solution compared to the same-sex control group. Comparisons of each exposure group to the control group indicated that only the high TCDD exposure group (100 ng/kg-day) different significantly ($p < 0.05$) compared to control in the consumption of 0.25% saccharin solution. In contrast, for the 0.50% saccharin solution, both the low and high TCDD dose groups differed significantly ($p < 0.05$ and $p < 0.01$, respectively) compared to the control group. The saccharin preference of TCDD-exposed male rats did not differ from that of the male control group. The TCDD-exposed females' preference for saccharin solution, however, was significantly reduced in both the 25 ($p < 0.05$) and the 100 ng/kg-day ($p < 0.005$) dose group compared to that of the female controls. The study authors state that the reduction in saccharin consumption and preference in females could be due to the anti-estrogenic action of TCDD and that recent research reports suggest that TCDD can decrease the level of estrogen receptor (ER) mRNA by blocking the ability of ER to transactivate from the estrogen response element.

A LOAEL for TCDD of 25 ng/kg-day for 7 days of gestational exposure is identified for significantly ($p < 0.05$) decreased preference in the consumption of 0.25% saccharin solution. A NOAEL cannot be determined for this study.

2.4.2.2.2. *Bell et al. (2007, 197041).*

Bell et al. (2007, 197041) examined the reproductive effects of TCDD in rats exposed during development. Female CRL:WI (Han) rats were treated with TCDD (99% purity; dissolved in acetone) in the diet at concentrations of 0 (acetone alone; $n = 75$), 28, 93, or 530 ($n = 65$/group) ng TCDD/kg diet, which provided average doses of 0, 2.4, 8, or 46 ng/kg-day, respectively. Rats were exposed to TCDD 12 weeks prior to mating, during mating, and through pregnancy. Dams were switched to the control diet after parturition. Litters from pregnant dams were reduced to a maximum size of eight on PND 4 and to five males (if possible) on PND 21. These males were left untreated until sacrificed (25/group, one/litter) on PND 70, while all remaining animals were sacrificed on PND 120. All sacrificed animals were

necropsied and received a seminology examination. Prior to sacrifice, during weeks 12 and 13, 20 animals from each dose group were tested for learning ability and motor activity, and were also administered a functional observation battery. During postnatal week 16, groups of 20 male F1 rats from each treatment group were paired with untreated virgin females for 7 days, and mated females were killed on GD 16 and examined for terminal body weights, pregnancy status, number of corpora lutea, and number of intrauterine implantations.

The study authors found no evidence of direct maternal toxicity from exposure to TCDD. In the high-dose groups, 8 of 27 dams suffered complete litter loss compared to 3 dams in the control group, but the difference was not statistically significant. Pup survival at PND 4 was also lower in the high-dose group, but the difference again was not statistically significant.

A dose-related decrease in mean pup body weight was observed on PND 1, and this trend continued throughout the lactation period. High-dose male pups had lower body weights when compared to controls at PND 21, with this trend continuing over the course of the study. Balanopreputial separation (BPS) was significantly ($p < 0.05$) delayed compared to controls in all three treatment groups by 1.8, 1.9, and 4.4 days in the low-, medium-, and high-dose groups, respectively. The study authors reported that adjustment for lower body weights observed at PND 21 and PND 42 did not affect the estimate of delay in BPS. No adverse effects from maternal treatment were observed on learning or in functional observational battery performance. Offspring in the high-dose group exhibited less activity when compared to controls ($p < 0.05$) when they were subjected to a test of motor activity for 30 minutes.

The median precoital time was 2−3 days for all 20 F1 males that were mated during postnatal week 16. The uterine and implantation data were similar in all dose groups and there were no significant differences in the proportion of male offspring between groups. Epididymal sperm counts and sperm motility did not differ significantly between dose groups in animals sacrificed during postnatal week 10. The mean number of spermatids was significantly lower (14%; $p < 0.05$) and the proportion of abnormal sperm was significantly ($p < 0.05$) higher in the high-dose group when compared to controls on PND 70. These effects, however, were not seen in animals sacrificed on PND 120.

Terminal body weights were significantly ($p < 0.05$) decreased in the high-dose group (6.9 %) compared to controls on PND 120, while the depression in body weight in the medium-dose group (5.5%) was not statistically significant. At PND 70, the relative and

absolute testis weight of the high-dose group was less than the controls (12 and 18%, respectively). Absolute spleen weight in the high-dose group was significantly higher (8%) on PND 70, and increased significantly ($p < 0.05$) by 1−3% on PND 120 in all dose groups compared to controls. Kidney weight in the low and medium-dose groups was significantly ($p < 0.05$) greater than in controls (~2%) at PND 120. In addition to these organs, ventral prostate (9.4%) and relative liver (~4.5%) weights were significantly ($p < 0.05$) higher than controls on PND 120 in the medium- and low- and high-dose groups, respectively. On PND 120, absolute brain weight was significantly ($p < 0.05$) less than the control in the medium-dose group, while relative brain weight was significantly ($p < 0.05$) higher than the control in the low- and high-dose group. Histological examination revealed no unusual findings.

A LOAEL for TCDD of 2.4 ng/kg-day following an estimated 17 week exposure duration of dams was identified in this study for significantly ($p < 0.05$) delayed BPS. A NOAEL was not identified in this study.

2.4.2.2.3. *Franczak et al. (2006, 197354).*

Franczak et al. (2006, 197354) examined the impact of chronic TCDD exposure on the onset of reproductive senescence in female rats. Pregnant Sprague-Dawley rats (n = 2-3/dose group) were fed 50 or 200 ng/kg TCDD (>99% purity) or corn oil vehicle (4 mL/kg) orally on GD 14 and 21 and PND 7 and 14 to provide in utero and lactational exposure to TCDD. On PND 21, female pups (n = 7/dose group) were weaned and were subsequently given weekly doses of 50 or 200 ng/kg-week TCDD by gavage (7.14 or 28.6 ng/kg-day adjusted for continuous exposure; administered doses divided by 7) or corn oil vehicle. Exposure continued for up to 8 months, and animals were observed for changes in estrus cycle at 4, 6, and 8 months. Rats were sacrificed at 8 months of age when the TCDD-treated animals had entered the transition to reproductive senescence. Following sacrifice, diestrus concentrations of serum LH, FSH, progesterone, and estradiol were measured, and the ovaries were collected for examination.

Estrus cycles at 4 months exhibited normal cyclicity in both TCDD-exposed groups and did not differ significantly from the control group. At 6 months, however, there was a tendency ($p < 0.1$) toward loss of normal estrus cyclicity in animals treated with TCDD. At the 8 month observation, estrus cyclicity was significantly ($p < 0.05$) different in both dioxin-exposed groups

compared to controls (cumulative TCDD exposure is reported as 1.7 and 8 μg/kg for the 50 and 200 ng/kg dose groups, respectively). The study authors noted that although the low-dose animals showed an increased prevalence of prolonged cycles, persistent estrus or diestrus was observed in only 10% of the rats. Conversely, approximately 50% of the rats exhibited loss of cyclicity in the high-dose group. There were no changes in the number and size distribution of ovarian follicles or the number of corpora lutea at either dose. Progesterone levels at 8 months tended to be higher ($p < 0.08$) in animals receiving either 7.14 or 28.6 ng/kg-day TCDD compared to controls, while serum estradiol concentrations were significantly ($p < 0.03$) lower at diestrus. Serum LH levels in TCDD-treated animals were comparable to those in the control group, while FSH levels were elevated in rats receiving 7.14 ng/kg-day TCDD, but not in the 28.6 ng/kg-day dose group.

A LOAEL for TCDD of 7.14 ng/kg-day for an 8-month exposure duration was identified for significantly ($p < 0.03$) decreased serum estradiol levels. A NOAEL cannot be determined for this study.

2.4.2.2.4. ***Hojo et al. (2002, 198785) (and related: Zareba et al. (2002, 197567)).***

Hojo et al. (2002, 198785) studied the impact of prenatal exposure to TCDD on sexually dimorphic behavior in rats. Thirty-six pregnant Sprague-Dawley rats were assigned according to a randomized block design to groups receiving 0, 20, 60, or 180 ng/kg TCDD (98% purity) on GD 8. Litters from pregnant dams were culled to 5 females and 5 males on PND 4 and allowed to wean normally, at which time 5, 5, 6, and 5 litters from the 0, 20, 60, and 180 ng/kg TCDD treatment groups, respectively, were maintained for examination of behavioral response. Offspring were exposed to TCDD (from a single maternal exposure) for about 35 days through gestation and lactation. After weaning at PND 21, offspring were fed ad libitum until PND 80, at which time a fixed amount of food was supplied daily to maintain constant body weights. At 90 days old, the rats in these treatment groups were trained to press a lever to obtain food pellets using two operant behavior procedures. Initially, each lever press was reinforced. The fixed-ratio (FR) requirement was then increased every fourth session from the initial setting of 1 to values between 6 and 71. The responses for 30 days were studied under a multiple schedule combining FR 11 and another schedule requiring a pause of at least 10 sec between responses (differential reinforcement of low rate, or DRL 10-sec)

Pup and dam body weights were not affected by TCDD exposure, and all pups were successfully trained in the lever-press response within 3–4 days. Analyses of the FR procedure data indicated that the male pups responded at a lower rate at all TCDD doses when compared to the control group. In case of female pups, all TCDD-treated groups responded at a higher rate than controls. None of these results were, by themselves, however, statistically significant. Examination of the FR 11 and DRL 10-second data indicated that when considering the FR component of this multiple procedure, males from all three treatment groups responded at lower rates when compared to the controls. Conversely, all female pups responded at a higher rate than controls. In addition, the treatment-by-sex interaction was significant ($p = 0.036$), with the 60 ng/kg female pups responding at a higher rate than the 60-ng/kg male pups. Examination of the delayed response component in the multiple FR 11 and DRL 10-sec procedures indicated that almost all TCDD treatment groups were affected. Like the FR component, male pups at all TCDD dose groups responded at a lower rate compared to controls, while female pups at all dose groups responded at a higher rate than controls. There was also a significant ($p = 0.001$) sex-by-treatment interaction for the DRL 10-sec similar to the FR component. Following behavioral testing, the animals were sacrificed and cortical depth measurements were taken in selected right and left brain regions. Reduced cortical thickness and altered brain morphometry were observed in both male and female offspring in the 180-ng/kg exposure group when compared to controls (reported in a separate article; Zareba et al., 2002, 197567).

A nominal LOAEL for TCDD of 20 ng/kg for a single exposure on GD 8 is established for this study based on abrogation of sexually dimorphic neurobehavioral responses. A NOAEL cannot be derived for this study.

2.4.2.2.5. *Kattainen et al. (2001, 198952).*

Pregnant Line A, B, and C rats derived from Han/Wistar and Long-Evans rats (4−8 pregnant dams/strain/treatment group) were administered a single gavage dose of 0, 30, 100, 300, or 1,000 ng/kg TCDD (purity >99%) in corn oil on GD 15 (Kattainen et al., 2001, 198952). On PND 1, the litters were culled to three males and three females. Offspring were weaned on PND 28. Female pups were sacrificed on PND 35 and male pups were sacrificed on PND 70. TCDD treatment did not affect body weight or cause clinical signs of toxicity in the dams. In Line B offspring, body weights in the 1,000 ng/kg group were slightly decreased

during PND 1−7, while Line C offspring had slightly decreased body weights throughout the study period (data were not provided). The development of the third molar was affected the most in Line C offspring. In 5 of 10 Line C females and 6 of 10 Line C males treated with 1,000 ng/kg TCDD, the lower third molar did not develop. In comparison, 1 of 19 Line A females and 1 of 18 Line B females administered 1,000 ng/kg TCDD lacked the third molar at sacrifice. Third molars were present in all the controls and all male Line A and B offspring administered 1,000 ng/kg. Due to the lack of eruption of the third molar in the majority of Line B and C control females (only 30% erupted), however, the effects of TCDD on third molar eruption could only be evaluated in Line A female offspring (with 94% eruption). There was a dose-dependent decrease in the eruption of the lower third molar in Line A female offspring with a significant ($p < 0.05$) decrease observed in the 300 and 1,000 ng/kg dose groups. In the male offspring, any third molar that developed erupted by PND 70. The mesiodistal length of the existing lower third molar was reduced in a dose-dependent manner in both genders of all three rat lines. In Line A and C females, the decrease was significant ($p < 0.05$) at all doses. The size of the second molars was also significantly decreased with 1,000 ng/kg ($p < 0.05$) in all but Line C males.

A developmental LOAEL for TCDD of 30 ng/kg for maternal exposure on GD 15 is established for this study, based on impaired tooth development (significantly reduced mesiodistal length of the lower third molar by approximately 12% to 38% [$p < 0.05$]). A NOAEL could not be determined.

2.4.2.2.6. *Keller et al. (2007, 198526; 2008, 198531; 2008, 198033).*

Keller et al. (2007, 198526; 2008, 198531; 2008, 198033) conducted three separate experiments to assess the impact of TCDD on molar tooth development using different mouse strains. In Experiment 1, Keller et al. (2007, 198526) used six inbred mouse strains (C57BL/6J, BALB/cByJ, A/J, CBA/J, C3H/HeJ, and C57BL/10J) known to possess high affinity ligand-binding aryl hydrocarbon receptor alleles (*b*), two with *b1* alleles (C57BL/6J and CBA/J), and four with *b2* alleles (BALB/cByJ, A/J, C3H/HeJ, and CBA/J). Females (number not specified) from each strain were mated with males of the same strain. On GD 13, each pregnant female was assigned to one of the four dose groups and treated with 0, 10, 100, or 1,000 ng TCDD/kg BW via oral gavage. The control group received corn oil. GD 13 was chosen for dosing because

the first morphological signs of tooth development occur on GD 11. The first visible signs of the M1 (molar) occur on GDs 13–14 followed by final cuspal morphology, which is determined on GD 15. The F1 offspring of females from each strain were weaned and separated by sex at PND 28 and were euthanized at PND 70. Each F1 mouse was examined for the presence or absence of both maxillary (M^3) and mandibular third molars (M_3) on both the left and right sides. In addition, all mice were scored as either normal or variant in M_1 morphology for both molar rows.

In Experiment 2 (Keller et al., 2008, 198531), dams from six inbred mouse strains (C57BL/6J, BALB/cByJ, A/J, CBA/J, C3H/HeJ, and C57BL/10J) were orally dosed on GD 13 with 0, 10, 100, or 1,000 ng TCDD/kg BW in corn oil. GD 13 was used as the dosing day because it coincided with the formation of Meckel's cartilage (a major signal center) in the mouse mandible that is followed shortly by intramembranous bone formation on GD 15. The A/J mouse strain was abandoned because the authors had difficulty rearing the offspring from this strain. All offspring (n = 4 or 5 per treatment group) from the remaining strains were euthanized at 70 days of age. Mandible size and shape from all selected offspring were examined using geometric morphometric methods to assess the impact of TCDD exposure.

In Experiment 3 (Keller et al., 2008, 198033), dams from six inbred mouse strains (C57BL/6J, BALB/cByJ, A/J, C3H/HeJ, CBA/J, and C57BL/10J) were treated with a single oral dose of 0, 10, 100, or 1,000 ng TCDD/kg-BW in corn oil. GD 13 was chosen as the dosing day because the first visible signs of the first molar (M1) occurs on GDs 13–14 and the final cuspal morphology (the pattern of projections on the chewing surface of the tooth) is not determined until after GD 15. Similar to Experiment 2, the A/J mouse strain was abandoned due to difficulty in rearing offspring. All offspring (n = 107–110 in each of the five strains for all treatment groups) were euthanized at 70 days of age and their molar size, shape, and asymmetry traits were examined using geometric morphometric methods.

In Experiment 1, all four M_3s were present in all dose groups in mice from C57BL/6J, BALB/cByJ, and C57BL/10J strains. A similar response was observed in the A/J strain mice with only 3 of 51 F1 mice exhibiting missing third molars. Approximately one-third of the mice from the CBA/J and C3H/HeJ strains, however, were missing at least one M^3 or M_3 molar. The numbers of CBA/J mice missing one or both M_3 or M^3 molars were 0/29, 2/21, 6/29, and 30/30 in the 0, 10, 100, and 1,000 ng/kg groups, respectively. In the C3H/Hej animals, the numbers missing one or both molars were 1/24, 3/28, 1/26, and 30/36, respectively.

Maternal TCDD exposure was also found to affect the frequency of M_1 variants, but only in the C57BL/10J strain, and the dose-response relationship was nonmonotonic. The proportions of variants observed in the 0, 10, 100, and 1,000 ng/kg dose groups were 33, 68, 59, and 58%, respectively.

A LOAEL for TCDD of 10 ng/kg maternal exposure on GD 13 is identified for this study for increased incidence (33%) of the M_1 variant in the C57BL/10J mouse strain. A NOAEL cannot be determined in this study.

In Experiment 2 TCDD exposure of dams did not affect offspring survival or 10-week body weight in any of the inbred mouse strains used. Analysis of variance (ANOVA) indicated that although mandible size in both male and female offspring varied significantly ($p < 0.0001$) among strains, it was not affected by TCDD exposure. In contrast, analysis of covariance indicated that TCDD exposure significantly ($p = 0.0033$) decreased the mandible size in male offspring in the C3H/HeJ strain at all treatment groups. The mean mandible size was similar across all treatment groups in both sexes in all strains with male offspring exhibiting larger mandibles compared to females. Males in the C3H/HeJ strain exhibited a significant (level not reported) downward trend in mandible size throughout all treatment groups. Females in the C3H strain also showed a similar trend in mandible size, but the trend was not significant. ANOVA on mandible shape indicated that males had significantly ($p < 0.0001$) different mandible shape in strain × treatment groups. In contrast, in female offspring, although the mandible shape was significantly ($p < 0.0001$) different due to strains, treatment groups, and litter, the strain × treatment interaction was not significant. Male offspring from the C3H/HeJ and C57BL/6J mouse strains appear to be more sensitive to TCDD than BALB/cByJ or CBA/J mice, with the C57BL/10J strain exhibiting intermediate sensitivity. In addition to these analyses, Procrustes distance analysis also indicated that C3H/HeJ mice had the greatest response to the highest dose of TCDD, followed by the C57BL/6J strain. Female offspring in the C3H/HeJ and C57BL/6J strains also exhibited the largest change in Procrustes distance with TCDD exposure. This trend, however, was not statistically significant ($p = 0.29$).

A LOAEL for TCDD of 10 ng/kg maternal exposure on GD 13 was identified for this study for significantly ($p = 0.0033$) decreased mandible shape and size in male C3H/HeJ mice. A NOAEL cannot be determined in this study.

In Experiment 3, effect of TCDD exposure on offspring survival or body weight was not reported. Three-way ANOVA results showed significant ($p < 0.0001$) differences in molar size among strains, sexes, and litters, but not between treatment groups. Molar size difference in sex × strain interaction was significant ($p = 0.03$), whereas differences in sex × treatment and sex × strain × treatment were not significant. Additionally, molar size in treatment × strain interaction also was not statistically significant. Based on these results, the authors reported that molar size varied significantly ($p < 0.0001$) among all five strains tested, with all strains exhibiting similar trends in all four treatment groups. Strain differences in molar size were more apparent in male offspring. A hormesis-like trend in molar size was observed in all strains (except in BALBc/ByJ) and sexes with an increase at the 100 ng/kg dose and a decrease in the 1,000 ng/kg dose. In addition to lack of difference in molar size for all treatment groups in all strains, fluctuating asymmetry in molar size also did not increase with increasing doses of TCDD.

In contrast to these results on molar size, the Procrustes ANOVA indicated that molar shape was significantly ($p < 0.0001$) affected by strain, sex, treatment, and litter size. Molar shape in sex × strain and sex × strain × treatment interactions was also highly significant ($p < 0.0001$). Based on these results, the authors concluded that differences between males and females varied based on the strain, and that the effect of TCDD exposure on each strain also differed for male and female offspring. Because molar shape in treatment × strain interaction was significant ($p < 0.0001$), differences in molar shape between the three treatment groups and the control group were analyzed for each strain using nonorthogonal contrasts. In male offspring, contrasts between the control group and 1,000 ng/kg were statistically significant only in the C3H/HeJ ($p < 0.0001$) and CBA/J ($p < 0.03$) strains. These results suggest that these two strains are most susceptible to TCDD effect on molar shape, and similar results were observed in female offspring of these two strains. The contrast in molar shape between the control and the 100 ng/kg treatment group for the female C57BL/6J mice also was statistically significant ($p = 0.0096$). On the whole, when considering Procrustes distance results for molar shape, the C3H/HeJ male offspring had the largest response at the low and high doses, while the female offspring had the largest response at low and mid doses. This observation in male C3H/HeJ mice is consistent with that of TCDD-induced changes in mandible size from Keller et al. (2008, 198531).

A LOAEL for TCDD of 10 ng/kg maternal exposure on GD 13 is identified for this study for significant ($p < 0.0001$) differences in molar shape in male C3H/HeJ mice. A NOAEL cannot be determined in this study.

2.4.2.2.7. *Kuchiiwa et al. (2002, 198355).*

Kuchiiwa et al. (2002, 198355) studied the impact of in utero and lactational TCDD exposure on serotonin-immunoreactive neurons in raphae nuclei on F1 male mouse offspring. Twenty-one adult female ddY mice (seven per treatment group) were administered TCDD (99.1% purity) by oral gavage once a week for 8 weeks at doses of 0, 4.9, or 490 ng/kg (0, 0.7, or 70 ng/kg-day average daily dose; administered doses divided by 7) or an equivalent volume of olive oil vehicle (6.7 mL/kg) by gavage. Immediately following the final treatment, the mice were housed with untreated male mice for mating. At approximately 20–21 days after mating, 3 female mice from each dose group, including the control group gave birth to 10–12 offspring. One day after birth, each litter was culled to 10 offspring to accommodate similar lactational TCDD exposure. On PND 28, the offspring were weaned, and three offspring from each TCDD exposed group and the control group were selected for an immunocytochemical examination at 42 days of age. Following sacrifice of these offspring, the brain of each animal was removed and every second serial section of the brain was processed for immunocytochemistry. In addition to the serial sections of the brain, cells from 18 offspring (6 males per treatment group) were used to assess the number of cells in the dorsal and median raphe nucleus, the supralemniscal area, and the Nucleus raphe magnus.

Examination of external morphology, birth, and postnatal body weights indicated that there were no differences between the male TCDD-exposed offspring and the control male offspring. TCDD-exposed males, however, were aggressive toward other normal mice and were also hypersensitive to soft touch.

Serotonin-immunoreactive neurons were found to be distributed throughout the entire brainstem in 42-day-old males, and the general pattern in the TCDD-exposed animals was consistent with those observed in control male offspring. Serotonergic neurons were identified and counted in the caudal linear nucleus, the median and dorsal raphe nucleus, Nucleus raphe pontis, interpeduncular nucleus, supralemniscal area, pedunculopontine segmental nuclei, deep mensencephalic nucleus, Nucleus raphe magnus, pallidus, and obscurus, dorsal and medial to the

facial nucleus and the ventrolateral medulla. Results from computerized cell counts ($n = 6$) showed an average of 1,573.3 immunoreactive neurons in the raphe nuclei from the control group versus 716.3 and 419.8 neurons in the low- and high-dose offspring, respectively. The numbers of immunoreactive neurons in the individual raphe nuclei (dorsalis, medianus, magnus, and B9) from the TCDD-exposed offspring were significantly ($p < 0.01$) lower than control values, with the degree of reduction being dose-related.

In the absence of other relevant neurotoxicity endpoints, reduced serotonin is not an adverse endpoint of toxicological significance in and of itself, thus, neither a NOAEL nor a LOAEL can be established for this study. A lowest-observed-effect level (LOEL) of 0.7 ng/kg-day for an 8-week exposure duration is identified in this study for a significantly ($p < 0.01$) lower number of serotonin-immunoreactive neurons in the raphe nuclei of male offspring. A no-observed-effect level (NOEL) cannot be determined for this study.

2.4.2.2.8. ***Li et al. (2006, 199059).***

Pregnant and pseudopregnant (obtained by mating normal estrous female mice with vasectomized male mice) NIH mice (10 per treatment group) were exposed to 0, 2, 50, or 100 ng/kg-day of TCDD (purity 99%) during early gestation (GDs 1–8), preimplantation (GDs 1−3), or peri-implantation to postimplantation (GDs 4–8) (Li et al., 2006). On GD 9, animals were evaluated. The two highest TCDD doses (50 and 100 ng/kg-day) caused significant ($p < 0.05$) early embryo loss independent of gestational exposure time. At 100 ng/kg-day, however, the embryo loss was greater when administered during GDs 1–8 or GDs 1–3 compared to GDs 4–8 ($p < 0.01$). Uterine weight was significantly decreased in the pseudopregnant mice when administered 50 or 100 ng/kg-day TCDD during GDs 1–8 ($p < 0.001$) or 1–3 ($p < 0.01$), but was only decreased at 100 ng/kg-day in pseudopregnant mice when administered during GDs 4–8 ($p < 0.01$). Estradiol levels were increased at all TCDD treatment levels (100% at the lowest dose), but statistical significance was not indicated. All doses at all treatment times resulted in a significant reduction ($p < 0.01$) in serum progesterone levels, with a 45% decrease at the lowest dose. Because the hormone effects were observed following 4 days of treatment, the nominal doses were averaged over the entire test period of 8 days prior to measurement. The resulting average daily doses of TCDD were 0, 1, 25, and 50 ng/kg-day.

A LOAEL of 2 ng/kg-day administered for 4 to 8 days is established in this study for a significant ($p < 0.01$) decrease in progesterone (45% above control) and an approximate 2-fold increase in estradiol levels (significance not indicated). A NOAEL cannot be determined.

2.4.2.2.9. *Markowski et al. (2001, 197442).*

Pregnant Holtzman rats (4–7 per treatment group) were administered a single gavage dose of 0, 20, 60, or 180 ng/kg TCDD (purity not specified) in olive oil on GD 18 (Markowski et al., 2001, 197442). One female rat from each liter (4–7 per treatment group) was assigned to training on a wheel apparatus to respond on a lever for brief opportunities to run. Once animals responded to an FR1 schedule of reinforcement, the requirement for lever pressing was increased to FR2, FR5, FR10, FR20, and FR30 schedules. After each training session, the estrous cycle stage was determined. Maternal body weight, length of gestation, number of pups per litter, and sex distribution within litters were unaffected by treatment. For each of the FR schedules, there was a significant dose-related ($p = 0.0001$) decrease in the number of earned run opportunities, lever response rate, and total number of revolutions in the wheel in the adult female offspring. There was no correlation between estrous cycle and responding for access to wheel running.

The developmental LOAEL for this study is a single dose of 20 ng/kg administered on GD 18 for neurobehavioral effects. A NOAEL cannot be determined for this study.

2.4.2.2.10. *Miettinen et al. (2006, 198266).*

Miettinen et al. (2006, 198266) administered a single oral dose of 0, 30, 100, 300, or 1,000 ng/kg TCDD (purity >99%) in corn oil on GD 15 to pregnant Line C rats. The offspring (24–32 per treatment group) were assigned to a sugar-rich cariogenic diet (via feed and drinking water) and were orally inoculated three separate times with fresh cultures of *Streptococcus mutans*. Three control groups varied with regard to TCDD exposure and administration of a cariogenic diet. Two of the control groups received no TCDD, and the offspring were either maintained on a normal diet without inoculation with *S. mutans* (C1; $n = 48$) or were given the cariogenic diet with *S. mutans* inoculation (C2; $n = 42$). The final control group was maternally exposed to 1,000 ng/kg TCDD with offspring fed a normal diet without *S. mutans* inoculation (C3; $n = 12$). TCDD did not affect the maternal or offspring body weight. Survival of the offspring was reduced in the 1,000 ng/kg dose group (50–58% survival compared to 83–95% in

C1 and C2, respectively). All offspring administered 1,000 ng/kg were missing all lower third molars. Two animals (8%) in the 100 ng/kg group were missing one of their lower third molars. All doses, except the 100 ng/kg dose, caused a significant ($p < 0.05$) increase in the number of caries lesions compared to group C2 (60, 79, 76, 83, and 91% in the C2, 30, 100, 300, and 1,000 ng/kg groups, respectively). Group C3 (1,000 ng/kg TCDD exposure, normal diet) animals also had increased caries lesions compared to C1 (8% versus 0%, respectively). There were no changes in tooth mineral composition that could explain the increase in caries susceptibility.

The developmental LOAEL from this study is a single dose of 30 ng/kg administered on GD 15 based on the significant ($p < 0.05$) increase in dental caries in pups (30% above control). A NOAEL cannot be determined from this study.

2.4.2.2.11. ***Nohara et al. (2000, 200027).***

Pregnant Holtzman rats were administered 0, 12.5, 50, 200, or 800 ng/kg TCDD in corn oil by gavage on GD 15 (Nohara et al., 2000, 200027). On PND 2, five males were randomly selected from each litter and dose group. TCDD was detected in the thymus, spleen, and bone marrow of the male pups on PND 21 and PND 49. TCDD was still detected in the thymus and spleen on PND 120 but the levels decreased over time. The TCDD concentration was highest in the thymus at all time points. There were no changes in the body, thymus, or spleen weights of the male offspring on PND 5, PND 21, PND 49, or PND 120. On PND 5, there was a 200-fold increase in CYP1A1 in the thymus of the high-dose male pups. CYP1A1 was only slightly increased in the spleen. This induction decreased through PND 49. There was a slight (not statistically significant) dose-dependent decrease in thymus cellularity in the male offspring at PND 120. Spleen cellularity at PND 49 decreased in a dose-dependent manner (15–50% of the control), with a statistically significant ($p < 0.05$) decrease observed in the high-dose group. A slight but not significant reduction in spleen cellularity was noted in the high-dose group at PND 21. The same effect was not observed at PND 120, nor was there any change in the percent of B or T cells in the spleen. No changes in cytokine levels were observed in the 800-ng/kg group.

Although a change in spleen cellularity on PND 49 (puberty) was observed, this effect was transient and there were no coexisting changes in the percentage of splenic lymphocytes,

spleen weight, and cytokine levels. Therefore, a developmental NOAEL of a single dose of 800 ng/kg administered on GD 15 is identified for this study. A LOAEL is not established.

2.4.2.2.12. *Ohsako et al. (2001, 198497).*

Pregnant Holtzman rats (6 per treatment group) were administered 0, 12.5, 50, 200, or 800 ng/kg TCDD (purity >99.5%) in corn oil by gavage on GD 15 (Ohsako et al., 2001, 198497). On PND 2, five males were randomly selected from each litter. Two male offspring from each litter were sacrificed on PND 49 and PND 120. Neither maternal nor male offspring body weight was affected by TCDD treatment. TCDD was detected in both fat and testes at all dose levels (including controls) with highest levels found in fat. There were no apparent treatment-related effects on testicular weight, epididymal weight, daily sperm production, cauda epididymal sperm reserves, luteinizing hormone, follicle stimulating hormone, or testosterone levels. There was, however, a clear dose-dependent decrease in urogenital complex weight and ventral prostate weight at both PND 49 and PND 120. For male offspring, statistically-significant ($p < 0.05$) decreases were noted in urogenital complex weight at PND 120 in the 200 and 800 ng/kg groups, in ventral prostate weight at PND 49 in 800 ng/kg group, and at PND 120 in the 200 and 800 ng/kg groups. There was also a dose-dependent decrease in anogenital distance (the length between the base of the genital tubercle and the anterior edge of the anus); the decrease was not statistically significant at PND 49. At PND 120, however, male offspring in all but the lowest dose group had significantly ($p < 0.05$) reduced anogenital distance compared to the control animals. There was also a dose-dependent increase in 5αR-II mRNA expression in the ventral prostate on PND 49 with significant increases ($p < 0.05$) in the 200 and 800 ng/kg animals. There was a significant ($p < 0.01$) decrease in the androgen receptor mRNA in the ventral prostate on PND 49 at all doses tested. Similar effects were not observed on PND 120 or in the caput epididymis on PND 49.

The developmental LOAEL for this study is a single dose of 50 ng/kg administered on GD 15 for significantly ($p < 0.01$) reduced anogenital distance in male offspring (approximately 14%). The NOAEL for this study is 12.5 ng/kg.

2.4.2.2.13. *Schantz et al. (1996, 198781).*

Schantz et al. (1996, 198781) studied the impact of in utero TCDD exposure on spatial learning in male and female pups. Groups of pregnant Harlan Sprague-Dawley rats (n = 108, divided into 4 cohorts; number of animals in each TCDD group approximately 4 per treatment group) were dosed via gavage with 0, 25, or 100 ng/kg-day TCDD (purity >98%) in corn oil on GDs 10−16. On the day of birth (post natal day [PND] 0), the pups were examined for gross abnormalities and the number of live pups, weight, and sex were recorded for each litter. On PND 2, litters were culled to eight animals and were balanced to include four males and four females whenever possible. To minimize litter-size effects, litters with fewer than five pups were excluded from the study. The exclusion of these litters resulted in 10−11 litters per treatment group. Pups were weaned on PND 21 and one male and one female pup from each litter were maintained for the learning tests. Pups were tested 5 days per week for spatial learning and memory in a radial arm maze and a T-maze. A radial arm maze working memory test and a T-maze DSA task were used a part of the testing process.

TCDD treatment did not affect dam gestational weight gain, dam liver weight, gestation length, litter size, percentage of live births, birth weight, or postnatal growth of the pups observed during the course of the study. Exposed pups, however, exhibited some signs of toxicity in all exposure groups. Thymus weight was decreased and liver weight was increased in the 100 ng/kg-day TCDD dose group. Also, liver microsomal 7-ethoxyresorufin-O-deethylase (EROD) activity was markedly induced in pups from both the 25 and 100 ng/kg-day dose groups. In the radial maze test, rats from all TCDD exposure groups displayed a significant ($p < 0.01$) learning behavior as shown by progressively fewer errors from the first block of sessions through the fourth session. The treatment by sex and treatment by session block interactions were not significant. Comparisons between the average number of errors per session block in the TCDD-exposed and control group indicated that both the 25 and the 100 ng/kg-day dose groups made significantly ($p < 0.05$ and $p < 0.001$, respectively) fewer errors compared to the control group. TCDD did not significantly affect adjacent arm selection behavior as measured by C statistic; hence the reduction in errors observed did not appear to be accounted for by an increased tendency to run into adjacent arms. Female pups had a significant ($p < 0.05$) shorter radial arm maze latency, however, compared to the male pups. In the T-maze test, TCDD did not significantly affect the percent of correct performance. All exposure groups

performed best at the shortest delay, which showed a decline as the length of the intertrial delay interval was increased. Additionally, all treated groups improved their performance over a three-block session period. This finding indicated that animals in all groups could learn the task. These observations were confirmed by a highly significant main effect of delay ($p < 0.001$) and highly significant main effect of session blocks ($p < 0.001$). At the shortest 15-second delay, average percent correct performance increased from 75 to 92%, while at the longest 40-second delay, the average percent correct performance increased from 62 to 82%. A significant ($p < 0.05$) main effect of exposure was evident in latency to respond in the T-maze. Comparisons of the exposed group to control group, however, indicated that none of the individual exposure groups differed significantly from the controls. Because no clear pattern was observed in the various exposure groups, differences in latency to respond had no impact on learning of the task.

Based on these results, the study authors state that the fact TCDD seems to have a facilitatory effect on radial arm maze learning in rats should be interpreted with caution and needs further evaluation using different and more varied learning tasks. No toxicologically adverse endpoints were concurrently examined. Thus, a LOAEL and a NOAEL cannot be determined for this study.

2.4.2.2.14. ***Seo et al. (1995, 197869).***

To study developmental effects of TCDD on thyroid hormone levels, time-mated female Sprague-Dawley rat dams (n = 10−14/treatment group) were administered 25 or 100 ng/kg-day of TCDD (>98% pure) in corn oil via gavage from GDs 10–16. Vehicle controls received equivalent amounts of corn oil. The study also investigated PCB treatment outcomes. At birth, pups were weighed and grossly examined for abnormalities. At 2 days of age, litters with fewer than 5 pups were excluded from the analysis and the remaining litters were culled to 4 males and 4 females. Each treatment group contained 10 or 11 litters. Pups remained with the dams until weaning. At weaning, 4−6 pups were retained for neurobehavioral tests (which were not reported as part of this study). The remaining offspring were sacrificed, which provided 5−9 litters per treatment group. Data were collected from one male and one female where possible. No signs of toxicity were evident in the dams; measurements on dams included gestational weight gain, liver weight, litter size, and live births. Pup birth weight and weaning

weight were unaffected by treatment. In pups sacrificed at weaning (21 days old), a significant ($p < 0.05$) decrease occurred in thymus weight for the high-dose group, but not in thyroid, liver, or brain weight. A significant ($p < 0.05$) decrease (20.4%) was observed in T4 in high-dose females. Thyroid stimulating hormone and T_3 were unaffected by treatment. Uridine diphosphate (UDP)-glucuronosyl transferase activity towards 4-nitrophenol significantly ($p < 0.05$) increased in both treatment groups over control values, and the increase in the high-dose group was significantly ($p < 0.05$) greater than in the low-dose group. Liver microsomal EROD activity was significantly ($p < 0.05$) increased in both treatment groups, but is considered to be an adaptive response and not adverse.

A LOAEL of 100 ng/kg-day for decreased thymus weights and decreased thyroxine is identified for this study. A NOAEL of 25 ng/kg-day is established.

2.4.2.2.15. *Simanainen et al. (2004, 198106).*

Simanainen et al. (2004, 198106) studied the impact of in utero and lactational TCDD exposure on the male reproductive system in three rat lines that are differentially sensitive to TCDD. Groups of 5 to 8 pregnant Line A, B, and C C57BL/6N CYP1A2 dams were given a single dose of 0, 30, 100, 300, or 1,000 ng/kg of TCDD (purity >99%) in corn oil on GD 15 via oral gavage. Control animals were similarly dosed with a corn oil vehicle. One day after birth, litters were randomly culled to include three males and three females to allow uniform postnatal exposure. Offspring were weaned on PND 28. Dam and pup viabilities were monitored throughout the study. Pup body weights were determined on PNDs 1, 4, 7, 14, and 28. Anogenital distance and crown-rump length were measured on PNDs 1 and 4. On day 70, pups were sacrificed and trunk blood was collected. Serum was collected for testosterone analysis. The testes, cauda of the right epididymis, ventral prostrate, seminal vesicles, and thymus was dissected and weighed. Absolute and relative organ weights were determined, and cauda epididymis and testes were also preserved for sperm count analysis.

TCDD caused no mortality or overt signs of toxicity to the dams. Pup survival from implantation to the day after birth also was not affected by TCDD exposure. Survival from the day of implantation to the day after birth, however, was uncharacteristically lower in control Line B rats (41%), resulting in a significant difference compared with the two lowest doses (30 and 100 ng/mg TCDD). The average survival percentage in the controls for Line A, B, and C

rats was 85% (range 80–86%); 64% (41–86%); and 74% (63–85%); respectively. Percentage of male pup survival in each line between PND 1 and PND 28 was 99% except for Line B males exposed to 30 ng/kg TCDD and Line C males exposed to 30 or 100 ng/kg, where male survival rate averaged 81% (range 81–83%). On PND 70, a significant ($p < 0.05$) reduction in body weight was observed only in Line B and C rats at 1,000 ng/kg. In pups exposed to 1,000 ng/kg TCDD, both absolute and relative weight of the ventral, anterior, and dorsolateral prostrate decreased in all three lines at most postnatal time points measured. The change was most consistent and significant ($p < 0.05$) in the ventral lobe. Animals exposed to 1,000 ng/kg TCDD had an average decrease in absolute weight of the anterior prostrate of 37, 32, and 34% in Lines A, B and C, respectively. Additionally, the average dorsolateral prostrate weight was also decreased by 34, 28, and 39% in Lines A, B, and C, respectively. The effect on the ventral prostrate was reversible with the only significant ($p < 0.05$) decrease in weight observed in Line B rats at PND 70 in the 1,000 ng/kg TCDD dose group. The authors reported that TCDD had no consistent effects on the weight of seminal vesicles. The absolute weights of the testis and epididymis showed a significant ($p < 0.05$) increase on PNDs 28–49, but the relative testis, epididymis, and cauda epididymis weights remained unchanged. In pups exposed to 1,000 ng/kg TCDD, severe malformation, including small caput and cauda and degeneration of corpus epididymis, was observed. Malformations in the epididymis were observed in 6 of 44 Line C male rat offspring and 3 of 47 Line A male rat offspring. In Line A, B, and C rats at PND 70in the 1,000 ng/kg TCDD dose group, daily sperm production was reduced by 9, 25, and 36% and cauda epididymal sperm reserves were reduced by 18, 42, and 49%, respectively. Daily sperm reduction (17%) was significant ($p < 0.05$) in Line C rats at a TCDD dose of 300 ng/kg and in Line B and C rats at 1,000 ng/kg. A reduction in cauda epididymal sperm reserves (25%) was significant ($p < 0.05$) in Line C rats at 300 and 1,000 ng/kg TCDD.

A LOAEL for TCDD of 300 ng/kg is identified for reduction in daily sperm production and cauda epididymal sperm reserves in Line C rats. A NOAEL of 100 ng/kg is identified for this study.

2.4.2.2.16. *Sugita-Konishi et al. (2003, 198375).*

Sugita-Konishi et al. (2003, 198375) examined the immunotoxic effects of lactational exposure to TCDD in newborn mice. Eight pregnant female C57BL/6NCji mice were

administered 0, 1.8, or 18 ng/L of TCDD via drinking water from parturition to weaning of the offspring (for a total of 17 days). Based on an average water intake of 14–16 mL/day, the average daily intake of TCDD for the dams was 1.14 and 11.3 ng/kg-day in the low- and high-dose groups, respectively. In male offspring sacrificed at weaning (21 days after birth), there was a statistically-significant ($p < 0.05$) decrease in relative spleen weight and a statistically-significant ($p < 0.005$) increase in thymic CD4+ cells in the high-dose group. The changes in relative spleen weight and thymic CD4+ cells were dose related, but effects in the low-dose group did not achieve statistical significance. Changes in spleen weight and CD4+ cell numbers were not observed in the female offspring. In a separate experiment, offspring infected with *Listeria monocytogenes* following lactational TCDD exposure exhibited a statistically significant increase in serum tumor necrosis factor alpha (TNF-α) 2 days after infection in both sexes in the low- ($p < 0.05$) and high-dose ($p < 0.005$) groups. There was also a statistically significant increase in serum interferon gamma in *Listeria*-infected high-dose females ($p < 0.05$). The number of bacteria in the spleen was also significantly increased ($p < 0.05$) 2 days after infection in the high-dose females compared to the controls, but not in males. *Listeria* levels in the spleen returned to control levels by 4 days after infection in both sexes.

Based on these results, a LOAEL for TCDD of 11.3 ng/kg-day following a 17 day exposure to dams was identified for significantly ($p < 0.05$) decreased spleen weight (in male pups), a significant ($p < 0.005$) increase in thymic CD4+ cells (in male pups), and for increased susceptibility to *Listeria monocytogenes* (in male and female pups). The NOAEL for this study is 1.14 ng/kg-day.

2.4.2.3. *Acute Studies*

2.4.2.3.1. *Burleson et al. (1996, 196998).*

Burleson et al. (1996, 196998) studied the impact of TCDD exposure on mice that were challenged with the influenza virus 7 days after treatment with TCDD. Groups of 8-week-old female B6C3F1 mice ($n = 20$, 2 replicate groups) were treated one time with 0, 1, 5, 10, 50, 100, or 6,000 ng/kg TCDD (purity >99%, dissolved in corn oil) via oral gavage. In addition to the treated groups, randomly selected animals were assigned as a sentinel group and screened for numerous pathogens. Results of all tests performed on this sentinel group were negative. Seven days after TCDD treatment, all animals were lightly anesthetized and infected intranasally

with a highly lethal influenza A/Hong Kong/8/68 virus (H3N1; passage 14). The animals were infected with sufficient H3N1 virus to achieve a 30% mortality rate in the control animals. Animals were observed for mortality and morbidity for 21 days following viral infection. Six mice from each treatment group were sacrificed on days 3, 9, and 12 postinfection, and body, thymus, and wet lung weights were recorded. Influenza viral titers were examined by sacrificing eight mice each at 2 hours and at 1, 4, 6, 7, 8, 9, 10, and 11 days post infection.

Exposure to TCDD resulted in significantly ($p < 0.05$) increased mortality in the 10, 50, and 100 ng/kg dose groups. No statistically significant difference in the percentage alive was observed between these dose groups. TCDD doses of 1 and 5 ng/kg did not alter mortality in influenza infected animals. A time-related increase in the wet weights of the lungs in infected mice as a result of increased edema also was reflected in an increase in the lung weight-to-body weight ratio. The study authors stated that this ratio was not altered as a result of TCDD exposure. TCDD-only exposures at 1, 10, or 100 ng/kg did not affect thymus weight. Similarly, animals infected with the influenza virus following TCDD exposure also showed no loss in thymic weight. Enhanced mortality in TCDD-treated animals was not correlated with an increase in influenza virus titers. Additionally, animals treated with 1, 10, 100, or 1,000 ng/kg did not affect pulmonary viral titer assays on days 6, 7, and 8 postinfection. The authors also concluded that TCDD did not alter Hong Kong virus replication or clearance.

Although these results support immunotoxic effects induced by TCDD, the findings were not reproduced by Nohara et al. (2002, 199021) using the identical study design, and the translation of these findings to humans is dubious. Thus, no LOAEL/NOAEL was established. A LOEL for TCDD of 10 ng/kg for a single exposure is identified for significantly ($p < 0.05$) increased mortality in mice infected 7 days later with the influenza virus. The NOEL for this study is 5 ng/kg.

2.4.2.3.2. *Crofton et al. (2005, 197381).*

Crofton et al. (2005, 197381) studied the impact of TCDD exposure in addition to the impact of mixtures of thyroid disrupting chemicals and PCBs on serum total thyroxine (TT4) concentration. Groups of female Long-Evans rats were dosed via oral gavage with 0, 0.1, 3, 10, 30, 100, 300, 1,000, 3,000, or 10,000 ng/kg-day TCDD (purity >99%) in corn oil (n = 14, 6, 12, 6, 6, 6, 6, 6, 6, and 4, respectively) for 4 consecutive days. On the day following the last dose,

animals were sacrificed, trunk blood was collected, and serum obtained via centrifugation was assayed for TT4 concentration using standard radioimmunoassay methods.

No visible signs of toxicity or changes in animal body weight as a result of TCDD exposure were observed. Serum T4 levels showed a dose-dependent decrease, with the levels dropping sharply beginning at 100 ng/kg-day dose. Percent serum T4 levels were 96.3, 98.6, 99.8, 93.3, 70.9, 62.5, 52.7, 54.7, and 49.1% in the 0.1, 3, 10, 30, 100, 300, 1,000, 3,000, and 10,000 ng /kg-day groups, respectively.

A LOAEL for TCDD of 100 ng/kg-day for 4 consecutive days of exposure is identified in this study for a reduction in serum T4 levels (70.9% compared to 100% in controls). The NOAEL for this study is 30 ng/kg-day.

2.4.2.3.3. *Kitchin and Woods (1979, 198750).*

Female Sprague-Dawley rats (nine per control and four per treatment group) were administered a single dose of 0, 0.6, 2, 4, 20, 60, 200, 600, 2,000, 5,000, or 20,000 ng/kg TCDD (purity >99%) in corn oil. Animals were sacrificed 3 days after treatment and CYP level and benzo(a)pyrene hydroxylase activity in the liver were measured. A significant ($p < 0.05$) increase in cytochrome P450 levels occurred with doses of 600 ng/kg or greater and in benzo(a)pyrene hydroxylase activity with doses of 2 ng/kg or greater. Cytochrome P450 was significantly ($p < 0.05$) higher 1 month after a single exposure of 2,000 ng/kg (the only dose measured), but not after 3 or 6 months. Aryl hydrocarbon hydralase (AHH; $p < 0.05$) and EROD ($p < 0.01$) were both significantly increased through 3 months after treatment, and although elevated at 6 months, the results were not significant.

CYP induction alone is not considered a significant toxicologically adverse effect given that CYPs are induced as a means of hepatic processing of xenobiotic agents. Thus, no LOAEL or NOAEL was established for this study because adverse endpoints (e.g., indicators of hepatotoxicity) were not measured. The acute LOEL, however, is 2 ng/kg based on a significant ($p < 0.05$) increase in benzo(a)pyrene hydroxylase activity (37% above control). The NOEL is 0.6 ng/kg.

2.4.2.3.4. *Li et al. (1997, 199060).*

Female Sprague-Dawley rats (22 days old; 10 per treatment) were administered a single oral dose of TCDD (>98% pure) in corn oil via gavage at doses of 3, 10, 30, 100, 300, 1,000, 3,000, 10,000, or 30,000 ng/kg. Vehicle controls received equivalent amounts of corn oil, while naïve controls were sham-treated only. In a preliminary time-course study, animals received a single dose of 10,000 ng/kg and were sacrificed at 1, 2, 4, 8, 16, 24, 48, and 72 hours. The time-course study showed two peaks in LH and FSH levels at 1 hour and 24 hours, with a decrease to control values by 48 hours. Thus, in the dose-response study, animals were sacrificed at 1 or 24 hours after treatment, blood was collected, and serum FSH and LH were measured. The dose-response study demonstrated that the peak at 1 hour was related to the vehicle as the peak also occurred in the vehicle controls, but did not occur in the naïve controls. At 24 hours, FSH was increased at 10 ng/kg and higher (>4-fold increase at 10 ng/kg). Doses of 10 to 1,000 ng/kg showed similar increases (not all reached statistical significance; $p < 0.05$). A dose-dependent increase occurred for doses ≥3000 ($p < 0.05$) with a maximum increase of 20-fold over the vehicle control. At 24 hours, the LH response significantly ($p < 0.05$) increased only for doses ≥300 ng/kg with a maximum increase of 15-fold above the vehicle control. The study authors calculated an ED_{50} of 500 ng/kg for gonadotropin increase. The dose-dependent release of LH was confirmed in in vitro studies, but did not occur with the same magnitude. The increase did not occur in calcium-free medium and was unrelated to gonadotropin releasing hormone.

Based on the increase in serum FSH, the LOAEL was 10 ng/kg and the NOAEL was 3 ng/kg.

2.4.2.3.5. *Lucier et al. (1986, 198398).*

Adult female Sprague-Dawley rats (six per treatment) were administered a single gavage dose of TCDD (purity not specified) in either corn oil or contaminated soil at doses of 15, 40, 100, 200, 500, 1,000, 2,000, 5,000 (corn oil), or 5,500 (contaminated soil) ng/kg. Animals were sacrificed 6 days later and livers were removed for analysis. No clinical signs of acute toxicity or changes in body weight were observed at any dose. AHH increased in a dose-dependent manner with significant ($p < 0.05$) increases observed at 15 ng/kg or greater in corn oil or 40 ng/kg or greater in contaminated soil. Cytochrome P450 was significantly ($p < 0.05$)

increased with doses of 1,000 ng/kg or greater in corn oil or 500 ng/kg or greater in contaminated soil. A dose-dependent increase was observed for UDP glucoronyltransferase (significance of individual doses not reported), with the results twice as high with corn oil than with contaminated soil. The authors state that the results indicate bioavailability from soils is 50%.

Because the association between AHH activity and TCDD-mediated hepatotoxicity is unknown and no adverse endpoints were measured, a LOAEL or NOAEL was not determined for this study. The acute LOEL for this study is 15 ng/kg, based on the significant ($p < 0.05$) increase (80% above control) in AHH. No NOEL is established.

2.4.2.3.6. *Nohara et al. (2002, 199021).*

Male and female B6C3F1 (C57BL/6 × C3H), BALB/c, C57BL/6N, and DBA2 mice (10−40 per treatment group) were administered a single dose of 0, 5, 20, 100, or 500 ng/kg TCDD in corn oil via gavage. Seven days following TCDD treatment, mice were infected with a mouse-adapted strain of influenza (A/PR/34/8; H1N1) at a plaque forming unit dose designed to target approximately 30% mortality in each strain. TCDD did not affect the body weight or survival in any of the infected mouse strains at any dose.

Therefore, no LOAEL is established in this study. The NOAEL is 500 ng/kg.

2.4.2.3.7. *Simanainen et al. (2003, 198582).*

Simanainen et al. (2003, 198582) studied the short-term effects of TCDD exposure to determine the efficacy and potency relationships among three differentially susceptible rat lines. The three rat lines used were A, B, and C, which were selectively bred from TCDD-resistant Han/Wistar and TCDD-sensitive Long-Evans rats. The study authors reported that Line A rats were most resistant to TCDD acute lethality followed by Line B and C. Groups of five or six randomly selected rats (sex not specified) were treated with a single oral dose of TCDD (purity >99%) in corn oil by oral gavage. The dose of TCDD was reported to range between 30 ng/kg and 3,000 µg/kg for Line A, 30 ng/kg and 1,000 µg/kg in Line B, and 30 ng/kg and 100 µg/kg for Line C. Control animals were similarly dosed with a corn oil vehicle. Rats were sacrificed on day 8 postexposure, and trunk blood was collected and serum separated. Liver and thymus were removed and weighed, and liver samples were collected and preserved. Liver

EROD activity, serum aspartate aminotransferase (ASAT) activity, free fatty acid (FFA) concentration, and total bilirubin concentration were determined. Teeth were also examined.

Relative thymus weights were reduced 25% at 300 ng/kg relative to controls in Line B rats. Liver enzyme (CYP1A1) induction, as measured by EROD activity, was evident at all exposure levels; CYP induction is considered to be an adaptive effect and not adverse in itself. No other endpoints were affected below 1 µg/kg in any of the three rat lines.

A LOAEL for TCDD of 300 ng/kg is identified for decreased relative thymus weight in Line B rats. A NOAEL of 100 ng/kg is identified for this study.

2.4.2.3.8. ***Simanainen et al. (2002, 201369).***

To study the short-term effects of TCDD on hormone levels, adult female Long-Evans (TCDD-sensitive) and Han/Wistar (TCDD-resistant) rats (n = 9−11/treatment) were administered a single dose of TCDD (>99% pure) in corn oil via gavage at doses ranging from 30 ng/kg to 100 µg/kg. Vehicle controls received an equivalent amount of corn oil. The study also examined other polychlorinated dibenzo-*p*-dioxins outcomes. Rats were sacrificed on day 8 postexposure, and trunk blood was collected and serum separated. Liver and thymus were removed and weighed, and liver samples were collected and preserved. Liver EROD activity, serum ASAT activity, FFA concentration, and total bilirubin concentration were determined. Teeth were also examined.

Neither FFA or ASAT levels in Han/Wistar rats showed a dose-response relationship. In Long-Evans rats, however, a significant ($p < 0.05$) dose-dependent increase in FFA occurred at 300 ng/kg TCDD. Serum ASAT sharply increased in Long-Evans rats between 3,000 and 10,000 ng/kg. Body weight change and relative thymus weights were significantly decreased ($p < 0.05$) in Han/Wistar rats with doses ≥10,000 ng/kg and in Long-Evans rats with doses ≥1,000 ng/kg. Liver EROD activity was significantly ($p < 0.05$) increased with all doses in both strains. Serum T4 was significantly ($p < 0.05$) decreased in Long-Evans rats at concentrations ≥300 ng/kg, but were not significantly affected in Han/Wistar rats. Serum bilirubin was significantly ($p < 0.05$) increased with doses ≥10,000 ng/kg in Long-Evans rats and ≥30,000 ng/kg in Hans/Wistar rats. Both strains of rat showed a dose-dependent increase in mean severity of incisor tooth defects. The results indicate that TCDD was the most potent congener tested in both rat strains.

A LOAEL of 300 ng/kg for decreased T4 in the Long-Evans rat is identified for this study. A NOAEL of 100 ng/kg is established.

2.4.2.3.9. *Smialowicz et al. (2004, 110937).*

Smialowicz et al. (2004, 110937) examined the impact of TCDD exposure on immunosuppression in mice. Groups of female (number not specified) C57BL/6N CYP1A2 (+/+) wild-type mice were administered a single dose of 0, 30, 100, 300, 1,000, 3,000, or 10,000 ng/kg TCDD (purity >99%) in corn oil via oral gavage. Control animals were similarly dosed with a corn oil vehicle. To assess immune function, 7 days after TCDD administration, all mice were immunized with sheep red blood cells (SRBCs) via injection into the lateral tail vein. Five days after immunization, mice were sacrificed, blood was collected, and enzyme-linked immunosorbant assays were performed. Additionally, spleen, thymus, and liver weights also were measured.

Body and spleen weights of the wild-type mice were unaffected by the TCDD exposure. A decrease in thymus weights of the mice appeared to be dose related. Only mice treated with 10,000 ng/kg TCDD, however, showed a statistically significant ($p < 0.05$) decrease in thymus weights compared to corresponding controls. Liver weights also showed a dose-related increase with only animals treated with 3,000 and 10,000 ng/kg TCDD showing statistical significance ($p < 0.05$) compared to the control group. The antibody response to SRBCs indicated a dose-related suppression in the wild-type mice, with animals treated with 1,000, 3,000, and 10,000 ng/kg TCDD showing statistically significant ($p < 0.05$) suppression compared to the controls.

A LOAEL for TCDD of 1,000 ng/kg is identified in female C57BL/6N CYP1A2 (+/+) wild-type mice for significant ($p < 0.05$) suppression of SRBCs. The NOAEL for this study is 300 ng/kg.

2.4.2.3.10. *Vanden Heuvel et al. (1994, 197551).*

Vanden Heuvel et al. (1994, 197551) examined the dose-response relationship between TCDD exposure and induction of hepatic mRNA. Groups of 10-week-old female Sprague-Dawley rats were administered TCDD (purity ~99%) in corn oil once at 0, 0.1, 0.05, 1, 10, 100, 1,000, or 10,000 ng/kg-BW. Four days after TCDD treatment, animals were sacrificed

and livers were excised and preserved. Total hepatic RNA was extracted using guanidine thiocyanate and DNA was removed using standard phenol-chloroform-isoamyl alcohol partitioning procedures. Quantitative competitive RNA-PCR method was used to analyze CYP1A1, UDP-glucuronosyltransferase I (UGT1), plasminogen activator inhibitor 2 (PAI2), β-actin, and transforming growth factor α (TGFα). In addition to hepatic mRNA levels, microsomal protein was assayed for EROD activity and livers were tested for TCDD concentration.

CYP1A1 mRNA induction levels in the TCDD-treated groups were low in the low-dose region and sharply increased to plateaus at higher doses. The lowest dose that showed a statistically significant ($p < 0.05$) difference compared to controls was the 1 ng/kg dose, which showed a three-fold increase in CYP1A1 mRNA levels. In contrast, a 130-fold increase occurred at 100 ng/kg and a 4,000- and 7,000-fold increase occurred at 1,000 and 10,000 ng/kg, respectively. A slight increase in the CYP1A1/β-actin levels was observed in the 0.1 ng/kg group, but this increase was not significant. EROD activity exhibited a pattern similar to CYP1A1 activity. EROD activity, however, was approximately 100-fold less sensitive compared to mRNA levels in TCDD-treated groups. Statistical significance (*p*-value not provided) in CYP1A1 level was observed at the 100 ng/kg dose compared to the 1 ng/kg dose. The study authors reported that, despite this difference in CYP1A1 and EROD activity, the correlation between CYP1A1 enzyme activity and mRNA levels was good. Dose-response relationships for the induction of UGT1, PAI2, and TGFα mRNA differed from what had been observed for CYP1A1 mRNA. UGT1 mRNA was induced, but at the much higher dose of 1,000 ng/kg. Additionally, the five-fold maximum induction of UGT1 mRNA was much less than the 7,000-fold induction observed for CYP1A1 mRNA at the 10,000 ng /kg dose. The authors state that this could be a result of the constitutive level of UGT1, which is much higher than CYP1A1, which makes detecting induction of UGT1 in the low dose regions more difficult. PAI2 and TGFα mRNA were not affected by TCDD in rat liver in the dose range tested. These results indicate that dioxin-inducible genes have a quite dissimilar dose-response relationship.

Induction of CYP1A1 expression is not considered an adverse effect, as the role of CYP1A1 in TCDD-mediated hepatotoxicity is unsettled. Therefore, in the absence of other indicators of hepatotoxicity, a NOAEL/LOAEL cannot be determined for this study. A LOEL

for TCDD of 1 ng/kg for a single exposure was identified for statistically significant ($p < 0.05$) increase in CYP1A1 mRNA levels. The NOEL for this study is 0.1 ng/kg.

2.4.2.4. *Subchronic Studies*

2.4.2.4.1. *Chu et al. (2001, 521829).*

Adult female Sprague-Dawley rats (five per treatment group) were administered TCDD (purity >99%) in corn oil by gavage at doses of 0, 2.5, 25, 250, or 1,000 ng/kg-day for 28 days (Chu et al., 2001, 521829). The 1,000 ng/kg-day dose of TCDD caused a significant ($p \leq 0.05$) decrease in body weight gain (36% lower than the control), increase in relative liver weight (40% greater than the control), and decrease in relative thymus weight (50% lower than the control). There was a significant ($p \leq 0.05$) increase in EROD activity, methoxy resoufin-O-deethylase (MROD) activity, and UDP-glucuronosyl transferase (UDPGT) activity in the liver of female rats receiving 250 or 1,000 ng/kg-day TCDD. In addition, significant ($p \leq 0.05$) increases in serum cholesterol were observed in the 250 and 1,000 ng/kg-day dose groups, and liver ascorbic acid (AA) also was significantly increased in the 1,000 ng/kg-day dose group. There was ~1.5-fold increase in liver glutathione-*S*-transferase (GST), which was not statistically significant. Other significant ($p \leq 0.05$) findings for the 1,000 ng/kg-day group included a decrease in liver vitamin A (51% lower than the control), an increase in kidney vitamin A (15.5-fold increase above the control), an increase in liver benzyloxy resoufin-O-deethylase (BROD, 30-fold increase above control), a decrease in liver pentoxyresoufin-O-deethylase (PROD, 37% lower than the control), increase in serum albumin (18% above the control), and a decrease in mean corpuscular hemoglobin (MCH, 7% below the control) and mean corpuscular volume (MCV, 7% below the control).

Based on the numerous significant ($p \leq 0.05$) liver-related biochemical changes and significant ($p \leq 0.05$) increased relative liver weight, as well as significantly decreased body weight and relative thymus weight, the LOAEL for 28 days of exposure in this study is 1,000 ng/kg-day and the NOAEL is 250 ng/kg-day.

2.4.2.4.2. *Chu et al., 2007.*

Chu et al. (2007) examined the potential impact of TCDD on various organs and the toxicological impacts as a result of interactions between TCDD and PCBs in rats. Groups of

female Sprague-Dawley rats (n = 5 per treatment group) were treated daily for 28 days via gavage with 0, 2.5, 25, 250, or 1,000 ng /kg-day TCDD (purity not specified) dissolved in corn oil. Body weights were determined three times per week, and clinical observations were made daily. At study termination, all animals were sacrificed and blood was analyzed for various biochemical and hematological parameters. Liver, spleen, heart, thymus, brain, and kidneys were removed and weighed. A small portion of the liver was homogenized and assayed for BROD; EROD; MROD; and PROD. UDPGT, GST, and ascorbic acid levels also were measured. Vitamin A levels in the liver, kidney, and lungs were analyzed as free retinol (vitamin A), and histopathological analysis was conducted on various tissues.

Growth rate and thymic weights in rats treated with 1,000 ng/kg-day TCDD were significantly ($p \leq 0.05$) inhibited compared to the control group. Enzyme analysis indicated that measured levels of TCDD in the liver correlated with hepatic microsomal enzyme activity. The authors reported that liver microsomal EROD and MROD activities were significantly ($p < 0.05$ for EROD activity, significance level for MROD not reported) increased in the 250 and 1,000 ng/kg-day TCDD dose groups compared to the control group. UDPGT levels were significantly (significance level not reported) increased in the 250 and 1,000 ng/kg-day TCDD dose groups compared to the controls. Serum albumin levels were significantly ($p < 0.05$) increased in the 1,000 ng/kg-day TCDD dose group compared to the control group. Serum cholesterol levels were significantly (level not reported) increased compared to the control group at 250 ng/kg-day TCDD dose, while liver ascorbic acid concentrations were significantly (level not reported) increased in the 1,000 ng/kg-day dose group. Hematological analysis indicated that hemoglobin, packed cell volume, MCH, MCV, and platelet values were decreased in the 1,000 ng/kg-day TCDD dose group. Significant ($p \leq 0.05$) differences were observed only in MCH and MCV levels compared to the control. Vitamin A levels in the liver and kidney were significantly ($p < 0.05$) lower in the 1,000 ng/kg-day TCDD group compared to the control group. Histopathological evaluation of various tissues indicated that liver, thyroid, and thymus were the target organs. No TCDD-related affects were found in other tissues. A dose-dependent alteration in the thymus consisted of reduced thymic cortex and increased medullar volume with more animals exhibiting these changes at the 250 and 1,000 ng/kg-day dose level compared to the control group. Alterations in thyroid included reduced follicles, reduced colloid density, and increased epithelial height. A dose-dependent change in the thyroid was observed, with the

highest impact evident in reduced follicles and reduced colloid density beginning at a dose of 25 ng/kg-day TCDD. Changes in liver were characterized by accentuated hepatic zones, anisokaryosis of hepatocytes, increased cytoplasmic density, and vacuolation. These changes were also dose dependent, with more animals exhibiting these histopathological changes with increasing TCDD dose. Based on these results, the study authors concluded that exposure to TCDD resulted in a wide range of adverse effects with the thyroid proving to be most sensitive.

A LOAEL for TCDD of 25 ng/kg for a 28-day exposure is identified for alterations in thyroid, thymus, and liver histopathology. The NOAEL for this study is 2.5 ng/kg-day.

2.4.2.4.3. ***DeCaprio et al. (1986, 197403).***

Hartley guinea pigs (10 per sex per dose) were administered TCDD (purity not specified) in the diet for 90 days at concentrations of 0, 2, 10, 76, or 430 ppt (equivalent to 0, 0.12, 0.61, 4.9, and 26 ng/kg-day in males and 0, 0.12, 0.68, 4.86, and 31 ng/kg-day in females calculated by the study authors using food consumption and body weights). Other animals were administered the high-dose diet (i.e., 430 ppt) for 11, 21, or 35 days and then administered the control diet (i.e., no exposure) for the remainder of the 90 days for recovery analysis. Four high-dose males died and two were sacrificed moribund by day 45; the remaining four animals were sacrificed on day 46 for necropsy. Four high-dose females also died and two were sacrificed moribund by day 55 with the remaining females sacrificed on day 60 for necropsy. Animals in the 76- and 430-ppt groups had significantly ($p < 0.05$) reduced body weights. Organ weights were not obtained in the 430-ppt group due to the early sacrifice, but in the 76-ppt group a significant decrease in relative thymus weight ($p < 0.05$) was observed, and relative liver ($p < 0.01$) and brain ($p < 0.05$) weights in males increased. Although a similar trend occurred in the females, the results were not statistically significant. Males administered 76 ppt in the diet also had a 53% increase in triglycerides ($p < 0.05$). The same increase was observed in females, but was not statistically significant. In the recovery groups, mortality during the recovery period after 11 or 21 days of treatment was 10% and after 35 days of treatment was 70%. Animals lost weight during the treatment period. Although the body weight increased during the recovery period, the body weight remained low compared to the control for the study duration.

The LOAEL from this study is 4.9 ng/kg-day for 90 days of exposure, based on decreased body weight (12−15%; $p < 0.05$) and changes in organ weights (10−30%, significant only in the males). The NOAEL is 0.61 ng/kg-day.

2.4.2.4.4. *Devito et al. (1994, 197278).*

Female B6C3F1 mice (5 per treatment) were administered 0, 1.5, 4.5, 15, 45, or 150 ng/kg TCDD (98% pure) in corn oil via gavage, 5 days a week for 13 weeks. This dose is equivalent to 0, 1.07, 3.21, 10.7, 32.1, 107 ng/kg-day (adjusted for continuous exposure, administered dose multiplied by 5 and divided by 7). Body weight was recorded weekly and animals were sacrificed 3 days after the last treatment. Examinations were performed on the lung, skin, uterus, and liver. No differences were observed in the liver or uterus weights or in the estrogen receptor levels in these two tissues. A dose-dependent increase in EROD activity (an indicator of CYP1A1 [CYP] induction) in the lung, skin, and liver was observed, with significant ($p < 0.05$) increases even at the lowest dose. The TCDD doses used did not achieve maximal EROD induction. A significant ($p < 0.05$) increase in liver acetanilide-4-hydroxylase (ACOH; an indicator of CYP1A2 induction) also was observed with all doses. A maximum induction of ACOH occurred with doses of 3.21 ng/kg-day and greater. A dose-dependent increase in specific phosphotyrosyl protein (pp) levels also was observed. Levels of pp34 and pp38 were significantly ($p < 0.05$) increased even at the lowest dose, while pp32 reached statistical significance ($p < 0.05$) with doses of 4.5 ng/kg-day and above.

The role of CYPs and phosphorylated pp32, pp34, and pp38 in TCDD-mediated toxicity is unknown, and changes in the activity or function of these proteins are not considered adverse Therefore, no LOAEL or NOAEL is established. The 13-week LOEL is 1.07 ng/kg-day, based on a significant ($p < 0.05$) increase in EROD, ACOH, pp34, and pp38 levels (all increased by at least 2-fold). No NOEL is established for this study.

2.4.2.4.5. *Fattore et al. (2000, 197446).*

Fattore et al. (2000, 197446) examined TCDD-induced reduction of hepatic vitamin A levels in a subchronic rat bioassay on Sprague-Dawley rats. Four experiments were conducted; Experiments 1, 2, and 3 were conducted in both male and female rats, while Experiment 4 was conducted only in female rats. The dosing regimens for each experiment were as follows

Experiment 1: Groups of six Iva:SIV 50 rats (male and female) were maintained on a diet consisting of 0, 200, 2,000, or 20,000 ng TCDD/kg diet and 3-μg vitamin A/kg diet for 13 weeks. Assuming food consumption of 10% of body weight per day, the average daily doses are 0, 20, 200, and 2,000 ng/kg-day TCDD.

Experiment 2: Groups of six male and female rats were treated with 0 or 200 ng TCDD/kg-day and 3 μg vitamin A/kg diet for 13 weeks.

Experiment 3: Groups of six male and female rats were fed 0, 200, or 1,000 ng TCDD/kg-day and 3 μg vitamin A/kg diet for 13 weeks.

Experiment 4: Groups of female rats (number not specified; IVA;SIV 50 Sprague-Dawley strain) were treated with TCDD for 26 and 39 weeks in addition to a 13-week dietary treatment with 0 or 100 ng TCDD/kg-day and 3 μg vitamin A/kg diet for 13 weeks.

For a 13-week exposure duration employed in all four experiments, male and female rats were treated at 0, 20, 100 (females only), 200, 1,000, or 2,000 ng/kg-day. In all four experiments, liver from control and treated animals was analyzed at termination for free retinol content to determine hepatic vitamin A levels.

***Results*:**

Experiment 1: Liver and body weights in both treated males and females were significantly affected at all but the lowest dose tested (20 ng/kg-day). Liver injury was severe, particularly in female rats treated with 2,000 ng TCDD/kg-day. Dietary intake of vitamin A in male rats was comparable to intake in controls, except in the 2,000 ng/kg-day group, which showed a reduction of 16% in the dietary intake of vitamin A compared to controls. There was no effect of TCDD on vitamin A intake in female rats. Hepatic vitamin A levels showed a dose-dependent reduction with levels dropping sharply in the 200 and 2,000 ng/kg-day dose groups, particularly in treated females. The reduction was significant at 200 ng/kg-day ($p < 0.05$) and 2,000 ng/kg-day ($p < 0.01$) in males, and at 200 ng/kg-day ($p < 0.5$) and 2,000 ng/kg-day ($p < 0.001$) in females. The reductions ranged from 68–99% in males and 72–99% in females when compared to corresponding controls.

Experiment 2: Changes in liver and body weights were not reported. Hepatic vitamin A level in males and females were reduced by 70% and 99%, respectively, compared to controls, in rats receiving 20 ng/kg-day (significance level in females: $p < 0.01$).

Experiment 3: Similar to the results of Experiments 1 and 2, a dose-related trend of significantly ($p < 0.001$) reduced hepatic vitamin A level was observed in both males and females, with males exhibiting a particularly sharp drop at the 1,000 ng/kg-day dose compared to controls.

Experiment 4: Females treated with 100 ng/kg-day showed significant reductions in hepatic vitamin A levels ($p < 0.05$–0.001) at all three treatment durations (13, 26, and 39 weeks).

A LOAEL for TCDD of 20 ng/kg-day for a 13-week subchronic exposure was identified in this study for decreased hepatic vitamin A levels (27 and 24 % lower than the corresponding control in female and male rats, respectively). This LOAEL is determined using data from Experiment 1. A NOAEL was not identified in this study.

2.4.2.4.6. *Fox et al. (1993, 197344).*

Sprague-Dawley rats (6 per sex per dose) were gavaged with TCDD (purity not specified) in corn oil using a dose-loading regime to achieve and maintain steady-state levels of 0.03, 30, or 150 ng/g in the liver. The regime consisted of an initial loading dose of 5, 2,500, or 12,000 ng/kg followed every 4 days with a maintenance dose of 0.9, 600, or 3,500 ng/kg. Averaging the doses over the 14 days provides average daily doses of 0.55, 307, and 1,607 ng/kg-day (e.g., 5 ng/kg-day on day 1 and 0.9 ng/kg-day on days 5, 9, and 13 is 5 + 0.9 + 0.9 + 0.9/14 = 0.55 ng/kg-day). Body weight, liver weight, and liver gene expression were measured at 7 and 14 days. A significant ($p < 0.05$) decrease in body weight occurred in high-dose males (at 14 weeks only) and females (at 7 and 14 days). A significant ($p < 0.05$) increase in absolute and relative liver weights was observed in mid- and high-dose males and females at both 7 and 14 days. Although the liver of treated animals indicated moderate vacuolization and swelling, there was no indication of necrosis. An increase in gene expression (clone 1, CYP1A1, CYP1A2, and albumin) was observed in the mid- and high-dose groups. A significant ($p < 0.05$) decrease in labeling index (indication of cell proliferation) occurred in both females (all doses) and males (high-dose only) during week 1, but not during week 2.

The 14-day LOAEL is 307 ng/kg-day for significant ($p < 0.05$) increases in absolute and relative liver weights (25—34%). The NOAEL is 0.55 ng/kg-day.

2.4.2.4.7. *Hassoun et al. (1998, 136626).*

Female B6C3F1 mice (number not specified) received TCDD (>98% pure) in corn oil 5 days per week for 13 weeks via gavage at doses of 0, 0.45, 1.5, 15, or 150 ng/kg (equivalent to 0, 0.321, 1.07, 10.7, and 107 ng/kg-day adjusted for continuous exposure; administered dose multiplied by 5 and divided by 7). Three days after the final dose, animals were sacrificed and brains were removed for oxidative stress testing. Biomarkers for oxidative stress included production of superoxide anion, lipid peroxidation, and DNA single-strand breaks. A significant

($p < 0.05$) increase was observed in superoxide anion production, lipid peroxidation as measured by thiobarbituric acid-reactive substances (TBARS), and DNA single-strand breaks with all doses tested.

No other indicators of brain pathology were assessed, and it is unfeasible to link the markers of oxidative stress to a TCDD-induced toxicological outcome in the brain. Thus, no LOAEL/NOAEL was established. The subchronic (13-week) LOEL is 0.32 ng/kg-day, based on significant ($p < 0.05$) increases in superoxide anion production (80% above control); lipid peroxide production (25% above the control); and DNA single-strand breaks (2-fold over the control). No NOEL is established.

2.4.2.4.8. *Hassoun et al. (2000, 197431).*

Hassoun et al. (2000, 197431) examined the effect of subchronic TCDD exposure on oxidative stress in hepatic and brain tissues. Groups of 8-week-old female Harlan Sprague-Dawley rats (6 rats/group) were administered TCDD (98% purity, dissolved in 1% acetone in corn oil) via gavage at 0, 3, 10, 22, 46, or 100 ng/kg-day, 5 days/week for 13 weeks (0, 2.14, 7.14, 15.7, 32.9, or 71.4 ng/kg-day adjusted for continuous exposure; administered doses were multiplied by 5 and divided by 7 days/week). Animals were sacrificed at the end of the study period, and brain and liver tissues were collected and used to determine the production of reactive oxygen species, lipid peroxidation, and DNA single-strand breaks (SSBs).

A dose-dependent effect was observed in both the liver and brain tissue as a result of TCDD treatment. Based on the maximal induction of superoxide anion by various doses, more production of superoxide anion was observed in the liver tissue when compared to the brain tissue with an observed increase of 3.1- and 2.2-fold respectively, when compared to the control group. A similar dose-dependent effect was observed in the induction of lipid peroxidation in TCDD-treated animals with an approximately 1.8-fold increase in lipid peroxidation in both tissues relative to the corresponding controls. A dose-dependent relationship was also observed for DNA SSBs in both the hepatic and brain tissues at all TCDD-treated doses compared to controls. Increases were statistically significant ($p \leq 0.05$) beginning at the lowest administered dose.

Similar to the statement above, because no adverse endpoints were measured, no LOAEL/NOAEL was established. However, a LOEL for TCDD of 2.14 ng/kg-day for a

13-week exposure duration was identified in this study for significant increases ($p \leq 0.05$) in superoxide anion, lipid peroxidation, and DNA SSBs in the liver and brain tissues. A NOEL cannot be determined for this study.

2.4.2.4.9. *Hassoun et al. (2003, 198726).*

Hassoun et al. (2003, 198726) examined the role of antioxidant enzymes in TCDD-induced oxidative stress in various regions of the rat brain after subchronic exposure. Groups of 8-week-old female Harlan Sprague-Dawley rats (12 rats/group) were administered TCDD (98% purity, dissolved in 1% acetone in corn oil) via gavage at 0, 10, 22, or 46 ng/kg-day (0, 7.14, 15.7, or 32.9 ng/kg-day adjusted for continuous exposure; administered doses were multiplied by 5 and divided by 7) daily for 13 weeks. Animals were sacrificed at the end of the study period and the brain was immediately removed and dissected to the following regions: cerebral cortex (Cc), hippocampus (H), cerebellum (C), and brain stem including midbrain, pons, and medulla. Four pooled samples from each region per dose (i.e., 3 animals/pooled sample) were used in the study. Dissected regions were subsequently assayed for lipid peroxidation (thiobarbituric acid reactive substances, or TBARS), superoxide dismutase, catalase, and glutathione peroxidase. Because the cytochrome c reduction method was used to determine superoxide anion (SA) production in brain tissues, superoxide dismutase (SOD) was added to some of the brain tissue samples that had the highest SA production (tissue homogenates from Cc and H from rats treated with 46 ng/kg-day TCDD).

A dose-dependent increase in the production of SA was observed in the Cc and H, but significant changes in SA production were not observed in either the C or the mid-brain, pons, or medulla brain stem cells. Similar to SA production, there was a dose-dependent increase in the production of TBARS in the Cc and H regions of the brain, but no significant changes were observed in either the C or the B sections of the brain. The study authors also measured the activities of various enzymes as a result of TCDD treatment and reported a dose-dependent increase in SOD activity in the C and B sections, while there was dose-dependent suppression in SOD activity in Cc and H. In contrast, catalase activity was significantly ($p < 0.05$) increased in H and Cc at the 10 ng/kg-day TCDD dose level compared to controls and the mid- and high-dose animals. Catalase activity also was increased in a dose-dependent manner in the C section, but no significant changes in the activity of this enzyme were observed in the B section at any of the

three TCDD tested doses. The effects of subchronic exposure to different doses of TCDD on glutathione stimulating hormone peroxidase (GSH-Px) showed a different response compared to other enzymes. There was a dose-dependent increase in the activity of this enzyme in the C and B regions of the brain, while a significant increase in the activity of GSH-Px occurred in Cc and H only at the 10 ng/kg-day TCDD dose. In addition, the activity of this enzyme was suppressed in a dose-dependent manner in the Cc and H at 22 and 46 ng/kg-day TCDD doses. Based on these results, the study authors concluded that induction of oxidative stress by TCDD in the rat brain occurs mainly in the Cc and H regions.

Similar to the statement above, because no adverse endpoints were measured, no LOAEL/NOAEL was established. However, a LOEL for TCDD of 7.14 ng/kg-day for a 13-week exposure duration was identified for this study for increases in superoxide anion and lipid peroxidation production, as well as increased activity in SOD, catalase, and GSH-Px.

2.4.2.4.10. ***Kociba et al. (1976, 198594).***

Adult Sprague-Dawley rats (12 per sex per treatment group) were administered TCDD (purity not reported) in corn oil via gavage 5 days per week at doses of 0, 1, 10, 100, or 1,000 ng/kg-day (equivalent to 0, 0.71, 7.14, 71.4, or 714 ng/kg-day averaged over 7 days; 5/7 of dose). Five animals per group were sacrificed at the end of treatment, and the remaining animals were observed over 13 weeks post treatment (only initial results for the post-treatment period were provided in the report). Body weights and food consumption were measured semiweekly. Hematology and clinical chemistry were measured after 36−37 or 85−86 days of treatment and 59−60 days after termination of treatment. Forty-eight hour urine samples were collected from select rats from 85−89 days of treatment and 52−56 days after cessation of treatment. Gross and histopathological exams were conducted on the tissues.

Four high-dose females died during treatment. Two high-dose females and two high-dose males died during the post-treatment period. Animals treated with 714 ng/kg-day were less active during the treatment period, which became less evident during the post-treatment period. Yellow discoloration of the external pinnae also was noted in this group, both during treatment and during the post-treatment period. A significant ($p < 0.05$) reduction in body weight and food consumption was observed in the 71.4 and 714 ng/kg-day groups. The following significant ($p < 0.05$) hematology changes were observed in the high-dose

(714 ng/kg-day) males at all measured time points: decreased packed cell volume, decreased red blood cells, decreased hemoglobin, increased reticulocytes, and decreased thrombocytes. Significant ($p < 0.05$) changes also occurred in the high-dose females, but the only consistent observation was a decrease in thrombocytes and increased leukocytes. Significant changes in clinical chemistry ($p < 0.05$) and urinalysis ($p < 0.05$) were more consistent between the sexes in the high-dose group and included increases in total and direct serum bilirubin; increase in serum alkaline phosphatase; decreased urinary creatinine; and increased urinary coproporphyrin, uroporphyrin, and delta-amino-levulinic. The following significant ($p < 0.05$) changes were observed in the 71.4 ng/kg-day group: decreased packed cell volume (4−9%) in males; decreased red blood cells (2−10%) in males; decreased hemoglobin (2−13%) in males; increased urinary coproporphyrin (2.2-fold increase during treatment) in females; increased urinary delta-amino-levulinic (47% increase during treatment) in females; increased total and direct serum bilirubin (48−61%) in females; and increased serum alkaline phosphatase (2-fold) in females. The following significant ($p < 0.05$) changes in relative organ weights were observed increased brain weight in 714 ng/kg-day males and females; increased liver weight in males (71.4 and 714 ng/kg-day) and females (7.14, 71.4, and 714 ng/kg-day); increased spleen weight in 714-ng/kg-day males and females; decreased thymus weight in 71.4 and 714 ng/kg males and females; and increased testes weight in 714 ng/kg-day males. Microscopic changes were observed in the thymus, and in other lymphoid tissues, and in the liver in rats treated with 71.4 ng/kg-day or greater.

The subchronic (13-week) LOAEL is 71.4 ng/kg-day, based on the numerous changes noted in body weight, hematology, clinical chemistry, urinalysis, and histopathology. The NOAEL is 7.14 ng/kg-day.

2.4.2.4.11. ***Mally and Chipman (2002, 198098).***

Female F344 rats (3 per treatment group) were administered TCDD at concentrations of 0, 2.5, 25, or 250 ng/kg in corn oil via gavage for either 3 consecutive days or 2 days per week for 28 days (Mally and Chipman, 2002, 198098). The average daily doses for the 28-day study when adjusted for 7 days a week were 0, 0.71, 7.1, and 71 ng/kg-day (i.e., 2/7 of administered dose). No clinical signs of toxicity were observed. Histological examination of the liver revealed no abnormalities. All doses of TCDD reduced the number of connexin (Cx) 32 plaques

and Cx32 plaque area in the liver, which was considered the target tissue. The reductions were not statistically significant after the 3-day treatment, but were significant after the 28-day treatment ($p < 0.05$). TCDD also caused a reduction in the Cx32 plaque number and area in the thyroid after 28 days, but the results were not statistically significant. Although the reduction in Cx32 plaque number and plaque area in the liver and thyroid occurred at all dose levels, there was no relation to dose. TCDD did not induce hepatocyte proliferation.

In the absence of additional indicators of hepatotoxicity, changes in Cx32 plaques are not clearly linked to TCDD-mediated hepatotoxicity, nor are they considered an adverse effect. Additionally, no toxicologically-relevant endpoints were examined. Therefore, a NOAEL or LOAEL cannot be determined. A 28-day LOEL at the lowest dose of 0.71 ng/kg-day for significantly ($p < 0.05$) decreased Cx32 plaque area is evident (approximately 70% of the controls).

2.4.2.4.12. *Slezak et al. (2000, 199022).*

Slezak et al. (2000, 199022) studied the impact of subchronic TCDD exposure on oxidative stress in various organs of B6C3F1 female mice. Groups of 8- to 10-week-old female B6C3F1 mice (number not specified) were administered TCDD (purity >98%, dissolved in corn oil) via gavage at 0, 0.15, 0.45, 1.5, 15, or 150 ng/kg-day (0, 0.11, 0.32, 1.07, 10.7, or 107.14 ng/kg-day adjusted for continuous exposure) 5 days per week for 13 weeks. Three days after the last treatment, the animals were sacrificed and organs were removed for the measurement of oxidative stress indicators including SA, lipid peroxidation (TBARS), and GSH-Px. Tissue TCDD concentrations also were measured.

The study authors reported that TCDD dose range resulted in overlapping tissue concentrations for liver, lung, kidney and spleen. Liver had the highest TCDD concentration, with each tissue demonstrating a dose-dependent increase in TCDD concentration. Compared to controls, SA production was significantly ($p < 0.05$) lower at the 0.15 ng/kg-day TCDD dose, while it was significantly ($p < 0.05$) higher at 15 and 150 ng/kg-day. A dose-dependent increase in hepatic TBARS production was observed, although the rate of production was significant ($p < 0.05$) only at the highest TCDD administered dose (150 ng/kg-day) compared to controls. AA also followed the same pattern observed for SA and TBARS with AA production significantly ($p < 0.05$) increased at the 15 and 150 ng/kg-day TCDD doses. Contrary to the SA,

TBARS, and AA responses, GSH levels were decreased at 0.15 ng/kg-day, were increased at 0.45 and 150 ng/kg-day, and did not change at 1.5 or 15 ng/kg-day when compared to the control group. Unlike the liver, there was no significant increase in SA production in the lung at any of the TCDD tested doses; a dose dependent reduction, however, was observed at 0.45, 15, and 150 ng/kg-day compared to controls. GSH and AA production was decreased at 0.15 ng/kg-day, while AA production was significantly ($p < 0.05$) increased at 15 and 150 ng/kg-day. Kidney SA production showed a statistically significant ($p < 0.05$) increase only at the 15 and 150 ng/kg-day doses. GSH, like the liver and the lung, exhibited a decrease in production following treatment at 0.15 ng/kg-day with this trend continuing at 0.45 and 1.5 ng/kg-day. AA levels were significantly ($p < 0.05$) lower at all subchronic doses, except at 1.5 ng/kg-day dose. SA levels in the spleen differed little from the control group at any of the TCDD doses. Total GSH was higher only at the 150 ng/kg-day dose level, while the AA levels were significantly ($p < 0.05$) decreased at 0.15, 1.5, and 150 ng/kg-day.

Similar to the statements regarding the Hassoun et al. studies above, because no adverse endpoints were measured, no LOAEL/NOAEL was established. Therefore, a NOAEL or LOAEL cannot be determined. However, a NOEL and LOEL of 1.07 and 10.7 ng/kg-day, respectively, are identified in this study for increases in superoxide anion in the liver.

2.4.2.4.13. *Smialowicz et al. (2008, 198341).*

Female B6C3F1 mice (8–15 per treatment group) were administered TCDD (purity >98%) in corn oil by gavage at doses of 0, 1.5, 15, 150, or 450 ng/kg-day, 5 days a week for 13 weeks (1.07, 10.7, 107, or 321 ng/kg-day, adjusted for continuous exposure; i.e., 5/7 of the dose) (Smialowicz et al., 2008, 198341). Mice were immunized 3 days after the final TCDD exposure with an intravenous injection of an optimal concentration of 4×10^7 SRBCs and sacrificed 4 days later. No TCDD-related effects on body weight were observed. There was a dose-related decrease in relative spleen weight (9–19% lower than control values) with statistically significant ($p < 0.05$) decreases at all but the lowest dose. Additionally, there was a statistically significant ($p < 0.05$) increase in relative liver weight (5–21%) in all treatment groups compared to controls. Statistically significant dose-dependent decreases were observed in the antibody response to SRBCs (24–89% lower than control values), as measured by both the number of plaque forming cells per 10^6 cells and plaque forming cells per spleen.

The 13-week LOAEL for this study is 1.07 ng/kg-day based on a significant ($p < 0.05$) increase in relative liver weight (10%) and a significant ($p < 0.05$) decrease in antibody response to SRBCs (24%). A NOAEL cannot be determined for this study.

2.4.2.4.14. ***Van Birgelen et al. (1995, 197096; 1995, 198052)***

Van Birgelen et al. (1995, 197096; 1995, 198052) studied the impact of TCDD exposure on various biochemical endpoints in rats. Groups of 7-week-old female Sprague-Dawley rats ($n = 8$ per treatment group) were treated with 0, 200, 400, 700, 5,000, or 20,000 ng/kg TCDD (purity >99%) in diet for 13 weeks. Daily TCDD intake based on food consumption, diet level, and mean weight was estimated to be 0, 14, 26, 47, 320, or 1,024 ng/kg-day. Blood samples were collected from treated animals and assayed for retinol (vitamin A), triiodothyronine, and total (TT4) and free (FT4) thyroxine. At study termination, the animals were sacrificed and the liver, thymus, spleen, and kidneys were removed and weighed. Parts of the liver were homogenized and assayed to determine EROD; CYP1A1; CYP1A2; and UDPGT activity. Liver samples also were analyzed for retinol content.

TCDD-treated animals showed a dose-related decrease in food consumption. Animals treated with 1,024 ng/kg-day TCDD consumed 32% less food compared to controls. Similarly, a dose-related decrease in body weight gain was observed in all animals treated with TCDD. Animals treated with ≥47 ng/kg-day of TCDD showed a statistically significant ($p < 0.05$) decrease in body weight gain. Relative liver weights were significantly ($p < 0.05$) increased in the 320 and 1,024 ng/kg-day TCDD dose groups compared to the controls. Absolute and relative thymus weights were significantly ($p < 0.05$) decreased at all TCDD dose groups compared to the control group. Relative kidney and spleen weights were significantly ($p < 0.05$) higher in animals dosed with ≥47 ng/kg-day of TCDD compared to the control group, with the greatest increase occurring in animals treated with 1,024 ng/kg-day TCDD (121 and 173% higher than controls for kidney and spleen, respectively). Cytochrome P450 enzymes, including EROD, CYP1A2, CYP1A1, and UDPGT, exhibited statistically significant ($p < 0.05$) increases in activity at all TCDD dose groups compared to the control group. TT4 and FT4 thyroid hormone concentrations were statistically significantly ($p < 0.05$) decreased only at TCDD doses ≥47 ng/kg-day. A dose-dependent increase was observed in the plasma retinol concentrations with significant ($p < 0.05$) increases occurring at ≥47 ng/kg-day TCDD after a 13-week

exposure. A dose-dependent reduction in liver retinoid levels also was observed after 13 weeks of TCDD exposure with the levels dropping significantly ($p < 0.05$) at all TCDD-treated doses compared to the control group.

A LOAEL for TCDD of 14 ng/kg for a 13-week exposure is identified for significantly ($p < 0.05$) decreased absolute and relative thymus weights and significantly ($p < 0.05$) decreased liver retinoid levels. A NOAEL cannot be determined for this study.

2.4.2.4.15. ***Vos et al., (1973, 198367).***

Vos et al. (1973, 198367) conducted a study to examine the immune response in laboratory animals treated with TCDD. In one experiment, 10 female Hartley strain guinea pigs were orally treated with 8 weekly doses of 0, 8, 40, 200, and 1,000 ng/kg TCDD in corn oil (purity of TCDD not specified) (0, 1.14, 5.71, 28.6, and 143 ng/kg-day adjusted for continuous exposure; administered dose divided by 7). At study termination, the animals were sacrificed, and heart blood was used to determine total leukocyte and differential leukocyte counts. In another experiment, the effect of TCDD on humoral immunity was determined by injecting 0.1 mL of tetanus toxoid into the right hind-foot pad on day 28 (1 left foot tetanus toxoid, aluminum phosphate-adsorbed) and again on day 42 (1 left foot tetanus toxoid, unadsorbed). Blood was collected ($n = 10$) on days 35 and 49, and the serum tetanus-antitoxin concentrations were determined using a modified single radial immunodiffusion technique.

All guinea pigs receiving 1,000 ng/kg-day TCDD either died or were killed when moribund between 24 and 32 days. These animals showed severe weight loss, lymphopenia, and depletion of the lymphoid organs, especially the thymus. Microscopic observations revealed severe atrophy of the thymic cortex with substantial destruction of lymphocytes, with the nuclear debris being engulfed by macrophages. Large cystic Hassall bodies, filled with polymorphonuclear leukocytes were observed in the medulla. All animals treated with 0, 8, 40, or 200 ng/kg-day TCDD survived until study termination. Body weight gain was significantly ($p < 0.01$) lower in the 200 ng/kg-day group. Absolute thymus weight was significantly reduced in the 40 and 200 ng/kg-day treatment groups ($p < 0.01$ and $p < 0.05$, respectively). In contrast, relative thymus weight was significantly ($p < 0.01$) reduced only in the 200 ng/kg-day dose group. The absolute weight of the superficial cervical lymph nodes was significantly ($p < 0.05$) decreased in the 200 ng/kg-day group, while the relative adrenal weight was significantly

($p < 0.05$) increased in the 200 ng/kg-day dose group. Total leukocyte count was significantly ($p < 0.05$) decreased in the 40 ng/kg-day dose group and total lymphocyte count was significantly decreased at 8, 40, and 200 ng/kg-day ($p < 0.01$, $p < 0.05$, and $p < 0.05$, respectively). A significant (p-values not provided) monotonic dose-response relationship was determined for body weight (decrease), relative thymus weight (decrease), relative adrenal weight (increase), and total leukocyte and lymphocyte count (decrease). Microscopic examination of the lymphoid organs and adrenals showed no effects, while slight cortical atrophy of the thymus was observed at the 200 ng/kg-day dose.

Animals receiving the tetanus toxoid injection showed a small but significant increase in serum tetanus antitoxin concentrations at the 8 and 40 ng/kg-day dose ($p < 0.05$ and $p < 0.01$, respectively). Measurement at days 49 and 56 indicated that serum antitoxin levels had decreased sharply and the significant ($p < 0.05$ on day 49 and $p < 0.01$ on day 56) effect was seen only at the 200 ng/kg-day dose level.

A LOAEL for TCDD of 5.71 ng/kg-day for an 8-week exposure is identified in this study for significantly ($p < 0.01$) reduced absolute thymus weight, significantly ($p < 0.05$) reduced leukocyte and lymphocyte count, and significantly ($p < 0.01$) increased serum tetanus antitoxin concentration. The NOAEL for this study is 1.14 ng/kg-day.

2.4.2.4.16. ***White et al. (1986, 197531).***

White et al. (1986, 197531) studied the impact of TCDD exposure on serum complement levels. Groups of female (C57BL/6 × C3H)F1(B6C3F1) mice were treated for 14 consecutive days with TCDD in corn oil (purity of TCDD not specified) at doses of 0, 10, 50, 100, 500, 1,000 or 2,000 ng/kg-day via gastric intubation ($n = 6–8$). At study termination, blood was collected from anesthetized animals and assayed for serum complement activity and complement component C3 levels.

Serum complement activity between the 10 and 100 ng/kg-day doses was between 69 and 59% compared to the vehicle control group, with all treatment groups being significantly ($p < 0.05$) low compared to the vehicle control. In contrast, C3 levels were comparable to the vehicle control with levels ranging between 98 and 94% of the control group. The higher doses of 500, 1,000, and 2,000 ng/kg-day, however, produced a marked decrease of the component

hemolytic activity (45, 35, and 19% of the vehicle control) and of C3 levels (91, 81, and 74 % of the vehicle control, respectively; significance level at $p < 0.05$).

A LOAEL for TCDD of 10 ng/kg-day for a 14-day exposure is identified in this study for significantly ($p < 0.05$) lower serum complement activity. A NOAEL cannot be determined for this study.

2.4.2.5. *Chronic Studies (Noncancer Endpoints)*

2.4.2.5.1. *Cantoni et al. (1981, 197092).*

CD-COBS rats (4 per treatment) were orally administered TCDD (purity not specified) dissolved in acetone:corn oil (1:6) at doses of 0 (vehicle alone), 10, 100, or 1,000 ng/kg per week (equivalent to 1.43, 14.3, and 143 ng/kg-day adjusted for continuous exposure, administered dose by dividing the dose by 7) for 45 weeks. Urine was collected several times during treatment and tested for porphyrin excretion. Twenty-four hours after the final dose, animals were sacrificed and their livers, spleens, and kidneys were removed for analysis of total porphyrins. All treatment groups had a significant ($p < 0.05$) increase in coproporphyrin excretion beginning at 6, 3, or 2 months, respectively. Uroporphyrin excretion was significantly ($p < 0.05$) increased in the 14.3 ng/kg-day group at 10 months and in the 143 ng/kg-day group beginning at 6 months. The high-dose group also had a significant ($p < 0.05$) increase in excretion of heptacarboxylic methyl ester beginning at 6 months. The high-dose group had a marked porphyric state beginning at 8 months as indicated by a 70-fold increase above controls in total urinary porphyrin excretion. This group also had a significant ($p < 0.05$) increase in total porphyrins in the liver, kidneys, and spleen.

The 45-week LOAEL for this study is 1.43 ng/kg-day, based on a 2- to 3-fold increase in urinary coproporphyrin excretion. No NOAEL was established for this study.

2.4.2.5.2. *Croutch et al. (2005, 197382).*

Croutch et al. (2005, 197382) examined the impact of TCDD exposure on body weight via insulin-like growth factor (IGF) signaling. Female Sprague-Dawley rats were randomly assigned in groups of five to initial loading doses of TCDD (purity >98.5%, dissolved in corn oil) at 0, 12.5, 50, 200, 800, or 3,200 ng/kg-day, followed by treatment with maintenance doses equivalent to 10% of the initial loading dose every third day to maintain a pharmacokinetic

steady state throughout the entire study (equivalent to: 14-day average = 0, 1.25, 5, 20, 80, or 320 ng/kg-day; 28-day average = 0, 0.85, 3.4, 13.6, 54.3, or 217 ng/kg-day; 63-day average = 0, 0.60, 2.4, 9.5, 38, or 152 ng/kg-day; and 128-day average dose = 0, 0.51, 2.0, 8.1, 32.5, or 130 ng/kg-day). Following 2, 4, 8, 16, 32, 64, or 128 days of initial dosing, the animals were sacrificed, livers were removed and weighed, and trunk blood was collected to analyze glucose content. Rat liver phosphoenolpyruvate carboxykinase (PEPCK) mRNA and protein levels also were analyzed, and PEPCK activity was measured.

Body weights of TCDD-treated animals decreased after the second week of the 3,200 ng/kg-day TCDD loading dose, with significant differences beginning at week 9. There was also a statistically significant ($p \leq 0.05$) difference in body weights at weeks 10, 11, 13, 18, and 19 at the highest loading dose (3,200 ng/kg-day). PEPCK activity in the liver was also decreased in a dose-dependent manner following TCDD administration at approximately 16 days. PEPCK inhibition was statistically significant ($p \leq 0.05$) on day 4 in rats treated with either 800 or 3,200 ng/kg-day TCDD when compared to animals treated with a loading dose of 200 ng/kg-day. A similar statistically significant change was observed in animals treated with 3,200 ng/kg-day on day 16 when compared to the 200 ng/kg-day treatment group. In contrast, differences in PEPCK activity at other doses or time points were not statistically significant. In TCDD-treated animals, there was also a dose-dependent decrease in PEPCK mRNA expression along with a decrease in PEPCK protein levels in the liver. In addition to body weight and PEPCK activity changes, animals treated with 3,200 ng/kg-day TCDD showed a sharp decline in circulating IGF-I levels on day 8 compared to the control group (corn oil) and TCDD-treated animals at lower doses. In the highest dose animals, IGF-I levels continued to decline to 42% of the control group by day 16 of the study. The IGF-I levels at the highest dose plateaued at an average decrease of 66% through day 128 when compared to controls. Beginning at day 8, the decrease in IGF-I was statistically significant at every time point through day 128 compared to the control group, as well as groups treated with either 12.5 or 50 ng/kg-day TCDD. Similar statistically significant decreases also were observed for the 800 ng/kg-day TCDD-treated groups with an initial decrease of 37% on day 16 followed by a further decline to approximately 45% thereafter compared to controls and the 12.5, 50, and 200 ng/kg-day dose groups. In contrast to these results, circulating levels of insulin and glucose were unaffected by TCDD treatment, while

the active or phosphorylated form of AMPK-α protein increased with dose as a result of TCDD treatment.

A LOAEL for TCDD of 217 ng/kg-day for a 28-day exposure duration (because this represented the most sensitive time for elicitation of effects) was identified in this study for decreased body weight, significant ($p \leq 0.05$) inhibition of PEPCK activity, and reduced IGF-I levels (42% lower than the control group). A NOAEL of 54.3 ng/kg-day was identified in this study.

2.4.2.5.3. *Hassoun et al. (2002, 543725).*

Hassoun et al. (2002, 543725) examined the potential of TCDD and other dioxin-like chemicals to induce oxidative stress in a chronic rat bioassay. Groups of six Harlan Sprague-Dawley female rats were treated with 0, 3, 10, 22, 46, or 100 ng/kg-day TCDD (98% purity), 5 days a week via gavage for 30 weeks. The administered doses adjusted for continuous exposure were 0, 2.14, 7.14, 15.7, 32.9, and 71.4 ng/kg-day, respectively (administered doses were multiplied by 5 and divided by 7). At study termination, hepatic and brain tissues from all treated rats were divided into two portions and examined for the production of reactive oxygen species and SSBs in DNA.

When compared to controls, there was a dose-dependent increase in the production of superoxide anion in TCDD-treated animals ranging from 21–998% and 66–257% in hepatic and brain tissues, respectively. Hepatic tissues had statistically significant ($p < 0.05$) increases in superoxide anion production at doses ≥7.14 ng/kg-day, while the brain tissue had a statistically significant ($p < 0.05$) increase over controls at all doses. Similarly, increases in lipid peroxidation were observed in hepatic and brain tissues with a 481% increase ($p < 0.05$) at 71.4 ng/kg-day in the hepatic tissue when compared to controls. The increase in lipid oxidation in brain tissue ranged from 33–188% ($p < 0.05$) in the 2.14–71.4 ng/kg-day dose groups. DNA SSBs were also observed in both hepatic and brain tissue in all treated groups. When compared to the control group, there was a dose-dependent statistically significant ($p < 0.05$) increase in DNA SSBs ranging from 58–322% and 29–137% in hepatic and brain tissues, respectively. Nonmonotonic dose-response relationships were observed for superoxide production and lipid peroxidation in liver tissues, with greater-than-linear increases in effect between the two highest dose levels.

As stated above, because no adverse endpoints were measured, no LOAEL/NOAEL was established. However, a LOEL for TCDD of 2.14 ng/kg-day for a 30-week exposure duration is identified in this study for significant ($p < 0.05$) increases in superoxide anion, lipid peroxidation production, and DNA SSBs in the liver and brain tissues. A NOEL cannot be determined for this study.

2.4.2.5.4. ***Kociba et al. (1978, 001818).***

Sprague-Dawley rats (50 per sex per treatment group) were administered TCDD (purity >99%) in the diet at doses of 0, 1, 10, or 100 ng/kg-day for 2 years. Body weights and food consumption were routinely measured. Hematology, clinical chemistry, and urinalysis were measured after 3, 12, or 23 months of treatment. Animals were routinely palpitated for tumors. Gross and histopathological exams were conducted on the tissues of dead or dying animals or at terminal sacrifice. Specific organs also were weighed.

The high-dose females had a statistically significant ($p < 0.05$) increase in mortality compared to the controls during the second half of the study. Mortality changes in males were variable and of questionable toxicological significance. A significant ($p < 0.05$) reduction in body weight occurred in the 100 ng/kg-day males and females beginning at 6 months. Mid-dose females also had reduced body weight, but to a lesser degree during the same time frame. There were no consistent changes in food consumption. The following significant ($p < 0.05$) hematology changes were observed in the high-dose animals: decreased packed cell volume in males after 3 months and in females after 1 year, decreased red blood cells in females after 1 year and in males at terminal sacrifice, decreased hemoglobin in males after 3 months and in females after 1 year, and decreased total white blood cell count in females after 1 year. Changes in clinical chemistry ($p < 0.05$) occurred only in high-dose females and consisted of an increase in serum alkaline phosphatase and gamma glutamyl transferase. Significant changes in urinalysis occurred only in females and included increased urinary coproporphyrin in the mid- and high-dose groups, increased urinary uroporphyrin in the mid- and high-dose groups, and increased urinary delta-amino-levulinic acid in the high-dose group. Significant ($p < 0.05$) changes in relative organ weights were observed, including increased liver weight in mid- and high-dose females and decreased thymus weight in high-dose females. Mid- and high-dose rats showed hepatocellular degeneration and inflammatory and necrotic changes in the liver. Thymic

and splenic atrophy were noted in high-dose females. An increase in non-neoplastic lung lesions was noted in mid-dose females and high-dose males and females. High-dose females had an increase in uterine changes. High-dose males had a significant ($p < 0.05$) increase in the incidence of stratified squamous cell carcinomas of the tongue. High-dose males and females had a significant ($p < 0.05$) increase in the incidence of squamous cell carcinomas of the hard palate/turbinates.

The chronic (2-year) LOAEL is 10 ng/kg-day, based on the numerous significant ($p < 0.05$) changes noted in coproporphyrin excretion (67% increase above control) and an increase in liver and lung lesions in female rats. The NOAEL is 1 ng/kg-day.

2.4.2.5.5. *Maronpot et al. (1993, 198386).*

An initiation-promotion study was performed in female Sprague-Dawley rats (8−10 rats per group). Rats were initiated with saline or diethylnitrosamine (DEN), followed 2 weeks later by promotion with biweekly administration of TCDD (purity not specified) in corn oil via gavage for 30 weeks. The doses were stated to be equivalent to 3.5, 10.7, 35.7, or 125 ng/kg-day. Rats were sacrificed 7 days after the final treatment. A significant ($p < 0.05$) decrease in body weight occurred in the 125 ng/kg-day group. A significant ($p < 0.05$) increase in relative liver weight occurred in the 35.7 and 125 ng/kg-day groups. There was a significant ($p < 0.05$) increase in the labeling index in the 125 ng/kg-day group, but only with DEN initiation. In the TCDD-alone group, a 2-fold increase in labeling index occurred in the 125 ng/kg-day group that did not reach statistical significance. A significant ($p < 0.05$) trend for increased alkaline phosphatase levels was observed in TCDD-treated animals, but despite a 50% increase in the highest dose group the increase was not statistically significant. Total cholesterol and triglycerides were significantly ($p < 0.05$) higher in the 125 ng/kg-day TCDD-alone group. A significant ($p < 0.05$) increase in 5'-nucleotidase occurred in the 35.7 and 125 ng/kg-day TCDD-alone groups. A dose-dependent increase in the incidence and severity of liver toxicity as measured by microscopic lesions was observed.

The 30-week LOAEL is 35.7 ng/kg-day, based on a significant ($p < 0.05$) increase in relative liver weight (12%, accompanied by increases in incidence and severity of liver lesions). The 30-week NOAEL is 10.7 ng/kg-day.

2.4.2.5.6. *National Toxicology Program (1982, 543764).*

National Toxicology Program (NTP, 1982, 543764) conducted a carcinogenic bioassay of TCDD on rats and mice. Fifty male and female Osborne-Mendel rats and male and female B6C3F1 mice were treated twice per week with TCDD (purity not specified) in corn oil via oral gavage at doses of 0, 5, 25, or 250 ng/kg for rats and male mice (1.4, 7.1, 71 ng/kg-day adjusted for continuous exposure; administered doses multiplied by 2 and divided by 7) and 0, 20, 100, or 1,000 ng/kg for female mice (5.7, 28.6, or 286 ng/kg-day adjusted for continuous dosing; administered doses multiplied by 2 and divided by 7) for 104 weeks. Seventy-five rats and mice of each sex served as vehicle controls. One untreated control group of 25 rats and mice of each sex was present in the TCDD treatment room and one untreated control group consisting of 25 rats and mice of each sex were present in the vehicle-control room. Animals surviving until study termination were sacrificed at 105 or 108 weeks. A complete histopathological evaluation was conducted on all animals.

Survival rates were not affected by TCDD exposure in rats or mice of either sex. Male rats exhibited a dose-related depression in mean body weight after week 55, while the females exhibited a dose-related body-weight depression after 45 weeks of TCDD exposure. However, the magnitude of the body weight response is not indicated. Mean body weights in male and female mice were comparable to the vehicle control group throughout the bioassay. Noncancer histopathologic findings included increased incidences of liver lesions (termed toxic hepatitis) from TCDD exposure, and were detected in the high-dose rats and high-dose mice of each sex.

A LOAEL for TCDD of 1.4 ng/kg-day for a 104-week exposure duration is identified for increased incidences of liver lesions in mice of both sexes. A NOAEL cannot be determined for this study.

2.4.2.5.7. *National Toxicology Program (2006, 197605).*

Female Sprague-Dawley rats (81 control; 82 treatment group) were administered TCDD (purity >98%) in corn oil:acetone (99:1) via gavage at doses of 0, 3, 10, 22, 46, or 100 ng/kg-day, 5 days per week for 105 weeks (0, 2.14, 7.14, 15.7, 32.9, or 71.4 ng/kg-day, adjusted for continuous exposure) (NTP, 2006, 197605). In addition to this primary group, a stop group of 50 animals was administered 100 ng/kg-day TCDD in corn oil:acetone (99:1) via gavage for 30 weeks and then just the vehicle for the remainder of the study. Up to 10 rats per

dose group were sacrificed and evaluated at 14, 31, or 53 ($n = 8$) weeks for biologically noteworthy changes in the incidences of neoplasms or non-neoplastic lesions in the liver, lung, oral mucosa, uterus, pancreas, thymus, adrenal cortex, heart, clitoral gland, ovary, kidney, forestomach, bone marrow, mesentery gland, and pituitary gland. All interim sacrifice animals also received a complete necropsy and microscopic examination, and the following organs were weighed: the left kidney, liver, lung, left ovary, spleen, thymus (14 weeks only), and thyroid gland. Out of 53 control animals and 53 or 54 animals per treatment group not used for interim sacrifice analyses, at study termination the number of surviving animals had declined to 25 in the control group and to 21, 23, 19, 22, and 21 in five treatment groups, respectively, due to accidental deaths, moribund animals, or death due to natural causes.

Survival rate was not affected by TCDD treatment. Mean body weights in the high dose primary study group and the 100 ng/kg stop group were less than the vehicle control group after week 13 of the study. The mean body weights of animals in the 46 ng/kg-day group were less than in the vehicle control at study termination (2 years), whereas animals in the 22 ng/kg-day had lower mean body weights compared to controls during the last 10 weeks of study. In addition to body weight changes, liver weights were also impacted as a result of TCDD exposure. Absolute and relative liver weights were significantly (either $p \leq 0.01$ or $p \leq 0.05$) higher in all dose groups compared to controls at the 14- and 31-week evaluation period, whereas the relative liver weights were significantly (either $p \leq 0.01$ or $p \leq 0.05$) higher only at ≥10 ng/kg-day at 53 weeks.

No clinical findings associated with TCDD treatment were observed. TCDD caused changes in thyroid hormone levels at 14, 31, and 53 weeks. The following changes were statistically significant ($p \leq 0.05$) compared to the vehicle control: decrease in TT4 at doses ≥22 ng/kg-day at 14 and 31 weeks and at doses ≥46 ng/kg-day at 53 weeks; decrease in FT4 at doses ≥22 ng/kg-day at 14 and 31 weeks; increase in total T_3 at doses ≥46 ng/kg-day at 14 and 31 weeks and at doses ≥10 ng/kg-day at 53 weeks; and increase in TSH at doses ≥46 ng/kg-day at 14 weeks. There was a statistically-significant ($p \leq 0.05$) increase in hepatocyte proliferation at 14 weeks (22 ng/kg-day group only); 31 weeks (all doses); and 53 weeks (≥46 ng/kg-day). There were statistically significant ($p \leq 0.01$) dose-dependent increases in liver (includes EROD [CYP1A1-associated] activity; 7-pentoxyresorufin-O-deethylase [PROD; CYP2B-associated] activity; and acetanilide-4-hydroxylase [CYP1A2-associated] activity) and lung (EROD)

cytochrome P450 enzyme activities in all treatment groups at all three evaluation periods compared to the vehicle control group. The largest effect was an 82-fold induction of hepatic EROD activity in the 46 ng/kg-day group at 31 weeks.

TCDD was detected at the greatest concentration in the liver, followed by fat tissue, with tissue concentration increasing in both of these tissues in a dose-dependent manner. TCDD tissue levels generally remained constant after the first measurement at week 14. Pathological examination at week 14 revealed increased incidences of hepatocellular hypertrophy in animals administered ≥10 ng/kg-day TCDD. Examinations at weeks 31 and 53 indicated that incidence and or severity of hepatocellular hypertrophy was increased at all treatment doses although incidences were statistically significant ($p \leq 0.05$) only at ≥10 ng/kg-day doses. The incidence of non-neoplastic hepatic lesions (including inflammation, necrosis, multiple eosinophilic focus, diffuse fatty change, pigmentation, toxic hepatopathy) in the liver increased at doses ≥22 ng/kg-day beginning at 14 weeks. Severity of the lesions increased at 14 weeks at doses ≥46 ng/kg-day and were also observed at lower dose levels during later evaluation periods (31 and 53 weeks). By terminal sacrifice, numerous non-neoplastic changes were noted in TCDD treated rats, even at the lowest dose tested.

Noncancer cardiovascular and pulmonary effects were evident after 2 years of TCDD exposure. Significantly increased incidences of minimal to mild cardiomyopathy were seen in male and female rats at ≥10 ng/kg-day. In the lung, there was a significant ($p \leq 0.01$) dose-dependent increase, when compared to the vehicle control, in the incidence of bronchiolar metaplasia of the alveolar epithelium at all dose groups in the primary study.

A LOAEL for TCDD of 2.14 ng/kg-day adjusted dose for a 105-week exposure duration is identified in this study for significantly (either $p \leq 0.01$ or $p \leq 0.05$) increased absolute and relative liver weights, increased incidence of hepatocellular hypertrophy, and increased incidence of alveolar to bronchiolar epithelial metaplasia. A NOAEL cannot be determined for this study.

2.4.2.5.8. ***Rier et al. (2001, 198776; 2001, 543773).***

Female rhesus monkeys (8 per treatment group) were administered 0, 5, or 25 ppt TCDD (purity not specified) in the diet for 4 years. Previously, Bowman et al. (1989, 543745) determined that these dietary concentrations were equivalent to 0, 0.15, and 0.67 ng/kg-day, respectively. Thirteen years after termination of TCDD treatment, serum concentrations of

TCDD and dioxin-like polyhalogenated aromatic hydrocarbons (PHAH) were measured in six control monkeys, six monkeys treated with 0.15 ng/kg-day, and three monkeys treated with 0.67 ng/kg-day (Rier et al., 2001, 198776). Even after 13 years without treatment, there was significantly ($p < 0.05$) elevated serum levels of TCDD and other dioxin-like compounds in treated monkeys. There was a significant increase in triglycerides and total lipids in the serum of monkeys treated with either 0.15 or 0.67 ng/kg-day, but not in cholesterol or phospholipids. In addition to these 15 animals, 8 other female monkeys (4 treated with 0.67 ng/kg-day TCDD that died 7 to 11 years after treatment and 4 lead-treated animals with no history of PHAH exposure) were evaluated for endometriosis. Elevated serum concentrations of TCDD were not correlated with endometriosis. Increased serum levels of 3,3',4,4'-tetrachlorobiphenyl (TCB), however, were associated with the presence and severity of endometriosis ($p < 0.05$). TCB was found in none of the animals without endometriosis, including TCDD-treated animals, nor was it found in control animals with endometriosis. Animals with elevated serum levels of TCB, pentachlorobiphenyl, and total serum analyte TCDD equivalents (TEQ) had an increased incidence of endometriosis, but severity was associated only with increased levels of TCB. EPA did not develop a LOAEL for TCDD for this study, because of DLC contamination.

In a separate study that evaluated the same 15 monkeys 13 years after exposure, Rier et al. (2001, 543773) examined effects on systemic immunity. Peripheral blood mononuclear cells (PBMC) obtained from untreated monkeys secreted no detectable levels of TNF-α in response to T-cell mitogen exposure. There was, however, a significant ($p < 0.05$) dose-dependent increase in TNF-α production in PBMC from the TCDD-treated monkeys. Although PBMC from treated monkeys with endometriosis produced more TNF-α than cells from unexposed controls without the disease (median 128 pg/mL compared to not detected; $p < 0.01$), PBMC from TCDD-treated animals without endometriosis also produced more TNF-α than controls (median 425 pg/mL, $p < 0.067$). TNF-α production from the animals without endometriosis, however, was much more variable and was not statistically significant compared to controls. In addition, there was a dose-related but statistically insignificant decrease in PBMC cytotoxicity against natural killer-sensitive RAJI cells in TCDD-treated animals compared to the unexposed controls. The results were again related to TCDD exposure and not the presence of endometriosis. TCDD alone was not associated with changes in PBMC surface antigen expression, but increased serum levels of TCDD. 1,2,3,6,7,8-Hexachlorodibenzofuran and

3,3',4,4',5-pentachlorobiphenyl were correlated with increased numbers of CD3+/CD25- and CD3-/CD25+ leukocytes, as well as increased secretion of TNF-α in response to T-cell mitogen exposure. Although TNF-α production is considered to be a general indicator of inflammation, relative adversity of increased TNF-α secreted by PBMCs in and of itself cannot be substantiated in the absence of concurrent physiological measurements of an inflammatory response. Therefore, neither a LOAEL nor NOAEL can be determined for this study.

2.4.2.5.9. ***Sewall et al. (1993, 197889).***

Sewall et al. (1993, 197889) examined the impact of TCDD exposure on the hepatic epidermal growth factor receptor (EGFR) as a critical effect in hepatocarcinogenicity. In two separate experiments, groups of 6- to 8-week-old female Sprague-Dawley rats were randomly assigned to the following groups: control group, receiving saline and corn oil; a promoted group that received four different doses of TCDD along with saline; a DEN-only initiated control group; and a DEN and TCDD initiated and promoted group that received four different doses of TCDD. DEN was administered via intraperitoneal injection at a dose of 175 mg/kg [saline (S) vehicle] as the initiating agent to animals that were 70 days old. The control animals received saline only. In the first experiment, each treatment group (S/TCDD and DEN/TCDD) that included sham-operated or ovariectomized and intact animals were treated with TCDD (purity >98%) at 125 ng/kg-day. In the second dose-response experiment, DEN-initiated and saline control treatment groups (intact animals, 84 days old) were administered TCDD (purity >98%) in corn oil via oral gavage once every 2 weeks for 30 weeks at doses equivalent to 0, 3.5, 10.7, 35.7, or 125 ng/kg-day ($n = 9$). A week after the last treatment, all animals were sacrificed and livers were harvested and fixed for immunohistochemistry. Sections of the fixed liver were tested for EGFR binding, EGFR autophosphorylation, immunolocalization of EGFR, and hepatic cell proliferation.

In the first experiment, intact animals treated with 125 ng/kg-day TCDD exhibited a 65% reduction in EGFR binding capacity. In contrast, the EGFR equilibrium maximum binding capacity (B_{max}) of the ovariectomized rats was not statistically different from the ovariectomized control rats, and no changes in the K_d were detected in any treatment group. In the dose-response experiment with intact animals, a significant ($p < 0.05$) TCDD dose-dependent decrease in the B_{max} of EGFR was shown. A two-factor, five-level ANOVA indicated that the

effect of TCDD exposure on EGFR B_{max} was significant ($p = 0.0001$), whereas, the effect of DEN treatment on EGFR B_{max} was not significant. Comparative analysis using Fisher's protected least significant difference indicated that the lowest TCDD dose resulting in a statistically significant ($p < 0.05$) decrease in the EGFR B_{max} was 10.7 ng/kg-day S/TCDD group. At the highest TCDD dose of 125 ng/kg-day, the EGFR B_{max} was reduced by 38% compared to controls in both the DEN initiated and noninitiated groups. A two-factor, five-level ANOVA showed no significant effect on EGFR K_d in either the DEN- or the TCDD-treated groups. The EGFR autophosphorylation assay indicated that, with increasing TCDD dose, the amount of EGFR autophosphorylation in DEN/TCDD-treated animals decreased. The study authors state that this decrease is similar to the dose-response alterations observed for the EGFR B_{max}. Additionally, EGFR autophosphorylation in control and 125 ng/kg-day noninitiated animals was similar to the corresponding dose levels for the DEN-treated animals, suggesting that DEN treatment did not affect the EGFR or the EGFR response to TCDD under the experimental conditions. The immunolocalization assay indicated that staining was more apparent in the centrilobular and midzonal regions of the liver in the DEN initiated control animals, whereas, the amount of hepatocyte plasma membrane staining in DEN/TCDD treated animals substantially decreased. The cell proliferation assay showed a decrease in the cell labeling index in the 3.5 ng/kg-day DEN/TCDD dose group that was statistically less ($p \leq 0.05$) than the labeling index for the control group. In contrast, the labeling index for the 125 ng/kg-day DEN/TCDD treatment group was significantly ($p \leq 0.05$) higher compared to controls. Except for the low-dose (3.5 ng/kg-day) group, a clear dose-response trend (two mid-level doses were not statistically significant) was observed in the other three TCDD treated groups.

The role of EGFR in TCDD-mediated hepatotoxicity is unknown, and as such, this endpoint cannot be unequivocally linked to TCDD-induced hepatotoxicity nor labeled as adverse. Thus, no LOAEL/NOAEL was established. A LOEL for TCDD of 3.5 ng/kg-day for a 30-week exposure duration was identified in this study for a significant ($p = 0.0001$ using ANOVA) decrease in EGFR B_{max} levels. A NOEL cannot be determined for this study.

2.4.2.5.10. *Sewall et al. (1995, 198145).*

Sewall et al. (1995, 198145) studied the dose-response relationship for thyroid function alterations in female rats as a result of TCDD exposure. Groups of female Sprague-Dawley rats were initiated with DEN at 70 days of age at a dose of 175 mg/kg in a saline vehicle via an i.p. injection. DEN was administered as a liver-initiating agent for a concurrent study to determine TCDD promotion of hepatic preneoplastic foci. Saline-treated animals served as controls. At 84 days of age, both the DEN-initiated and the saline-noninitiated groups of animals were administered TCDD (purity >98%) or corn oil vehicle via oral gavage once every 2 weeks for 30 weeks at dose levels equivalent to 0, 0.1, 0.35, 1.0, 3.5, 10.7, 35.7, or 125 ng/kg-day ($n = 9$ per group). One week after the last TCDD treatment, the animals were sacrificed and the thyroid was removed and fixed for further analysis. Blood was drawn from the abdominal aortic vein, and the serum was isolated and preserved for hormone analysis. Liver was also removed and prepped for further analysis. Thyroid hormone analysis was performed to determine serum TSH, T3, and T4 levels using radioimmunoassay kits. Histological examination was conducted on eosin-stained sections of the thyroid tissue. RNA level in the hepatic tissue was determined using a reverse transcription polymerase chain reaction (RT-PCR) technique.

TCDD treatment did not affect thyroid weight. A dose-dependent decrease in serum T4 levels was observed in both noninitiated and DEN-initiated animals with T4 levels dropping significantly ($p < 0.05$) at the 35 and 125 ng/kg-day TCDD doses in the noninitiated group. Compared to the noninitiated control group, DEN alone did not significantly affect T4 levels. Serum T3 level in the 125 ng/kg-day treatment group was slightly elevated but was not significantly different from levels in the control group. TSH levels in DEN initiated rats were increased at a dose of 3.5 ng/kg-day. In the noninitiated group, TSH level in the 125 ng TCDD/kg-day group was 3.27 ± 0.34 ng/mL ($n = 9$) compared to 1.3 ± 0.18 ng/mL in the corn oil control group ($n = 7$). This result, in conjunction with the T4 data, demonstrates that TCDD had a similar effect on thyroid hormone levels in both the noninitiated and DEN initiated groups. Histological sections examined for nodular lesions or neoplasms exhibited thyroid follicular adenoma in one DEN/corn oil control animal. The DEN/TCDD-treated animals exhibited diffuse follicular hyperplasia, with the size of colloidal follicles decreasing with TCDD treatment. Other qualitative DEN/TCDD-related changes included increased frequency of abnormally shaped follicles. The study authors reported that image analysis demonstrated a

significant ($p = 0.013$) TCDD dose-related decrease in mean follicle size along with a significant ($p = 0.001$) TCDD dose-related increase in parenchymal area. Additionally, like T4 and TSH levels, DEN treatment alone or in combination with TCDD did not influence thyroid follicular or C-cell morphology.

RT-PCR results for UGT1 and CYP1A1 mRNA levels indicated that the amount of UGT1 mRNA at the 125 ng/kg-day dose was approximately 2.5-fold higher compared to the concurrent controls. The study authors also stated that the maximal response for the UGT1 mRNA levels was reached at a dose between 1.0 and 3.5 ng TCDD/kg-day. In contrast, the maximum induction of CYP1A1 mRNA was 260-fold higher at the 125 ng/kg-day compared to the concurrent controls.

A LOAEL for TCDD of 35 ng/kg-day for a 30-week exposure duration was identified in this study for a significant ($p < 0.05$) decrease in T4 levels. The NOAEL for this study is 10.7 ng/kg-day.

2.4.2.5.11. ***Toth et al. (1979, 197109).***

Toth et al. (1979, 197109) examined the impact of TCDD exposure on the formation of liver tumors in male mice. Ten-week-old, outbred Swiss/H/Riop male mice were administered sunflower oil or TCDD (purity not specified; in sunflower oil) at 0, 7, 700 or 7,000 ng/kg (0, 1, 100, or 1,000 ng/kg-day adjusted for continuous dosing; administered dose divided by 7; $n = 38$, 44, 44, and 43, respectively) once per week via gastric tube for 1 year. Once exposure had ceased, animals were followed for the rest of their lives. After spontaneous death or when mice were moribund, autopsies were performed and all organs were examined histologically.

Average life span in the 1,000 ng/kg-day dose group decreased considerably (72%) when compared to the control group. TCDD also caused dose-dependent, severe chronic and ulcerous skin lesions (12, 30, and 58% in the 1, 100, and 1,000 ng/kg-day dose groups, respectively) that was followed by generalized lethal amyloidosis (12, 23, and 40% in the 1, 100, and 1,000 ng/kg-day dose groups, respectively).

A LOAEL for TCDD of 1 ng/kg-day for 1-year exposure duration was identified in this study for severe chronic and ulcerous skin lesions (12% higher than controls), and generalized lethal amyloidosis (12% higher than controls). A NOAEL cannot be determined for this study.

2.4.2.6. *Chronic Studies (Cancer Endpoints)*

2.4.2.6.1. *Della Porta et al. (1987, 197405).*

Della Porta et al. (1987, 197405) studied the long-term carcinogenic effects of TCDD in B6C3F1 (C57BL/6JDp × C3Hf/Dp) mice. Six-week-old male and female mice (initially about 15/sex/dose, and increased by approximately 30 to 40 per group within a few weeks) were administered 0, 2,500, and 5,000 ng/kg TCDD (purity not provided) in corn oil by oral gavage once per week for 52 weeks (0, 357, and 714 ng/kg-day adjusted for continuous exposure). At ages 31 to 39 weeks, 41 male mice and 32 female mice in the 2,500 ng/kg dose group were mistakenly administered a single dose of 25,000 ng/kg TCDD. TCDD treatment for the 2,500 ng/kg dose group was halted for 5 weeks (beginning the week after the 25,000 ng/kg dose was administered in error) and resumed until exposure was terminated at 57 weeks. Mortality was observed and body weights recorded at unspecified intervals until 110 weeks of age, when all surviving animals were sacrificed and necropsied. Histopathological analysis was conducted on the following organs and tissues: Harderian glands, pituitary, thyroid, adrenals, tongue, esophagus, and trachea; lungs, liver, pancreas; spleen, kidneys, and bladder; testes, ovaries, and uterus, mesenteric lymph nodes, small intestine, and all other organs with presumed pathological changes.

Body weights of both male and female mice exposed to 2,500 and 5,000 ng/kg TCDD were markedly lower than in the corresponding control groups (statistical significance not reported). Relative to the controls, a significant ($p < 0.001$), dose-related decrease in survival occurred in animals treated with either dose of TCDD. In the subset of animals treated inadvertently with a single dose of 25,000 ng/kg TCDD, mortality in male mice increased shortly after this treatment; females, however, did not show a mortality increase following the inadvertent treatment. This mortality in male mice was associated with subcutaneous edema, degenerative hepatocyte changes, and bile duct hyperplasia. The incidence of non-neoplastic lesions (such as amyloidosis of the liver, spleen, adrenals, and pancreas), liver necrosis, and nephrosclerosis, was increased in mice exposed to TCDD compared to controls (statistical significance not reported).

The study authors used two statistical tests to analyze tumor incidence. Because of the increased mortality in treated groups compared to controls, one test, which assumes all tumors are fatal, overestimated the differences between the treated and control groups. The second test

assumes that all tumors are incidental and resulted in an underestimation of TCDD effects. Both tests were used to analyze the results for nonthymic lymphomas and hepatic adenomas and carcinomas. Incidence of nonthymic lymphomas (6/45, 4/51, and 3/50 in the 0, 2,500, and 5,000 ng/kg dose groups, respectively in males and 17/49, 21/42, and 17/48 in the 0, 2,500, and 5,000 ng/kg dose groups, respectively in females) was significantly ($p < 0.05$ in males and $p < 0.01$ in females) higher in TCDD-treated animals compared to the corresponding controls using the fatal tumor test. However, the incidental tumor test showed that this higher incidence was not significant. Similarly, a significantly ($p < 0.001$) higher incidence of hepatocellular adenomas occurred in male mice using the fatal tumor test (10/43, 11/51, and 10/50 in the 0, 2,500, and 5,000 ng/kg dose groups, respectively), but the incidence was not significant when assessed using the incidental tumor test. Hepatocellular carcinomas in males were significant ($p < 0.001$) using either the fatal or incidental tumor tests (5/43, 15/51, and 33/50 in the 0, 2,500, and 5,000 ng/kg dose groups, respectively). In female mice, hepatocellular adenomas were significant using both the fatal ($p < 0.01$) and incidental ($p < 0.001$) tumor tests (2/49, 4/42, and 11/48 in the 0, 2,500, and 5,000 ng/kg dose groups, respectively). Similar results for female mice were obtained for incidence of hepatocellular carcinomas (1/49, 12/42, and 9/48 in the 0, 2,500, and 5,000 ng/kg dose groups, respectively), which also were significant using both the fatal ($p < 0.01$) and incidental ($p < 0.05$) tumor tests. TCDD-related incidences of other tumor types in both sexes were uniformly low and comparable in the treatment and control groups.

These results indicate that TCDD is carcinogenic in male and female B6C3F1 mice, causing hepatocellular adenomas and carcinomas in both sexes.

In addition to the long term bioassay results in mice described by Della Porta et al. (1987, 197405), carcinogenic effects of TCDD in a neonatal bioassay were reported in the same publication. Briefly, groups of male and female B6C3F1 and B6CF1 (C57/BL6J × BALB/c) mice were treated with 0, 1000, 30,000 or 60,000 ng/kg BW TCDD via intraperitoneal (i.p.) injection beginning at postnatal day 10. Animals were treated once weekly for 5 weeks and then observed until 78 weeks of age. However, because this study utilized i.p. injection as the route of TCDD exposure, it does not qualify for further consideration based on the study selection criterion that the study design consist of orally administered TCDD.

2.4.2.6.2. *Kociba et al. (1978, 001818).*

As discussed above, Kociba et al. (1978, 001818) conducted a lifetime (2-year) feeding study of male and female Sprague-Dawley rats using doses of 0, 1, 10, and 100 ng/kg-day. There were 50 males and 50 females in each group.

With respect to the cancer endpoints examined, the most significant finding was an increase in hepatocellular hyperplastic nodules and hepatocellular carcinomas in female rats. The incidence of hepatocellular carcinomas was significantly elevated above the control incidence at the 100 ng/kg-day dose, whereas increased incidence of hyperplastic nodules was evident in the 10 ng/kg-day dose group.

There have been two reevaluations of slides of liver sections from the Kociba et al. study (Goodman and Sauer, 1992, 197667; Sauer, 1990, 198829; Squire, 1990, 548781). The Squire Review was requested by EPA as an independent review of the slides. The Sauer Review was carried out using refined criteria for the diagnosis of proliferative hepatocellular lesions (Maronpot et al., 1986, 013967; Maronpot et al., 1989, 548778). Liver tumor incidences for the three evaluations are compared in Appendix F. Although there are some quantitative differences between the evaluations, the lowest detectable effect for liver tumor incidence is consistently observed at 10 ng/kg-day.

In the 10 ng/kg-day dose group, significant increases in the incidence of hyperplastic nodules of the liver were observed in female rats (18/50 in the Kociba evaluation, 27/50 in the Squire evaluation). Two females (2/50) had hepatocellular carcinomas. In the 1990 reevaluation (Goodman and Sauer, 1992, 197667; Sauer, 1990, 198829), nine females (9/50) were identified with hepatocellular adenomas and none with carcinomas; thus only one-third of the previously observed "tumors" were identified when using the refined diagnostic criteria. As discussed below, the tumor reclassification of Goodman and Sauer (1992, 197667) was used in the dose-response modeling for the Kociba et al. (1978, 001818) data set.

In addition to nodules in the liver, increased incidence of stratified squamous cell carcinoma of the tongue and nasal turbinates/hard palate, and keratinizing squamous cell carcinoma of the lung were also observed in female rats in the 100 ng/kg-day dose group. One possible cause for the induction of lung tumors in the Kociba feeding study may have been the aspiration of dosed feed into the lungs. However the promotion of lung tumors has been observed in mice treated systemically by intraperitoneal (i.p.) injections of TCDD (Beebe et al.,

1995, 548754). In addition the induction of hyperplastic and metaplastic lesions in rats has been observed following chronic oral gavage treatment with TCDD (Tritscher et al., 2000, 197265). More recently, chronic oral exposure to HCDD resulted in the induction of lung tumors in treated female rats (Rozman, 2000, 548758). These data indicate that the induction of lung tumors in the Kociba was most likely primarily the result of systemic chronic dietary exposure to TCDD rather than due to a localized exposure to aspired dosed feed.

There was no detectable increase in liver tumor incidences in male rats in any of the dose groups. The mechanism responsible for dioxin-mediated sex specificity for hepatocarcinogenesis in rats is not clear, but may involve ovarian hormones (Lucier et al., 1991, 199007).

Although there was no increase in liver tumors in male rats in this study, in the 100 ng/kg-day group, there was an increased incidence of stratified squamous cell carcinoma of the hard palate/nasal turbinate, stratified squamous cell carcinoma of the tongue, and adenoma of the adrenal cortex.

Kociba et al. (1978, 001818) had reported that chemically related increases in preneoplastic or neoplastic lesions were not found in the 1 ng/kg-day dose group. However, Squire identified two male rats in the 1 ng/kg-day dose group with squamous cell carcinoma of the nasal turbinates/hard palate, and one of these male rats had a squamous cell carcinoma of the tongue. These are both rare tumors in Sprague-Dawley rats, and these sites are targets for TCDD, implying that 1 ng/kg-day may not represent a NOEL. However, no dose-response relationships were evident for tumors at these sites (Huff et al., 1991, 197981)

There is considerable controversy concerning the possibility that TCDD-induced liver tumors are a consequence of cytotoxicity. Goodman and Sauer (1992, 197667) have extended the reevaluation of the Kociba slides to include liver toxicity data and have reported a correlation between the presence of overt hepatotoxicity and the development of hepatocellular neoplasms in female rats. With the exception of two tumors in controls and one each in the low- and mid-dose groups, all liver tumors occurred in livers showing clear signs of toxicity. However, male rat livers exhibit cytotoxicity in response to high TCDD doses, yet they do not develop liver tumors. Moreover, both intact and ovariectomized female rats exhibit liver toxicity in response to TCDD, yet TCDD is a more potent promoter in intact but not ovariectomized rats (Lucier et al., 1991, 199007). Therefore, if cytotoxicity is playing a role in liver tumorigenesis, other factors must

also be involved. Also, there is little information on the role of cytotoxicity in TCDD-mediated cancer at other sites such as the lung and thyroid.

2.4.2.6.3. *Toth et al. (1979, 197109).*

In a study of 10-week-old outbred male Swiss/H/Riop mice, Toth et al. (1979, 197109) administered oral gavage TCDD doses of 0, 7, 700, and 7,000 ng/kg-day in sunflower oil weekly for 1 year (0, 1, 100, or 1,000 ng/kg-day adjusted for continuous dosing; see details above). All mice (100/group) were followed for their entire lives. The study authors identified the effective number of mice in each group to be the number of surviving animals when the first tumor-bearing animal was identified. The average lifespan of the control, low, mid and high dose groups was 588, 649, 633, and 424 days, respectively.

In the 100 ng/kg-day dose group, liver tumor incidence was twice that of the control group and was statistically significant ($p < 0.01\%$). A dose-related increase in liver tumor incidence was observed (18, 29, 48, and 30% in the control and three TCDD-treated groups, respectively) in all treated mice. Increases were not statistically significant, however, at 1 and 1,000 ng/kg-day. The study authors also stated that spontaneous and induced liver tumors were not histologically different. Additionally, the ratio of benign hepatomas to hepatocellular carcinomas in the control group was not affected by treatment and an increase was observed only in the absolute number of liver tumors. Cirrhosis was not observed with the tumors.

2.4.2.6.4. *NTP (1982, 543764).*

As discussed above, the NTP (1982, 543764) study was conducted using Osborne-Mendel rats and B6C3F1 mice (NTP, 1982, 543764). Groups of 50 male rats, 50 female rats, and 50 male mice received TCDD as a suspension in corn oil:acteone (9:1) by gavage twice each week at doses of 0, 5, 25, or 250 ng/kg-day (daily averaged doses of 0, 1.4, 7.1, or 71 ng/kg-day for rats and male mice and doses of 0, 5.7, 28.6, or 286 ng/kg-day for female mice.

There were no statistically significant dose-related decreases in survival in any sex-species group. TCDD-induced malignant liver tumors occurred in the high-dose female rats and in male and female mice. These can be considered to result from TCDD exposure because they are relatively uncommon lesions in control Osborne-Mendel rats (male, 1/208; female,

3/208), are seen in female rats and mice of both sexes, and their increasing incidence with increasing dose is statistically significant (Cochran-Armitage trend test, $p = 0.004$). Because liver tumors were increased in both sexes of mice, this effect is not female-specific as was observed in rats. Interestingly, liver tumor incidences were decreased in female rats in both the NTP and Kociba low doses (not statistically significant compared with controls). For example, the combined control incidence data were 11/161 (7%) compared with 4/99 (4%) in the low-dose group.

The incidences of thyroid gland (follicular cell) tumors were increased in all three dose groups in male rats. Because the responses in the two highest dose groups are highly significant, the statistically significant elevation of incidence in the lowest dose group (Fisher exact p-value = 0.042) is considered to be caused by exposure to TCDD, suggesting that thyroid tumor incidence may be the most sensitive site for TCDD-mediated carcinogenesis. Because 71 ng/kg-day is above the maximum tolerated dose (MTD) (Huff et al., 1991, 197981), thyroid tumors occur at doses more than 50 times lower than the MTD.

TCDD-induced neoplasms of the adrenal gland were observed in the 7.1 ng/kg-day/dose group in male rats and in high-dose female rats. Fibrosarcomas of the subcutaneous tissue were significantly elevated in high-dose female mice and female rats. One additional tumor type, lymphoma, was seen in high-dose female mice. Lung tumors were elevated in high-dose female mice; the increase was not statistically significant when compared with concurrent controls, but the increase was dose related (Cochran-Armitage trend test, $p = 0.004$).

Huff (1992, 548757) concluded, based on the NTP bioassay results, that TCDD was a complete carcinogen and induced neoplasms in rats and mice of both sexes. As was observed in the Kociba study (1978, 001818), liver tumors were observed with greater frequency in treated female rats, but in male rats the thyroid appears to be the most sensitive (increased tumor incidence at doses as low as 1.4 ng/kg-day).

2.4.2.6.5. *NTP (2006, 197605).*

As discussed above, female Sprague-Dawley rats (53 control; 53 or 54 animals per treatment group) were administered TCDD (purity >98%) in corn oil:acetone (99:1) via gavage at doses of 0, 3, 10, 22, 46, or 100 ng/kg-day, 5 days per week for 105 weeks (0, 2.14, 7.14, 15.7, 32.9, or 71.4 ng/kg-day, adjusted for continuous exposure) (NTP, 2006, 197605). In addition to

this primary group, a stop-dose group of 50 animals was administered 100 ng/kg-day TCDD in corn oil:acetone (99:1) via gavage for 30 weeks and then just the vehicle for the remainder of the study. At study termination, the number of surviving animals had declined to 25 in the control group and to 21, 23, 19, 22, and 21 in five treatment groups, respectively, due to accidental deaths, moribund animals, or death due to natural causes.

Incidence of hepatocellular adenomas was significantly ($p < 0.001$) increased in the 100 ng/kg-day dose group in the primary study and exceeded incidences seen in historical vehicle control range at study termination. A dose-related increase in the incidence of cholangiosarcoma was seen in the primary study group in animals receiving 22 ng/kg-day or higher doses of TCDD. The high dose group of 100 ng/kg-day had the highest incidence of cholangiosarcoma with a significant ($p < 0.001$) number of animals exhibiting multiple cholangiosarcomas. Such an incidence was not seen in historical vehicle controls. In contrast, only two cholangiosarcomas and hepatocellular adenomas were seen in the 100 ng/kg-day group in the stop-exposure study.

In the lung, at 2 years, there was a significantly ($p = 0.002$) increased incidence of cystic keratinizing epithelioma in the 100 ng/kg-day dose group of the primary study, while there were no epitheliomas in the 100 ng/kg-day group of the stop-exposure study. There was also a significant ($p \leq 0.01$) dose-dependent increase, when compared to the vehicle control, in the incidence of bronchiolar metaplasia of the alveolar epithelium at all dose groups in the primary study. Squamous metaplasia was also present in the 46 and 100 ng/kg-day dose groups in the primary study, and was also observed in the 100 ng/kg-day dose group in the stop-exposure study.

A positive trend in the incidence of gingival squamous cell carcinoma of the oral cavity was seen at all doses (except 22 ng/kg-day), with the incidence significantly ($p = 0.007$) high in the 100 ng/kg-day dose group. In addition, the occurrence of this lesion in the 46 and 100 ng/kg-day group of the primary study and 100 ng/kg-day group of the stop-exposure study exceeded the historical control range. The incidence of gingival squamous hyperplasia was significantly (either $p \leq 0.01$ or $p \leq 0.05$) increased in all dose groups of the primary study as well as the 100 ng/kg-day group of the stop-exposure study.

In the uterus, at 2 years, there was a significantly ($p = 0.032$) higher rate of squamous cell carcinoma in the 46 ng/kg-day group compared to vehicle controls. In addition there were

two squamous cell carcinomas in the 100 ng/kg-day group of the stop-exposure study. No squamous cell carcinomas have been reported in historical vehicle controls.

These results indicate that TCDD is carcinogenic to female Sprague-Dawley rats and causes tumors at multiple sites.

2.4.3. Summary of Key Data Set Selection for TCDD Dose-Response Modeling

To meet the NAS' concerns regarding transparency and clarity in the identification of TCDD studies for dose-response assessment, EPA has, in this section, developed and applied two sets of criteria for animal bioassays and epidemiologic studies. EPA has collected and evaluated these studies, including studies from the 2003 Reassessment and newer studies found via literature searches and through public submissions. Tables 2-4 and 2-5 contain the final lists of key cancer and noncancer studies, respectively, that have met EPA's inclusion criteria for epidemiologic data. Tables 2-6 and 2-7 provide the final lists of key studies that have met EPA's inclusion criteria for animal bioassay data for cancer and noncancer studies, respectively. Collectively, these four tables contain the final set of key studies that EPA has used to develop noncancer and cancer dose-response assessments for TCDD in Sections 4 and 5 of this document, respectively. In Sections 4 and 5, additional evaluations are made to determine which study/endpoint data sets are the most appropriate for development of the RfD and OSF for TCDD, using statistical criteria, dose-response modeling results and decisions regarding toxicological relevance of the endpoints. The approaches taken to select the final candidate study/endpoint data sets are discussed in Sections 4 and 5 and are illustrated in Figures 4-1, 4-2 and and 5-3 of those sections.

Table 2-1. Summary of epidemiological cancer studies (key characteristics)

Publication	Length of follow-up	Latency period	Half-life for TCDD	Fraction of TEQs accounted for by TCDD
NIOSH cohort studies				
Fingerhut et al. (1991, 197375)	1942–1987	0, 20 years	N/A	N/A
Steenland et al. (1999, 197437)	1942–1993	0, 15 years	N/A	N/A
Steenland et al. (2001, 197433)	1942–1993	0, 15 years	8.7 years (Michalek et al., 1996, 198893)	TCDD accounted for all occupational TEQ; 10% of background
Cheng et al. (2006, 523122)	1942–1993	0, 10, 15 years	8.7 years (Michalek et al., 1996, 198893), and CADM (Aylward et al., 2005, 197114)	N/A
Collins et al. (2009, 197627)	1942–2003	None	7.2 years (Flesch-Janys et al., 1996, 197351)	N/A
BASF cohort studies				
Thiess et al. (1982, 064999)	1953–1980	None	N/A	N/A
Zober et al. (1990, 197604)	1953–1987	Years since first exposure: 0–9, 10–19, and 20+	N/A	N/A
Ott and Sober (1996, 198101)	1953–1991	None	5.8 years	N/A
Hamburg cohort studies				
Manz et al. (1991, 199061)	1952–1989	None, used duration of employment (<20, >20 years)	N/A	N/A
Flesch-Janys et al. (1995, 197261)	1952–1992	None	7.2 years Flesch-Janys et al. (1994, 197372)	Mean TEQ without TCDD was 155 ng/kg; mean TEQ with TCDD was 296.5 ng/kg
Flesch-Janys et al. (1998, 197339)	1952–1992	None	7.2 years Flesch-Janys et al. (1996, 197351), also used decay rates that were function of age and fat composition	Mean concentration of TCDD was 101.3 ng/kg; for TEQ (without TCDD) mean exposure was 89.3 ng/kg
Becher et al. (1998, 197173)	1952–1992	0, 5, 10, 15 and 20 years	7.2 years Flesch-Janys et al. (1996, 197351) took into account age and fat composition	Not described

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Table 2-1. Summary of epidemiological cancer studies (key characteristics) (continued)

Publication	Length of follow-up	Latency period	Half-life for TCDD	Fraction of TEQs accounted for byTCDD
Seveso cohort studies				
Bertazzi et al. (2001, 197005)	1976−1996	Periods postexposure: 0, 0−4, 5−9, 10−14, 15−19 years	N/A	N/A
Warner et al. (2002, 197489)	1976−1998	None	8 years (Pirkle et al., 1989, 197861)	N/A
Pesatori et al. (2003, 197001)	1976−1996	Period postexposure: 20 years	N/A	N/A
Baccarelli et al. (2006, 197036)	1976-1998	Period postexposure: 22 years	N/A	N/A
Consonni et al. (2008, 524825)	1976−2001	Periods postexposure: 0, 0−4, 5−9, 10−14, 15−19, 20−24 years	N/A	N/A
Chapaevsk cohort studies				
Revich et al. (2001, 199843)	Cross-sectional study (1995−1998)	N/A	N/A	N/A
Ranch Hand cohort studies				
Akhtar et al. (2004, 197141)	1962−1999	None	N/A	N/A
Michalek and Pavuk (2008, 199573)	1962−2004	None, but stratified by period of service	7.6 years	N/A

Table 2-1. Summary of epidemiological cancer studies (key characteristics) (continued)

Publication	Length of follow-up	Latency period	Half-life for TCDD	Fraction of TEQs accounted for byTCDD
New Zealand cohort studies				
t'Mannetje et al. (2005, 197593)	1969–2000 (herbicide producers); 1973–2000 (herbicide sprayers)	N/A	N/A	N/A
McBride (2009, 198490)	1969–2004	None	N/A	N/A
McBride et al. (2009, 197296)	1969–2004	None	7 years	N/A
Dutch cohort study				
Hoooiveld et al. (1998, 197829)	1955-1991	Periods postexposure: 0–19 years, >19 years	7.1 years	N/A

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Table 2-2. Epidemiological cancer study selection considerations and criteria

	Considerations					Criteria			
Cancer	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationship can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined.	Pass for dose-response analyses? Y/N
NIOSH Cohort Studies									
Fingerhut et al. (1991, 197375) all cancer sites, site-specific analyses	√	X	X	X	√	√	X	√	N
Steenland et al. (1999, 197437) all cancer sites combined, site-specific analyses	√	√	√	√	√	√	√	√	N[a]
Steenland et al. (2001, 197433) all cancer sites combined	√	√	√	√	√	√	√	√	Y
Cheng et al. (2006, 523122) all cancer sites combined	√	√	√	√	√	√	√	√	Y
Collins et al. (2009, 197627) all cancer sites combined, site-specific analyses	√	√	√	√	√	√	√	√	Y
BASF Cohort Studies									
Thiess et al. (1982, 064999)									
all cancer sites combined, site-specific analyses	√	X	X	X	X	√	X	X	N
Zober et al. (1990, 197604) all cancer sites combined, site-specific analyses	√	√	X	X	X	√	X	X	N

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Table 2-2. Epidemiological cancer study selection considerations and criteria (continued)

	Considerations					Criteria			
Cancer	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationship can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined.	Pass for dose-response analyses? Y/N
Ott and Zober (1996, 198101) all cancer sites combined	√	√	√	√	√	√	√	√	Y
Hamburg Cohort									
Manz et al. (1991, 199061) all cancer sites combines, site-specific analyses	√	√	√	√	√	√	X	√	N
Flesh-Janys et al. (2006, 197621) all cancer sites combined	√	√	√	√	√	√	√	X	N
Flesh-Janys et al. (1998, 197339) all cancer sites combined, site-specific analyses	√	√	√	√	√	√	√	√	N[b]
Becher et al. (1998, 197173) all cancer sites combined	√	√	√	√	√	√	√	√	Y
Seveso Cohort									
Bertazzi et al. (2001, 197005) all cancer sites combined, site-specific analyses	√	√	√	X	√	√	X	X	N
Pesatori et al. (2003, 197001) all cancer sites combined, site-specific analyses	√	√	X	X	√	√	X	X	N

Table 2-2. Epidemiological cancer study selection considerations and criteria (continued)

Cancer	Considerations: Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Considerations: Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Considerations: Association between TCDD and adverse health effect, with exposure-response relationship.	Considerations: Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Considerations: Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Criteria: Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Criteria: Exposure primarily TCDD and quantified so that dose-response relationship can be assessed.	Criteria: Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined.	Pass for dose-response analyses? Y/N
Consonni et al. (2008, 524825) all cancer sites combined, site-specific analyses	√	√	√	X	√	√	X	X	N
Seveso Cohort–Women's Health Study									
Baccarelli et al. (2006, 197036) site specific analysis	√	√	X	√	√	√	√	√	N[c]
Warner et al. (2002, 197489) breast cancer incidence	√	√	√	√	√	√	√	√	Y
Chapaevsk Study									
Revich et al. (2001, 199843) all cancer sites combined, site-specific analyses	X	X	X	X	√	X	X	X	N
Ranch Hands Cohort									
Akhtar et al. (2004, 197141) all cancer sites combined, site-specific analyses	√	X	√	√	√	√	X	√	N
Michalek and Pavuk (2008, 199573) all cancer sites combined	√	X	√	√	√	√	X	√	N

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Table 2-2. Epidemiological cancer study selection considerations and criteria (continued)

Cancer	Considerations: Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Considerations: Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Considerations: Association between TCDD and adverse health effect, with exposure-response relationship.	Considerations: Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Considerations: Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Criteria: Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Criteria: Exposure primarily TCDD and quantified so that dose-response relationship can be assessed.	Criteria: Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined.	Pass for dose-response analyses? Y/N
Others									
Hooiveld et al. (1998, 197829) all cancer sites combined, site-specific analyses	√	√	√	√	X	√	√	X	N
t'Mannetje et al. (2005, 197593) all cancer sites combined, site-specific analyses	√	X	√	√	√	X	X	X	N
McBride et al. (2009, 197296)									
all cancer sites combined, site-specific analyses	√	X	X	√	X	√	X	X	N
McBride et al. (2009, 198490) all cancer sites combined, site-specific analyses	√	√	X	√	X	√	√	√	N[d]

[a]This study has been superseded and updated by Steenland et al. (2001, 197433).
[b]Becher et al. (1998, 197173)) assessed this same cohort taking cancer latency into account, thereby superseding this study.
[c]It is unknown whether the frequency of t(14;18)translocations in lymphocytes relates specifically to an increased risk of non-Hodgkin's lymphoma. Given this lack of obvious adverse effect, dose-response analyses for this outcome were not conducted.
[d]No dose-response associations were noted.

√ = Consideration/criteria satisfied; X= Consideration/criteria not satisfied.

Table 2-3. Epidemiological noncancer study selection considerations and criteria

Noncancer	Considerations: Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Considerations: Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Considerations: Association between TCDD and adverse health effect, with exposure-response relationship.	Considerations: Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Considerations: Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Criteria: Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Criteria: Exposure primarily TCDD and quantified so that dose-response relationships can be assessed.	Criteria: Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined for a Nonfatal endpoint.	Pass for dose-response analyses? Y/N
NIOSH Cohort									
Steenland et al. (1999, 197437) mortality (noncancer) -ischemic heart disease	√	X	√	√	√	√	X	X	N
Collins et al. (2009, 197627) mortality (noncancer)	√	√	X	√	√	√	√	X	N
BASF Cohort									
Ott and Zober (1996, 198101) mortality (noncancer)	√	√	X	√	√	√	√	X	N
Hamburg Cohort									
Flesch-Janys et al. (1995, 197261) mortality (noncancer)	√	√	√	√	√	√	√	X	N
Seveso Cohort–Women's Health Study									
Eskenazi et al. (2002, 197168) menstrual cycle characteristics	√	√	√	√	√	√	√	√	Y
Eskenazi et al. (2002, 197164) endometriosis	X	X	X	√	X	√	√	X	N
Eskenazi et al. (2003, 197158) birth outcomes	X	X	X	√	√	√	√	X	N

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Table 2-3. Epidemiological noncancer study selection considerations and criteria (continued)

	Considerations					Criteria			
Noncancer	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationships can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined for a Nonfatal endpoint.	Pass for dose-response analyses? **Y/N**
Warner et al. (2004, 197490) age at menarche	√	√	X	√	√	√	√	X	N
Eskenazi et al. (2005, 197166) age at menopause	√	√	X	√	√	√	√	X	N
Warner et al. (2007, 197486) ovarian function	√	√	X	√	√	√	√	X	N
Eskenazi et al. (2007, 197170) uterine leiomyoma	√	√	√	√	√	√	√	X	N[a]
Seveso Cohort–Other Studies									
Bertazzi et al. (2001, 197005) mortality (noncancer)	√	√	X	X	√	√	X	X	N
Consonni et al. (2008, 524825) mortality (noncancer)	√	√	X	X	√	√	X	X	N
Mocarelli et al. (2000, 197448) sex ratio	√	√	√	√	√	X	√	X	N[b]

Table 2-3. Epidemiological noncancer study selection considerations and criteria (continued)

	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationships can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined for a Nonfatal endpoint.	Pass for dose-response analyses?
Baccarelli et al. (2002, 197062; 2004, 197045) immunological effects	√	√	√	√	√	√	√	X	N
Landi et al. (2003, 198362) gene expression	√	√	X	√	X	√	X	X	N
Alaluusua et al. (2004, 197142) oral hygiene	√	√	√	√	√	√	√	√	Y
Baccarelli et al. (2005, 197053) chloracne	√	√	√	√	√	√	√	√	N[c]
Baccarelli et al. (2008, 197059) neonatal thyroid function	√	√	√	X	√	√	√	√	Y
Mocarelli et al. (2008, 199595) semen quality	√	√	√	√	√	√	√	√	Y
Chapaevsk Study									
Revich et al. (2001, 199843) mortality (noncancer) and reproductive health	√	X	X	X	√	√	X	X	N
Ranch Hands Cohort									
Michalek and Pavuk (2008, 199573) diabetes	√	X	√	√	√	√	X	√	N

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Table 2-3. Epidemiological noncancer study selection considerations and criteria (continued)

	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationships can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined for a Nonfatal endpoint.	Pass for dose-response analyses?
Other									
Ryan et al. (2002, 198508) sex ratio	X	X	X	X	√	√	X	X	N
Kang et al. (2006, 199133) long-term health consequences	X	X	X	√	√	√	X	X	N
McBride et al. (2009, 198490) mortality (noncancer)	X	X	X	√	X	√	√	X	N
McBride et al. (2009, 197296) mortality (noncancer)	X	√	X	√	X	√	X	X	N

[a]Categorical measures of TCDD suggest an inverse association between TCDD exposure and uterine fibroids. The observed direction of the reported associations precluded quantitative dose-response modeling.

[b]The somewhat arbitrary cut off age of 19 for statistically significant exposure associations results in a highly uncertain critical exposure window. It is difficult to determine whether effects are a consequence of the initial high exposure during childhood or a function of the cumulative exposure for this entire exposure window. The differences between these two dose estimates are quite large.

[c]Chloracne is recognized to occur following high TCDD exposure levels. This study provides limited relevance to TCDD RfD development, as exposure levels observed in the general population are much lower.

√ = Consideration/criteria satisfied. X = Consideration/criteria not satisfied.

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Table 2-4. Epidemiological studies selected for TCDD cancer dose-response modeling

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/ deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Mortality from all cancers	USA, 1942–1993	NIOSH cohort including 3,538 occupationally exposed male workers at 8 plants in the United States; 256 cancer deaths	Cumulative serum lipid TCDD concentrations (CSLC) based on work histories, job-exposure matrix, and concentration and age-dependent two-compartment model of elimination kinetics	No exposure categories provided	256 cancer deaths	The slope (β) was 3.3×10^{-6} for lag of 15 years excluding upper 5% of TCDD exposures. The slopes ranged two orders of magnitude depending on modeling assumption	Available: age, year of birth, and race Risks adjusted for: year of birth, age, and race	Confounding by smoking was considered indirectly by analysis of smoking-related and smoking-unrelated cancers. Other occupational exposures were considered indirectly by repeated analyses removing one plant at a time. Based on indirect evaluation, there was no clear evidence of confounding.	Cheng et al. (2006, 523122)
Mortality from all cancers	USA, 1942–1993	NIOSH cohort including 3,538 male workers, 256 cancer deaths	CSLC based on work histories, job-exposure matrix, and a simple one-compartment first-order pharmacokinetic elimination model with 8.7-year half-life	CSLC (ppt-years) <335 335–520 520–1,212 1,212–2,896 2,896–7,568 7,568–20,455 ≥20,455	 64 29 22 30 31 32 48	 1.00 1.26 (0.79–2.00) 1.02 (0.62–1.65) 1.43 (0.91–2.25) 1.46 (0.93–2.30) 1.82 (1.18–2,82) 1.62 (1.03–2,56)	Available: date of birth and age Adjusted for: date of birth, and age was used as time scale in Cox model	Included in U.S. EPA (2003, 537122)	Steenland et al. (2001, 197433)

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Table 2-4. Epidemiological studies selected for TCDD cancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/ deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Mortality from all cancers combined	Hamburg, Germany, production period was 1950−1984 and mortality follow-up extended through 1992	Boehringer cohort including approximately 1,189 workers employed in the production of herbicides	Cumulative TCDD serum lipid concentrations based on area under curve (in µg/kg years); back-extrapolation to date of last employment	Categorical exposures (Cox model) 0− <1 1− <4 4− <8 8− <16 16− <64 64+	124	 1.0 1.12 (0.70−1.80) 1.42 (0.70−2.85) 1.77 (0.81−3.86) 1.63 (0.73−3.64) 2.19 (0.76−6.29)	 Available: year of entry, age of entry, duration of employment, birth cohort, β-HCH; TEQ other than TCDD	Included in U.S. EPA (2003, 537122) A large number of models were fitted. These included models for 5 different latency intervals (0, 5, 10, 15, and 20 years), as well as multiplicative, additive and power models, and different offset variables (person years and expected deaths)	Becher et al. (1998, 197173)
			took into account age and percent body fat; half-life value was 7.2 years	Continuous exposure TCDD (µg/kg years)	124	β = 0.0089, p = 0.0047	Available: year of entry, age of entry, duration of employment, birth cohort, β-HCH; TEQ other than TCDD		

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Table 2-4. Epidemiological studies selected for TCDD cancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/ deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Mortality and incidence for all cancers combined, as well as for specific cancer sites	Ludwig-shafen, Germany, 1954−1992	BASF cohort, 243 men exposed from accidental release that occurred in 1953 during production of trichlorophenol, or who were involved in clean-up activities	Cumulative TCDD serum lipid concentrations expressed in µg/kg based on TCDD half-life of 5.1-8.9 years, Cox regression model	Internal comparisons based on continuous measure of TCDD. External comparisons exposure categories: <0.1, 0.1−0.99, 1.0−1.99 >2 µg/kg	*Internal cohort analysis* 31 cancer deaths 47 incident cancers *External cohort analyses* Deaths 8 8 8 7	Date of 1st TCDD exposure 1.22 (95% CI: 1.00−1.50) 1.11 (95% CI: 0.91−1.35) SMRs 0.8 (0.4−1.6) 1.2 (0.5−2.3) 1.4 (0.6−2.7) 2.0 (0.8−4.0)	Available: age, BMI, smoking status and history of occupational exposure to aromatic amines and asbestos	Included in U.S. EPA (2003, 537122) Positive associations noted for digestive cancer, but not for respiratory cancer Associated between TCDD and increased SMRs found only among current smokers Last published account of this cohort	Ott and Zober (1996, 198101)

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Table 2-4. Epidemiological studies selected for TCDD cancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/ deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Breast cancer incidence	Italy 1976–1998	981 women from zones A and B with available archive serum samples, 15 breast cancer cases	TCDD serum lipid concentrations (ppt) collected between 1976 and 1981. For most samples collected after 1977, serum TCDD levels were back-extrapolated using a first-order kinetic model with a 9-year half-life.	<20 ppt 20.1−44 ppt 44.1−100 ppt >100 ppt Log_{10}TCDD also modeled as continuous variable	Cases 1 2 7 5 15	 1.0 1.0 (0.1−10.8) 4.5 (0.6−36.8) 3.3 (0.4−28.0) 2.1 (1.0−4.6)	Available: gravidity, parity, age at first pregnancy, age at last pregnancy, lactation, family history of breast cancer, age at menarche, current body mass index, oral contraceptive use, menarcheal status at explosion, menopause status at diagnosis, height, smoking, alcohol consumption. Adjusted for age, which was used as time scale in Cox model; other covariates were evaluated but were not identified as confounders.	Included in U.S. EPA (2003, 537122)	Warner et al. (2002, 197489)

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Table 2-4. Epidemiological studies selected for TCDD cancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/ deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Mortality from all cancers and specific cancer types	Midland, Michigan, USA. Follow-up period: 1942–2003. Serum collection period: 2004–2005	Subset of NIOSH cohort including 1,615 occupationally exposed male workers at 1 plant in the United States; 177 cancer deaths	Cumulative serum lipid TCDD concentrations based on work histories, job-exposure matrix, and concentration and age-dependent two-compartment model of elimination kinetics. Serum samples were obtained from 280 former workers collected during 2004–2005.	Part per billion-year estimates of cumulative TCDD exposure	177 cancer deaths	The slope of a proportional hazards regression model for fatal soft tissue sarcoma was 0.05872 (95% CI not provided but for Chi-square $p = 0.0060$) for every 1-part per billion-year increase in cumulative exposure of TCDD. Slope estimates for all fatal cancers, fatal lung, fatal prostate, fatal leukemias and fatal non-Hodgkin lymphomas were not statistically significant	Hazard ratios adjused for age, year of birth, and hire year. Stratified analyses used to examine potential impact of pentachlorophenol exposure on mortality.	Confounding by smoking was not considered directly due to a lack of data. Relatively long follow-up period (average = 36 years). Potential outcome misclassification for soft tissue sarcoma due to potential inaccuracies on death certificates. Data analyzed from one plant reduces heterogeneity associated with multiplant analyses. More serum samples ($n = 280$) analyzed than used to derive TCDD estimates for other NIOSH cohort analyses.	Collins et al. (2009, 197627)

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Table 2-5. Epidemiological studies selected for TCDD noncancer dose-response modeling

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/ deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
b-TSH measured 72 hours after birth from a heel pick (routine screening for all newborns in the region	Italy, 1976; children, 1994–2005	*Population-based study:* 1,041 singletons (56 from zone A, 425 from zone B and 533 from reference) born between Jan. 1, 1994-June 30, 2005. *Plasma dioxin study:* 51 children born to 38 women of fertile age who were part of the Seveso Chloracne Study.	Based on zone of residence, estimated mean values from a previous study. Maternal plasma TCDD levels estimated at the date of delivery using a first-order pharmacokinetic model and elimination rate estimated in Seveso women (half-life =9.8 years).	*Population-based study:* Reference Zone B Zone A *Plasma dioxin study:* Continuous maternal plasma TCDD	 533 births 425 births 56 births	*Population-based study* Mean b-TSH Reference: 0.98 (95% CI: 0.90−1.08) Zone B: 1.66 (95% CI: 1.19−2.31) Zone A: 1.35 (95% CI: 1.22−1.49) Association between neonatal b-TSH with plasma TCDD: adjusted $\beta = 0.75$ ($p < 0.001$)	Available: gender, birth weight, birth order, maternal age at delivery, hospital, type of delivery. There was limited evidence of confounding, so mean TSH results presented here are unadjusted.	An association with serum TCDD levels of mothers was found with b-TSH among the 51 births in the plasma dioxin study.	Baccarelli et al. (2008, 197059)

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Table 2-5. Epidemiological studies selected for TCDD noncancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/ deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Sperm conc. (million/mL) Progressive motility (%) Serum E_2 (pmol/L)	Italy, 1976, 1998	135 exposed (from zone A) and 184 nonexposed men aged 1−26 in 1976 were included. These subjects were selected from the cohort of 257 exposed and 372 unexposed people.	Serum TCDD (in ppt) from 1976-1977 samples (for exposed men); background values were assumed for unexposed men based on serum analysis of residents in uncontaminated areas.	TCDD quartiles		Mean values were compared between the exposed and comparison groups for sperm concentration, volume, motility and count, FSH, E_2, LH, and Inhibin B.	Available: age, abstinence time, smoking status, education, alcohol use, maternal smoking during pregnancy, employment status, BMI, chronic exposure to solvents and other toxic substances. Adjusted for smoking status, organic solvents, age at time of tests, BMI, alcohol use, education, employment status and abstinence (days) for sperm data. Hormone data not adjusted for education level, employment status, and abstinence time.	Results stratified by timing of exposure (1−9 yrs old vs. 10−17 yrs old in 1976).	Mocarelli et al. (2008, 199595)

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Table 2-5. Epidemiological studies selected for TCDD noncancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/ deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Dental defects	Seveso, Italy, Dental exams administered in 2001 among those exposed to TCDD in 1976	65 subjects <9.5 years old at time of Seveso explosion and residing in zones ABR; 130 subjects recruited from the non-ABR region (unexposed)	Serum TCDD (ng/kg) from 1976 samples for those who resided in Zone ABR; no serum levels for non-ABR residents (unexposed). TCDD exposure represent levels as of 1976 (after accident)	Non-ABR Zone 31−226 ng/kg 238−592 ng/kg 700−26000 ng/kg <5 years of age at time of accident Non-ABR Zone or 31−226 ng/kg serum TCDD 238-26,000 ng/kg serum TCDD	10/39 1/10 5/11 9/15 25/75	Dental defect % 26% 10% 45% 60% Odds Ratios (among those <5 years of age at time of accident) 1.0 2.4 (1.3−4.5)	Available: medical history, age, sex, education, smoking	Dose-response pattern observed with dental defects in the ABR zone; however, the control population had a much higher prevalence of dental defects (26%) than those in the lowest exposure group (10%). Also assessed hypodontia and other dental and oral aberrations, but these were too rare to allow modeling by ABR zone.	Alaluusua et al. (2004, 197142)

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Table 2-5. Epidemiological studies selected for TCDD noncancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/ deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Menstrual cycle characteristics: menstrual cycle length.	Seveso, Italy, follow-up interview conducted in 1996-1997 of women exposed to TCDD in the 1976 accident	Women who were <40 years from zones A or B in 1976, A positive association found among women who were pre-menarcheal at the time of accident (*n* = 134)	Serum TCDD (ng/kg) from 1976 samples. TCDD exposure level was back-extrapolated to 1976 using the Filser or the first-order kinetic models.	Interquartile range was 64-322 ppt TCDD examined as continuous measure (per 10-fold increase in serum levels).		Lengthening of the menstrual cycle by 0.93 days (95% CI: -0.01, 1.86)	Interview data: medical history, personal habits, work history, reproductive history, age, smoking, body mass index, alcohol and coffee consumption, exercise, illness, abdominal surgeries.		Eskenazi et al. (2002, 197168)

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Table 2-6. Animal bioassays selected for cancer dose-response modeling

Species/strain	Sex exposure route/duration	*n*	Average daily dose levels (ng/kg-day)	Cancer types	Statistical significant tumors (pairwise with controls or trend tests)	Reference
Mouse/ B6C3F1	Male/Female Oral gavage once per week; 52 weeks	Approximately 40 to 50 in each dose group including controls	0, 351, and 714	Females and males: hepatocellular adenomas and carcinomas	Liver: adenomas and carcinomas in females and carcinomas in males (using incidental tumor statistical test)	Della Porta et al. (1987, 197405)
Rat/Sprague-Dawley	Male/female Oral-lifetime feeding; 2 years	50 each (86 each in vehicle control group)	0, 1, 10, or 100	Females: liver, lung, oral cavity Males: adrenal, oral cavity, tongue	Adrenal cortex: adenoma Liver: hepatocellular adenoma(s) or carcinoma(s); hyperplastic nodules Lung: keratinizing squamous cell carcinoma Oral cavity: stratified squamous cell carcinoma of hard palate or nasal turbinates Tongue: stratified squamous cell carcinoma	Kociba et al. (1978, 001818); (Female liver tumors analysis updated in Goodman and Sauer, 1992, 197667)
Mouse/ B6C3F1	Male/female Oral-gavage twice per week; 104 weeks	50 each (75 each in vehicle control group)	0, 1.4, 7.1, or 71 for males; 0, 5.7, 28.6, or 286 for females	Females: hematopoietic system, liver, subcutaneous tissue, thyroid Males: liver, lung	Hematopoietic system: lymphoma or leukemia Liver: hepatocellular adenoma or carcinoma Lung: alveolar/bronchiolar adenoma or carcinoma Subcutaneous tissue: fibrosarcoma Thyroid: follicular-cell adenoma	NTP (1982, 543764)
Rat/Osborne-Mendel	Male/female Oral-gavage twice per week; 104 weeks	50 each (75 each in vehicle control group)	0, 1.4, 7.1, or 71	Females: adrenal, liver, subcutaneous tissue, thyroid Males: adrenal, liver, thyroid	Adrenal: cortical adenoma, or carcinoma or adenoma, NOS Liver: neoplastic nodule or hepatocellular carcinoma Subcutaneous tissue: fibrosarcoma Liver: neoplastic nodule or hepatocellular carcinoma Thyroid: follicular-cell adenoma or carcinoma	NTP (1982, 543764)

Table 2-6. Animal bioassays selected for cancer dose-response modeling (continued)

Species/strain	Sex exposure route/duration	*n*	Average daily dose levels (ng/kg-day)	Cancer types	Statistical significant tumors (pairwise with controls or trend tests)	Reference
Rat/Harlan Sprague-Dawley	Female Oral-gavage 5 days per week; 2 years	53 or 54	0, 2.14, 7.14, 15.7, 32.9, or 71.4	Liver Lung Oral mucosa Pancreas	Liver: hepatocellular adenoma Liver: cholangiocarcinoma Lung: cystic keratinizing epithelioma Oral mucosa: squamous cell carcinoma Pancreas: adenoma or carcinoma	NTP (2006, 197605)
Mouse/ Outbred Swiss/H/Riop	Male Gastric intubation once per week; 1 year	43 or 44 (vehicle control group = 38)	0, 1, 100, or 1,000	Liver	Liver: tumors	Toth et al. (1979, 197109)

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Table 2-7. Animal bioassay studies selected for noncancer dose-response modeling

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Reproductive toxicity studies									
Monkey/ Rhesus	Daily dietary exposure in female monkeys (3.5–4 years)	F (F0, F1, F2, F3)	3 to 7 (F1)	0, 0.15, or 0.67	0.15	0.67	Reproductive and developmental effects	Neurobehavioral effects (e.g., discrimination-reversal learning affected)	Bowman et al.(1989, 543744; 1989, 543745); Schantz and Bowman (1989, 198104); Schantz et al. (1986, 088206)
Rat/Sprague-Dawley, Long-Evans, Han/Wistar	Biweekly oral gavage (22 weeks)	Female	8	0, 10, 30 or 100	10	30	Body weight, relative liver weight, relative thymus weight	Increased relative liver weight in Sprague-Dawley and Long-Evans Rats; Increased relative thymus weight in Sprague-Dawley, Han/Wistar and Long-Evans Rats	Franc et al. (2001, 197353)
Mink	Daily dietary exposure (132 days)	F	12	0.03 (control), 0.8, 2.65, 9, or 70	None	2.65	Reproductive effects	Reduced kit survival	Hochstein et al (2001, 197544)
Rat/Sprague-Dawley	Oral gavage (GD 14 and 21, postpartum days 7 and 14), (Pups: once per week for 3 months)	Female (F0 and F1)	3 (F0 and F1)	0 or 7.14	None	7.14	Developmental effects	Lower proportion of morphologically normal pre-implantation embryos during compaction stage	Hutt et al. (2008, 198268)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Reproductive toxicity studies (continued)									
Rat/Holtzman	Corn oil gavage (initial loading dose followed by weekly dose during mating, pregnancy, and lactation– about 10 weeks)	F (F0) F and M (F1 and F2)	12 (F0) Not specified (F1 and F2)	0 or 16.5	None	16.5 (maternal exposure)	Reproductive and developmental effects	Decreased development of the ventral prostrate (F1), decreased sex ratio (percentage of males) (F2)	Ikeda et al. (2005, 197834)
Mouse/ICR	Sesame oil gavage (initial loading dose followed by weekly doses for 5 weeks)	M (F0)	42 or 43	0, 0.095, or 950	0.1	100	Reproductive effects	Decreased male/female sex ratio (percentage of males) (F1)	Ishihara et al. (2007, 197677)
Rat/Wistar albino	Olive oil gavage (daily for 45 days)	M	6	0, 1, 10, or 100	None	1	Reproductive effects	Reduced sperm production, decreased reproductive organ weights	Latchoumycandane and Mathur (2007, 197298) and related Latchoumycandane et al. (2002, 198365; 2002, 197839; 2003, 543746)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Reproductive toxicity studies (continued)									
Rat/Sprague-Dawley	Daily dietary exposure (3 generations)	F and M, (F0) F and M, (F1 and F2)	10–32 (F0) 22 (F1) 28 (F2)	0, 1, 10, or 100	1	10	Reproductive and developmental effects	Decrease in fertility, decrease in the number of live pups, decrease in gestational survival; decrease in postnatal survival, decreased postnatal body weight in one or more generations	Murray et al. (1979, 197983)
Monkey/ Rhesus	Daily dietary exposure (4 years)	F	8	0, 0.15, or 0.67	None	0.15	Reproductive effects	Increased incidence of endometriosis (disease ranged from moderate to severe)	Rier et al. (1993, 199987; 1995, 198566)
Rat/Sprague-Dawley	Maternal corn oil gavage (weekly on GD 14 and 21; PND 7 and 14) Offspring corn oil gavage (weekly for 11 months)	F (F0) F (F1)	3 (F0) 10 (F1)	0, 0.14, 0.71, 7.14, or 28.6	0.14	0.71	Reproductive effects	Decrease serum estradiol levels (F1)	Shi et al. (2007, 198147)
Rhesus monkey/ Cynomolgus	Fed gelatin capsules (5 days/week for 12 months)	F	6 (treatment) 5 (controls)	0, 0.71, 3.57, or 17.86	17.86	None	Endometriosis effects	Increased endometrial implant survival, increased maximum and minimum implant diameters, growth regulatory cytokine dysregulation	Yang et al. (2000, 198590)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Developmental toxicity studies									
Rat/Harlan Sprague-Dawley	Corn oil gavage (GD 10−16	F (F0)	80−88 (F1)	0, 25, or 100	None	25	Developmental effects	Decreased preference in the consumption of 0.25% saccharin solution (F1)	Amin et al. (2000, 197169)
Rat/CRL:WI (Han)	Maternal daily dietary exposure for an estimated 20 weeks (12 weeks prior to mating through parturition)	F (F0) M (F1)	65 (F0 treatments) 75 (F0 controls) at study initiation; following interim sacrifice ~30 animals were allowed to litter; F1 on PND 21 was ~7	0, 2.4, 8, or 46	None	2.4 (maternal exposure)	Reproductive and developmental effects	Delayed BPS (F1)	Bell et al. (2007, 197041)
Rat/Sprague-Dawley	Maternal corn oil gavage (GD 14 and 21; PND 7 and 14) Offspring corn oil gavage (weekly for 8 months)	F (F0 and F1)	2 or 3 (F0) 7 (F1)	0, 7.14, or 28.6	None	7.14	Developmental effects	Decreased serum estradiol levels (F1)	Franczak et al. (2006, 197354)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Developmental toxicity studies									
Rat/Sprague-Dawley	Maternal single corn oil gavage (GD 8) Offspring exposed during gestation and lactation (35 days)	F (F0) F and M (F1)	12 (F0) 50 or 60 (F1)	0, 20, 60, or 180	None	20 (maternal exposure)	Developmental effects	Abrogation of sexually dimorphic neuro-behavioral responses (F1)	Hojo et al. (2002, 198785) and related Zareba et al. (2002, 197567)
Rat/ Han/Wistar and Long-Evans	Maternal single corn oil gavage (GD 15)	F (F0) F and M (F1)	4 to 8 (F0) 3F/3M per treatment group (F1)	0, 30, 100, 300, or 1,000	None	30 (maternal exposure)	Developmental effects	Reduced mesiodistal length of the lower third molar (F1)	Kattainen et al. (2001, 198952)
Mouse/ C57BL/6J, BALB/cByJ, A/J, CBA/J, C3H/HeJ, and C57BL/10J	Maternal single corn oil gavage (GD 13)	F (F0) F and M (F1a, b, c)	Dams not specified (F0); 23–36 (F1a); 4–5 (F1b); 107–110 (F1c)	0, 10, 100, or 1,000	None	10 (maternal exposure)	Developmental effects	Variation in M1 morphology in C57BL/10J males and females (F1a); decreased mandible shape and size in C3H/HeJ males (F1b); variation in molar shape in C3H/HeJ males (F1c)	Keller et al. (2007, 198526; 2008, 198531; 2008, 198033)
Mouse/ddY	Maternal olive oil gavage (weekly for 8 weeks prior to mating)	F (F0) M (F1)	7 (F0) 3 (F1 immuno-cytochemical analysis) 6 (F1 cell number count)	0, 0.7, or 70	None	0.7 (LOEL) (maternal exposure)	Neurotoxicity	Decreased serotonin-immunoreactive neurons in raphe nuclei of male offspring (F1)	Kuchiiwa et al. (2002, 198355)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Developmental toxicity studies									
Mouse/NIH (pregnant and pseudo-pregnant)	Maternal sesame oil gavage daily for 8 days (GD 1−8)	F	10	0, 2, 50, or 100	None	2	Developmental effects	Decreased progesterone and increased serum estradiol levels	Li et al. (2006, 199059)
Rat/Holtzman	Maternal single olive oil gavage (GD 18)	F (F0 and F1)	4−7 (F0 and F1)	0, 20, 60, or 180	None	20 (maternal exposure)	Behavioral effects	Decreased training responses (F1)	Markowski et al. (2001, 197442)
Rat/Line C	Maternal single corn oil gavage (GD 15)	F (F0) F and M (F1)	24−32 (treatment) 12−48 (controls)	0, 30, 100, 300, or 1,000	None	30 (maternal exposure)	Developmental effects	Increase in dental caries (F1)	Miettinen et al. (2006, 198266)
Rat/Holtzman	Maternal single corn oil gavage (GD 15)	F (F0) M (F1)	Not specified (F0) 5 males and 3 females (F1)	0, 12.5, 50, 200, or 800	800 (maternal exposure)	None	Immunotoxicity	Decreased spleen cellularity (F1)	Nohara et al. (2000, 200027)
Rat/Holtzman	Maternal single corn oil gavage (GD 15)	F (F0) M (F1)	6 (F0) 5 males and 3 females (F1)	0, 12.5, 50, 200, or 800	12.5 (maternal exposure)	50 (maternal exposure)	Developmental effects	Decreased anogenital distance (F1)	Ohsako et al. (2001, 198497)
Rat/Harlan Sprague-Dawley	Maternal corn oil gavage (GD 10−16	F(F0)	~4 (F0); 80−88 (F1)	0, 25, or 100	None	None	Developmental effects	Facilitatory effect on radial arm maze learning (F1)	Schantz et al. (1996, 198781)
Rat/Sprague-Dawley	Maternal corn oil gavage (GD 10−16)	F and M (F1)	~15 (F0); 5−9 (F1)	0, 25, or 100	25	100	Developmental effects	Decreased thymus weight	Seo et al. (1995, 197869)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Developmental toxicity studies									
Rat/TCDD-resistant Han/Wistar bred with TCDD-sensitive Long-Evans	Maternal corn oil gavage (GD 15)	F (F0) M (F1)	5−8 (F0)	0, 30, 100, 300, or 1,000	100	300	Reproductive effects	Reduction in daily sperm production and cauda epididymal sperm reserves	Simanainen et al. (2004, 198106)
Mouse/C57/6 NCji	Maternal drinking water exposure (daily for 17-day lactational period)	F (F0) F and M (F1)	8 (F0) Not specified (F1)	0, 1.14, or 11.3	1.14 (NOEL) (maternal exposure)	11.3 (LOEL) (maternal exposure)	Immunotoxicity	Increased susceptibility to *Listeria* (F1 males and females); increase in thymic CD4+ cells (F1 males); decreased spleen weight (F1 males)	Sugita-Konishi et al. (2003, 198375)
Acute toxicity studies									
Mouse/B6C3F1	Corn oil gavage (single exposure)	F	20	0, 1, 5, 10, 50, 100, or 6,000	5	10	Immunotoxicity	Increased mortality from influenza infection 7 days after a single TCDD exposure	Burleson et al. (1996, 196998)
Rat/Long-Evans	Corn oil gavage (4 consecutive days)	F	14, 6, 12, 6, 6, 6, 6, 6, 6, and 4, respectively in control and treated groups	0, 0.1, 3, 10, 30, 100, 300, 1,000, 3,000, or 10,000	30	100	Thyroid effects	Reduction in serum T4 levels	Crofton et al. (2005, 197381)
Rat/Sprague-Dawley	Corn oil gavage (single dose)	F	4 (treated); 9 (control)	0, 0.6, 2, 4, 20, 60, 200, 600, 2,000, 5,000, or 20,000	0.6 (NOEL)	2 (LOEL)	Enzyme induction	Increased benzo(a)pyrene hydroxylase (BPH)	Kitchin and Woods (1979, 198750)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Acute toxicity studies (continued)									
Rat/Sprague-Dawley	Corn oil dose via oral gastric intubation (single dose)	F	10	0, 3, 10, 30, 100, 300, 1,000, 3,000, 10,000, or 30,000	3	10	Hormonal effects	Increased serum FSH	Li et al. (1997, 199060)
Rat/Sprague-Dawley	Corn oil gavage or TCDD-contaminated soil (single dose)	F	6	0, 15, 40, 100, 200, 500, 1,000, 2,000, or 5,000 in corn oil 0, 15, 44, 100, 220, 500, 1,100, 2,000, or 5,500 in contaminated soil	None	15 (LOEL)	Enzyme induction	Induction of aryl hydrocarbon hydroxylase (at low dose in both treatment protocols)	Lucier et al. (1986, 198398)
Mouse/ B6C3F1 (BALB/c (C57BL/6N (and DBA2	Corn oil gavage (single dose)	M, F	10–40	0, 5, 20, 100, or 500	500	None	Mortality and body weight changes	No increased mortality of virus-infected mice or treatment-related changes in body weight	Nohara et al. (2002, 199021)
Rat/TCDD-resistant Han/Wistar bred; TCDD-sensitive Long-Evans	Corn oil gavage (single dose)	M, F	9–11	30–100,000	100	300	General toxicological endpoints, organ weights, dental defects	Reduction in serum T4 levels	Simanainen et al. (2002, 201369)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Acute toxicity studies (continued)									
Rat/TCDD-resistant Han/Wistar bred with TCDD-sensitive Long-Evans	Corn oil gavage (single dose)	M, F	5–6	Line A: 30–3,000,000 Line B: 30–1,000,000 Line C: 30–100,000	100	300	General toxicological endpoints, organ weights, dental defects	Decreased thymus weight	Simanainen et al. (2003, 198582)
Mouse/ C57BL/6N CYP1A2 (+/+) wild-type	Corn oil gavage (single dose)	F	Not specified	0, 30, 100, 300, 1000, 3000, or 10,000	300	1,000	Immunotoxicity	Decreased antibody response to SRBCs	Smialowicz et al. (2004, 110937)
Rat/Sprague-Dawley	Corn oil gavage (single dose)	F	5–15	0, 0.05, 0.1, 1, 10, 100, 1,000, or 10,000	0.1 (NOEL)	1 (LOEL)	Liver effects	Increase in hepatic EROD activity and CYP1A1 mRNA levels	Vanden et al. (1994, 197551)
Subchronic toxicity studies									
Rat/Sprague-Dawley	Corn oil gavage (daily for 28 days)	F	5	0, 2.5, 25, 250, or 1,000	250	1,000	Body and organ weight changes	Decreased body weight, increased relative liver weight and related biochemical changes, decreased relative thymus weight	Chu et al. (2001, 521829)
Rat/Sprague-Dawley	Corn oil gavage (daily for 28 days)	F	5	0, 2.5, 25, 250, or 1,000	2.5	25	Liver effects	Alterations in thyroid, thymus, and liver histopathology	Chu et al., 2007
Guinea pig/ Hartley	Daily dietary exposure (90 days)	M, F	10/sex	0, 0.12, 0.61, 4.9, or 26 (males); 0, 0.12, 0.68, 4.86, or 31 (females)	0.61	4.9	Body and organ weight changes	Decreased body weight (male and females); increased relative liver weights (males); decreased relative thymus weight (males)	DeCaprio et al. (1986, 197403)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Subchronic toxicity studies (continued)									
Mice/B6C3F1	Corn oil gavage (5 days/week for 13 weeks)	F	5	0, 1.07, 3.21, 10.7, 32.1, or 107	None	1.07 (LOEL)	Body and organ weight changes; enzyme induction	Increased EROD, ACOH and phosphotyrosyl proteins at all doses	DeVito et al. (1994, 197278)
Rat/Iva:SIV 50-Sprague-Dawley	Daily dietary exposure (13 weeks)	M, F	6	0, 20, 200, or 2,000	None	20	Liver effects	Reduced hepatic vitamin A levels	Fattore et al. (2000, 197446)
	Daily dietary exposure (13 weeks)	M, F	6	0 or 200					
	Daily dietary exposure (13 weeks)	M, F	6	0, 200, or 1,000					
	Daily dietary exposure (13 weeks, 26, and 39 weeks)	F	6	0 or 100					
Rat/Sprague-Dawley	Gavage loading/ maintenance doses (every 4 days for 14 days)	M, F	6	0, 0.55, 307, or 1,607	0.57	327	Body and liver weight changes; hepatic cell proliferation	Increased absolute and relative liver weight	Fox et al. (1993, 197344)
Mouse/ B6C3F1	Corn oil gavage (5 days/week for 13 weeks)	F	Not specified	0, 0.32, 1.07, 10.7, or 107	None	0.32 (LOEL)	Brain effects	Induction of biomarkers of oxidative stress at all doses	Hassoun et al. (1998, 136626)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Subchronic toxicity studies (continued)									
Rat/Harlan Sprague-Dawley	Corn oil gavage (5 days/week for 13 weeks)	F	6	0, 2.14, 7.14, 15.7, 32.9, or 71.4	None	2.14 (LOEL)	Liver and brain effects	Induction of biomarkers of oxidative stress at all doses in liver and brain	Hassoun et al. (2000, 197431)
Rat/Harlan Sprague-Dawley	Corn oil gavage (5 days/week for 13 weeks)	F	12	0, 7.14, 15.7, or 32.9	None	7.14 (LOEL)	Brain effects	Induction of biomarkers of oxidative stress at all doses	Hassoun et al. (2003, 198726)
Rat/Sprague-Dawley	Corn oil gavage (5 days/week for 13 weeks)	M, F	12	0, 0.71, 7.14, 71.4, or 714	7.14	71.4	Liver effects, body weight changes, and hematologic and clinical effects	Reduced body weight and food consumption, slight liver degeneration, lymphoid depletion, increased urinary porphyrins and delta aminolevulinic acid, increased serum alkaline phosphatase and bilirubin	Kociba et al. (1976, 198594)
Rat/F344	Corn oil gavage (2 days/week for 28 days)	F	3	0, 0.71, 7.14, or 71.4	None	0.71 (LOEL)	Clinical signs and histopathology	Decreased Cx32 plaque number and area in the liver	Mally and Chipman (2002, 198098)
Mouse/ B6C3F1	Corn oil gavage (5 days/week for 13 weeks)	F	Not specified	0, 0.11, 0.32, 1.07, 10.7, or 107.14	1.07 (NOEL)	10.7 (LOEL)	Liver, lung, kidney, and spleen effects	Increased hepatic superoxide anion	Slezak et al. (2000, 199022)
Mouse/ B6C3F1	Corn oil gavage (5 days/week for 13 weeks)	F	8−15	0, 1.07, 10.7, 107, or 321	None	1.07	Immunotoxicity and organ weight	Reduced antibody response to SRBC, increased relative liver weight	Smialowicz et al. (2008, 198341)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Subchronic toxicity studies (continued)									
Rat/Sprague-Dawley	TCDD in diet (13 weeks)	F	8	0, 14, 26, 47, 320, or 1,024	None	14	Multiple end-points	Decreased absolute and relative thymus weights, decreased liver retinoid levels	Van Birgelen (1995, 197096; 1995, 198052)
Guinea pig/ Hartley	Corn oil gavage (weekly for 8 weeks)	F	10	0, 1.14, 5.71, 28.6, or 143	1.14	5.71	Immunotoxicity	Decreased total leukocytes and lymphocyte count, decreased absolute thymus and weight, increase in primary serum tetanus antitoxin	Vos et al. (1973, 198367)
Mouse/ B6C3F1	Corn oil gavage (daily for 14 days)	F	6–8	0, 10, 50, 100, 500, 1,000, or 2,000	None	10	Immunotoxicity	Reduction of serum complement activity	White et al. (1986, 197531)
Chronic toxicity studies									
Rat/CD-COBS	Corn oil gavage (weekly for 45 weeks)	F	4	0, 1.43, 14.3, or 143	None	1.43	Hepatic porphyria	Increased urinary porphyrin excretion	Cantoni et al. (1981, 197092)
Rat/Sprague-Dawley	Loading/ maintenance dose (every 3 days for different durations up to 128 days)	F	5	0, 0.85, 3.4, 13.6, 54.3, or 217 (28-day duration)	54.3 (28-day duration)	217 (28-day duration)	Body weight changes and changes in PEPCK activity and IGF-I levels	Decreased body weight, decreased PEPCK activity, and reduced IGF-I levels	Croutch et al. (2005, 197382)
Rat/Sprague-Dawley	Corn oil gavage (5 days/week for 30 weeks)	F	6	0, 2.14, 7.14, 15.7, 32.9, or 71.4	None	2.14 (LOEL)	Brain effects	Induction of biomarkers of oxidative stress at all doses	Hassoun et al. (2002, 543725)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Chronic toxicity studies (continued)									
Rat/Sprague-Dawley	Daily dietary exposure (2 years)	M, F	50	0, 1, 10, or 100	1	10	Multiple endpoints measured	Increased urinary porphyrins, hepatocellular nodules, and focal alveolar hyperplasia	Kociba et al. (1978, 001818)
Rat/Sprague-Dawley	Biweekly gavage (30 weeks)	F	9	0, 3.5, 10.7, 35, or 125	10.7	35	Body and organ weight changes, clinical chemistry, hepatocellular proliferation	Increased relative liver weight	Maronpot et al. (1993, 198386)
Mouse/ B6C3F1; Rat/Osborne Mendel	Corn oil gavage (2 days/week for 104 weeks)	M, F	50	0, 1.4, 7.1, or 71 for rats and male mice; 0, 5.7, 28.6, or 286 for female mice	None	1.4	Liver and body weight changes	Increased incidences of liver lesions in mice (males and females)	NTP (1982, 543764)
Rat/Sprague-Dawley	Corn oil gavage (5 days/week for 105 weeks)	F	53	0, 2.14, 7.14, 15.7, 32.9, or 71.4	None	2.14	Liver and lung effects	Increased absolute and relative liver weights, increased incidence of hepatocellular hypertrophy, increased incidence of alveolar to bronchiolar epithelial metaplasia	NTP (2006, 197605)
Monkey/ Rhesus	Daily dietary exposure (4 years)	F	8	0, 0.15, or 0.67	None	0.15	General toxicological endpoints and reproductive effects	Elevated serum triglycerides and total lipids	Rier et al. (2001, 198776; 2001, 543773)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Chronic toxicity studies (continued)									
Rat/Sprague-Dawley	Biweekly gavage (30 weeks)	F	9	0, 3.5, 10.7, 35, or 125	None	3.5 (LOEL)	EGFR kinetics and auto-phosphorylation, hepatocellular proliferation	Decrease in EGFR maximum binding capacity	Sewall et al. (1993, 197889)
Rat/Sprague-Dawley	Biweekly gavage (30 weeks)	F	9	0, 0.1, 0.35, 1, 3.5, 10.7, 35, or 125	10.7	35	Thyroid function	Decreased serum T^4 levels	Sewall et al. (1995, 198145)
Mouse/Swiss/ H/Riop	Sunflower oil gavage (weekly for 1 year)	M	38−44	0, 1, 100, or 1,000	None	1	Skin effects	Dermal amyloidosis and skin lesions	Toth et al. (1979, 197109)

ND = not determined.

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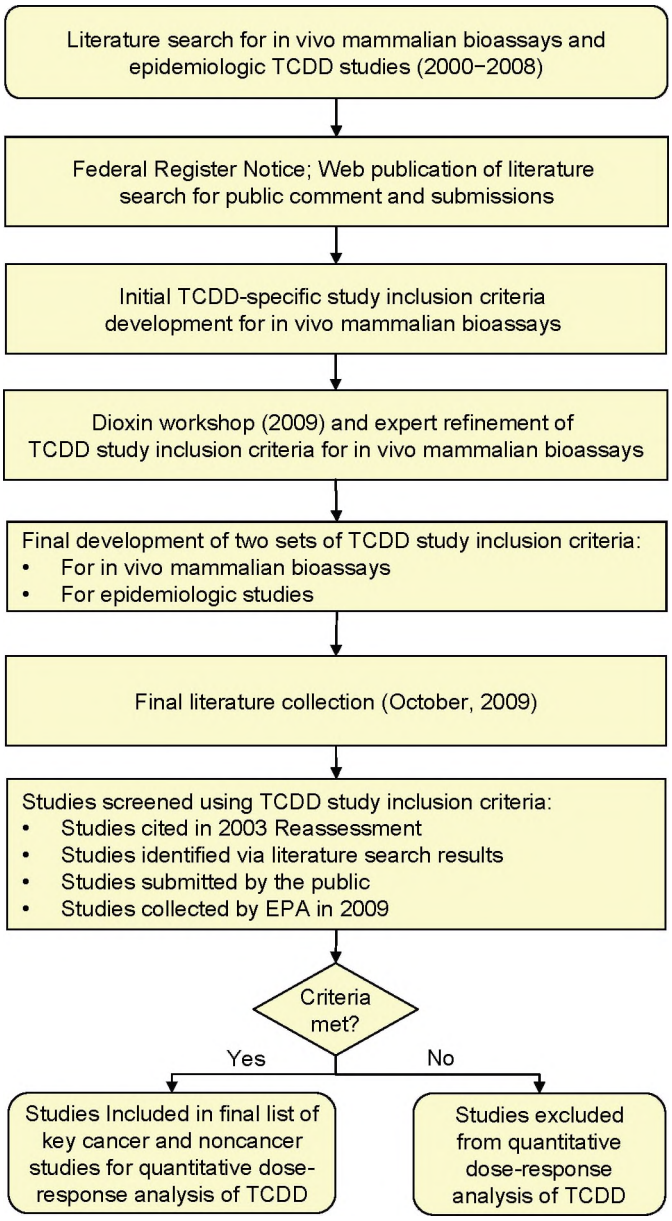


Figure 2-1. EPA's process to select and identify in vivo mammalian and epidemiologic studies for use in the dose-response analysis of TCDD. EPA first conducted a literature search to identify studies published since the 2003 Reassessment. Results were published and additional study submissions were accepted from the public. Next EPA developed TCDD-specific study inclusion criteria for in vivo mammalian studies and held a Dioxin Workshop where these criteria were discussed and refined. Third, EPA developed two final sets of study inclusion criteria, one for in vivo mammalian studies and another for epidemiologic studies. Finally, EPA applied these two sets of criteria to all studies from the literature search, public submissions, 2003 Reassessment, and additional studies identified by EPA after the Dioxin Workshop through October 2009. The studies that met these criteria formed a list of key studies for EPA's consideration in TCDD dose-response assessment.

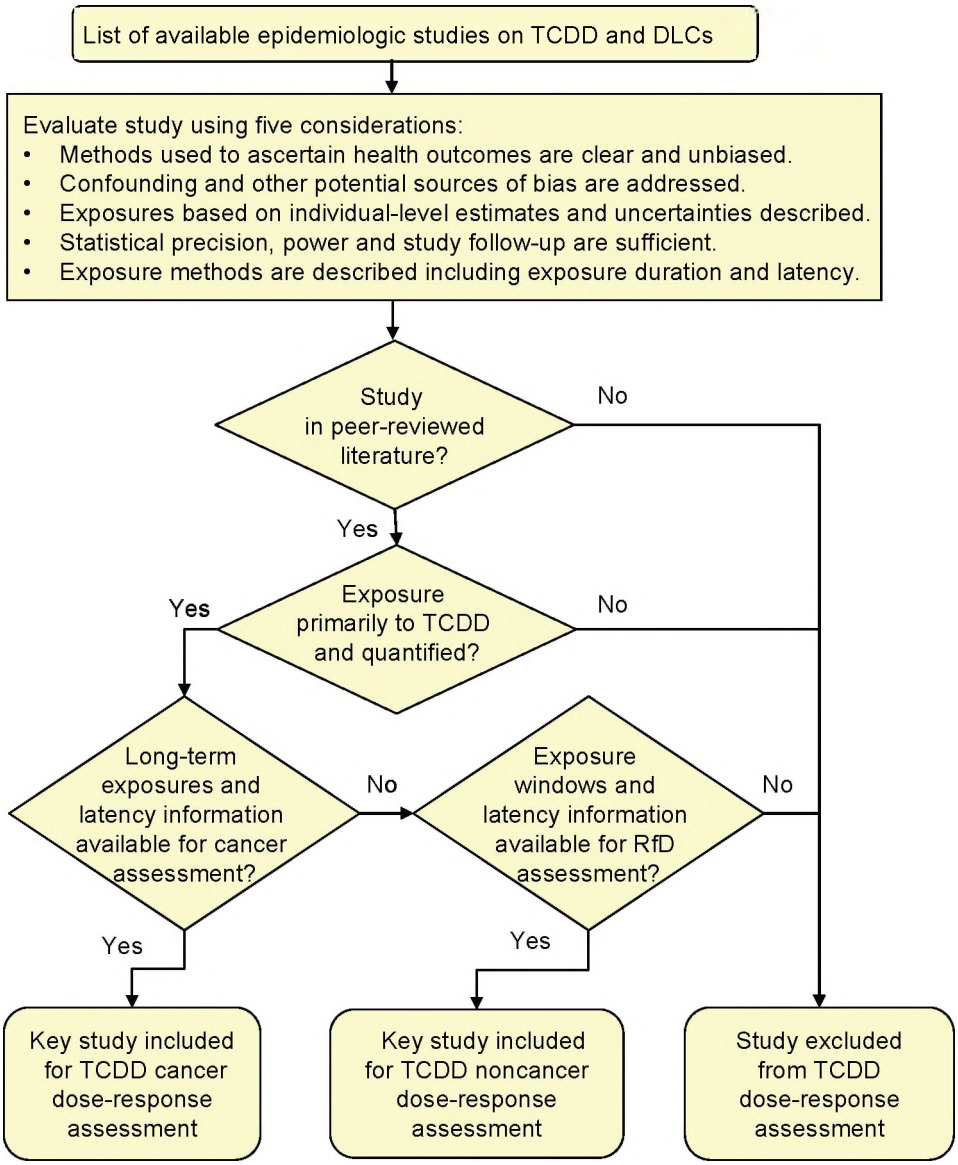


Figure 2-2. EPA's process to evaluate available epidemiologic studies using study inclusion criteria for use in the dose-response analysis of TCDD. EPA applied its TCDD-specific epidemiologic study inclusion criteria to all studies published on TCDD and DLCs. The studies were initially evaluated using five considerations regarded as providing the most relevant kind of information needed for quantitative human health risk analyses. For each study that was published in the peer-reviewed literature, EPA then examined whether the exposures were primarily to TCDD and if the TCDD exposures could be quantified so that dose-response analyses could be conducted. Finally, EPA required that the effective dose and oral exposure be estimable: (1) for cancer, information is required on long-term exposures, (2) for noncancer, information is required regarding the appropriate time window of exposure that is relevant for a specific, nonfatal health endpoint, and (3) for all endpoints, the latency period between TCDD exposure and the onset of the effect is needed. Only studies meeting these criteria were included in EPA's TCDD dose-response analysis.

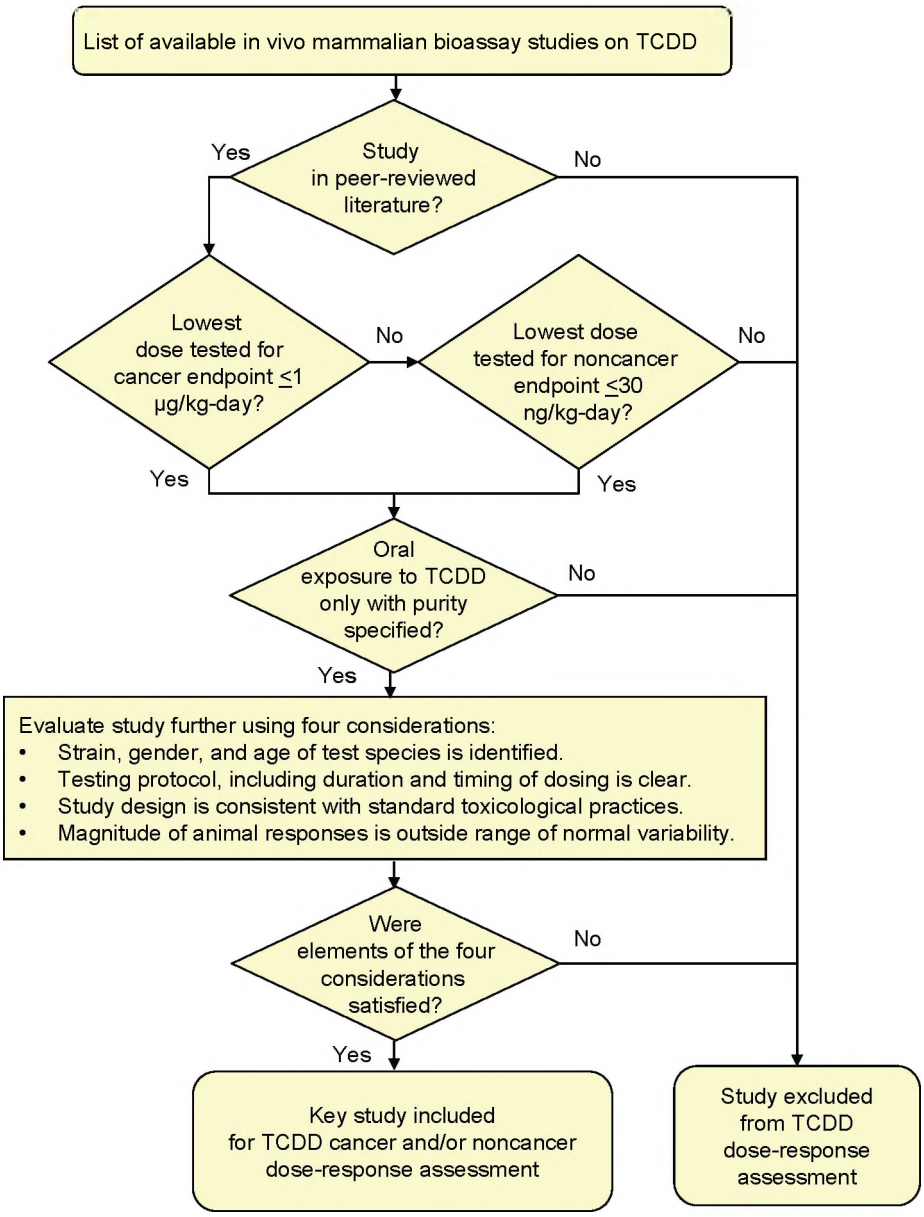


Figure 2-3. EPA's process to evaluate available animal bioassay studies using study inclusion criteria for use in the dose-response analysis of TCDD. EPA evaluated all available in vivo mammalian bioassay studies on TCDD. Studies had to be published in the peer-reviewed literature. Next, to ensure working in the low-dose range for TCDD dose-response analysis, EPA applied dose requirements to the lowest tested average daily doses in each study, with specific requirements for cancer (≤1 µg/kg-day) and noncancer (≤30 ng/kg-day) studies. Third, EPA required that the animals were exposed via the oral route to only TCDD and that the purity of the TCDD was specified. Finally, the studies were evaluated using four considerations regarded as providing the most relevant kind of information needed for quantitative human health risk analyses from animal bioassay data. Only studies meeting all of these criteria and considerations were included in EPA's TCDD dose-response analysis.

3. THE USE OF TOXICOKINETICS IN THE DOSE-RESPONSE MODELING FOR CANCER AND NONCANCER ENDPOINTS

A key recommendation from the National Academy of Sciences (NAS) for improving the 2003 Reassessment was that U.S. Environmental Protection Agency (EPA) should justify its approaches to dose-response modeling for cancer and noncancer endpoints. Further, the NAS suggested that EPA incorporate the most up-to-date and relevant state of the science for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) dose-response assessment.

While EPA believes that at the time of its release, the 2003 Reassessment offered a substantial improvement over the general state-of-the-science regarding dose-response modeling, EPA agrees with the NAS that the justification of the approaches to dose-response modeling can be improved and the methodologies updated to reflect the most current EPA practices and science. In Section 3, EPA describes the use of toxicokinetic (TK)[11] information in the dose-response modeling of TCDD. Section 3.1 summarizes the NAS comments regarding the use of TK in the dose-response approaches for TCDD. Section 3.2 overviews EPA's responses to the NAS comments. Section 3.3 discusses TCDD kinetics, including TK models developed to simulate disposition of this compound in rodents and humans (see Section 3.3.4), alternative measures of dose that could be used in a TCDD dose-response analysis and uncertainties in the TCDD dose estimates (see Section 3.3.5). Sections 4 and 5 of this document incorporate the TK information into noncancer and cancer dose response modeling, respectively.

3.1. SUMMARY OF NAS COMMENTS ON THE USE OF TOXICOKINETICS IN DOSE-RESPONSE MODELING APPROACHES FOR TCDD

The NAS commented on the appropriate use of TK models in dose-response modeling for TCDD. Specifically, the committee requested that EPA consider using such models to provide refined estimates of dose, for example, as the underlying science and predictive capabilities of these models improved.

> [Discussing Kinetic models]…the committee encourages further development and use of these models as data become available to validate and further develop them (NAS, 2006, 198441p. 59).

[11]Toxicokinetics (TK) is that part of the pharmacokinetics (PK) where toxicity is resulted in the organism.

Although the NAS basically agreed with EPA's use of body burden as a dose metric in the 2003 Reassessment (e.g., see NAS, 2006, 198441, p. 7), the NAS was concerned about the limitations of first order kinetic models, such as the one used in the 2003 Reassessment, to estimate TCDD body burdens.

> TCDD, other dioxins, and DLCs act as potent inducers of CYP, a property that can affect both the hepatic sequestration of these compounds and their half-lives. Hepatic sequestration of dioxin may influence the quantitative extrapolation of the rodent liver tumor results because the body-burden distribution pattern in highly dosed rats would differ from the corresponding distribution in humans subject to background levels of exposure. EPA should consider the possible quantitative influence of dose-dependent toxicokinetics on the interpretation of animal toxicological data (NAS, 2006, 198441, p. 129).

The NAS also asked EPA to evaluate the impact of kinetic uncertainty and variability on dose-response assessment. The NAS committee asked EPA to use TK models to examine both interspecies and human interindividual differences in the disposition of TCDD, which would better justify EPA dose-response modeling choices.

> The Reassessment does not adequately consider the use of a PBPK model to define species differences in tissue distribution in relation to total body burden for either cancer or noncancer end points (NAS, 2006, 198441, p. 62).
>
> EPA …should consider physiologically based pharmacokinetic modeling as a means to adjust for differences in body fat composition and for other differences between rodents and humans (NAS, 2006, 198441, p. 10).
>
> The Reassessment does not provide details about the magnitudes of the various uncertainties surrounding the decisions EPA makes in relation to dose metrics (e.g., the impact of species differences in percentage of body fat on the steady-state concentrations present in nonadipose tissues). The committee recommends that EPA use simple PBPK models to define the magnitude of any differences between humans and rodents in the relationship between total body burden at steady-state concentrations (as calculated from the intake, half-life, bioavailability) and tissue concentrations. The same model could be used to explore human variability in kinetics in relation to elimination half-life. EPA should modify the estimated human equivalent intakes when necessary (NAS, 2006, 198441, p. 73).

Finally, the NAS asked EPA to use TK considerations to better justify its choice of dose metric.

> EPA makes a number of assumptions about the appropriate dose metric and mathematical functions to use in the Reassessment's dose-response analysis … but does not adequately comment on the extent to which each of these assumptions could affect the resulting risk estimates…EPA did not quantitatively describe how this particular selection affected its estimates of exposure and therefore provided no overall quantitative perspective on the relative importance of the selection (NAS, 2006, 198441, p. 51).

3.2. OVERVIEW OF EPA'S RESPONSE TO THE NAS COMMENTS ON THE USE OF TOXICOKINETICS IN DOSE-RESPONSE MODELING APPROACHES FOR TCDD

In response to the NAS recommendations regarding TCDD kinetics and choice of dose metrics, this document presents an in depth evaluation of TCDD TK models, exploring their differences and commonalities and their possible application for the derivation of dose metrics relevant to TCDD. Initially, EPA discusses the application of first order kinetics to estimate body burden as a dose metric for TCDD. This first order kinetic model is used to predict TCDD body burden for all of the studies identified as Key Studies (see Section 2.4); this model uses a constant half-life to simulate the elimination of TCDD from the body. However, given the observed data indicating early influence of cytochrome P450 1A2 (CYP1A2) induction and binding to TCDD in the liver and later redistribution of TCDD to fat tissue, the use of a constant half-life for TCDD clearance following long term or chronic TCDD exposure is not biologically supported. Therefore, using half-life estimates based on observed terminal steady state levels of TCDD will not account for the possibility of an accelerated dose-dependent clearance of the chemical during early stages following elevated TCDD exposures. The biological processes leading to dose-dependent TCDD excretion are better described using physiologically based pharmacokinetic (PBPK) models than by simple first order kinetic models. Additionally, as part of its preparation for developing this document, EPA evaluated recent TCDD kinetic studies as NAS advocated. Although the NAS agreed with continued use of body burden metric as the dose metric of choice, EPA believes that the state-of-the-practice has advanced sufficiently to justify the consideration of alternative dose metrics (other than administered dose) based on an application of a physiologically-based TK model.

EPA identified a number of advances in the overall scientific understanding of TCDD disposition; many of these are documented in a summary discussion introducing the section on TCDD kinetics (see Section 3.3). The increased understanding warranted an evaluation of current kinetic modeling of TCDD to determine if the use of such models would improve the dose-response assessment for TCDD. Justification of the final PBPK model choice is detailed in Section 3.3. Through the choice of a published PBPK model to estimate dose metrics for dioxin, EPA has addressed several of the NAS concerns. The PBPK model can be applied to estimate dose metrics other than body burden that may be more directly related to response, e.g., tissue levels, serum levels, blood concentrations, or dose metrics related to TCDD-protein receptor binding. The selected PBPK model included explicit description of physiological and biochemical parameters, therefore, it can also provide an excellent tool for investigating differences in species uptake and disposition of TCDD. One of the criteria used to select a PBPK model for TCDD kinetics was the availability of both human and animal models so that differences in species uptake and disposition of TCDD can be investigated. Additionally, the PBPK model includes quantitative information that is suitable for addressing the impact of physiological (e.g., body weight [BW] or fat tissue volume), or biochemical (e.g., induction of CYP1A2) variability on overall risk of TCDD between species, in response to another area of concern in the NAS report. The sensitivity analysis and uncertainty in dose metrics derived for the risk assessment of TCDD are also presented in Section 3.3. Detailed discussion on the uncertainty in choice of PBPK model-driven dose metrics is also provided in Section 3.3.

3.3. PHARMACOKINETICS (PK) AND PK MODELING

3.3.1. PK Data and Models in TCDD Dose-Response Modeling: Overview and Scope

In general, the use of measures of internal dose in dose-response modeling is considered to be superior to that of administered dose (or uptake) because the former is more closely related to the response. The evaluation of internal dose, or dose metric, in exposed humans and other animals is facilitated by an understanding of pharmacokinetics (i.e., absorption, distribution, metabolism, and excretion). When measurements of internal dose (e.g., blood concentration, tissue concentration) are not available in animals and humans, pharmacokinetic models can be used to estimate them. The available data on the pharmacokinetics of TCDD in animals and

humans have been reviewed (NAS, 2006, 198441; U.S. EPA, 2003, 537122; van Birgelen and van, 2000, 523248).

It is evident based on these reviews and other analyses that three distinctive features of TCDD play important roles in determining its pharmacokinetic behavior, as discussed below:

- **TCDD is very highly lipophilic** and thus is more soluble in fat or other relatively nonpolar organic media than in water. The *n*-octanol/water partition coefficient is a commonly-used measure of lipophilicity equal to the equilibrium ratio of a substance's concentration in *n*–octanol (a surrogate for biotic lipid) to the substance's concentration in water (Leo et al., 1971, 019600). For TCDD, this coefficient is on the order of 10,000,000 or more (ATSDR, 1998, 197033). It follows that the solubility of TCDD in the body's lipid fraction, i.e., the fatty portions of various tissues, including adipose, organs, and blood, is extremely high.
- **TCDD is very slowly metabolized** compared to many other organic compounds, with an elimination half life in humans on the order of years following an initial period of distribution in the body (Carrier et al., 1995, 197618; Michalek et al., 2002, 199579). Most laboratory animals used for toxicologic testing tend to eliminate TCDD much more quickly than people, although even in animals TCDD is eliminated much more slowly than most other chemicals.
- **TCDD induces binding proteins in the liver** that have the effect of sequestering some of the TCDD. The ability of TCDD to alter gene expression and the demonstration that the induction of CYP1A2 is responsible for hepatic TCDD sequestration suggest that both pharmacokinetic and pharmacodynamic events must be incorporated for a quantitative description of TCDD disposition (Santostefano et al., 1998, 200001). The induction of these proteins implies that TCDD tends to be eliminated more rapidly in the early years following short-term, high-level exposures than it is after those initial levels have declined. Leung et al. (1988, 198815) and Andersen et al. (1993, 196991), in their PBPK modeling, had taken into consideration the issue of liver protein binding. Recent efforts of pharmacokinetic modeling have supported the concentration-dependent elimination of TCDD in animals and humans (Aylward et al., 2005, 197014; Emond et al., 2006, 197316).

Sections 3.3.2 and 3.3.3 present the salient features of TCDD pharmacokinetics in animals and humans, with particular focus on mechanisms and data of relevance to interspecies and intraspecies variability. Section 3.3.4 describes the various dose metrics for the dose-response modeling of TCDD and the characteristics of pharmacokinetic models potentially useful for estimating these metrics. Finally, Sections 3.3.5 and 3.3.6 summarize the results of application of pharmacokinetic models to derive dose metrics as well as the uncertainty associated with the predictions of dose metrics used in dose-response modeling. Dose metrics

derived via PBPK modeling approaches are utilized in Sections 4 and 5 of this document for noncancer and cancer TCDD dose-response modeling, respectively.

3.3.2. PK of TCDD in Animals and Humans

3.3.2.1. *Absorption and Bioavailability*

When administered via the oral route in the dissolved form, TCDD appears to be well absorbed. Animal studies indicate that oral exposure to TCDD in the diet or in an oil vehicle results in the absorption of >50% of the administered dose (Nolan et al., 1979, 543785; Olson et al., 1980, 197976). Human data from Poiger and Schlatter (1986, 197336) indicate that >87% of the oral dose (after ingestion of 105 ng [^{3}H]−2,3,7,8−TCDD [1.14 ng/kg BW] in 6 mL corn oil) was absorbed from the gastrointestinal tract. Lakshmanan et al. (1986, 548729), investigating the oral absorption of TCDD, suggested that it is absorbed primarily by the lymphatic route and transported predominantly by chylomicrons.

Oral absorption is generally less efficient when TCDD is more tightly bound in soil matrices. Based on experiments in miniature swine, Wittsiepe et al. (2007, 548736) reported an approximately 70% reduction in bioavailability when TCDD was administered in the form of contaminated soil, relative to TCDD after extraction from the same soil matrix with solvents. Working with soil from the prominent contamination site at Times Beach, Missouri, Shu et al. (1988, 548739) reported an oral bioavailability of approximately 43% based on experiments in rats. Percent dose absorbed by the dermal route is reported to be less than the oral route, whereas absorption of TCDD by the transpulmonary route appears to be efficient (Banks and Birnbaum, 1991, 548742; see, for example; Banks et al., 1990, 548741; Diliberto et al., 1996, 143712; Nessel et al., 1992, 548743; Roy et al., 2008, 548747; U.S. EPA, 2003, 537122).

3.3.2.2. *Distribution*

TCDD in systemic circulation equilibrates and partitions into the tissues where it is then accumulated, bound, or eliminated. Whereas the bulk of the body tissues are expected to equilibrate in a matter of hours, the adipose tissue will approach equilibrium concentrations with blood much more slowly. Consistent with these assertions, a number of experimental and modeling studies in rats and humans have shown that TCDD has a large volume of distribution (Vd), i.e., the apparent volume in which it is distributed. The Vd corresponds to the volume of

blood plus the product of internal tissue volumes and the corresponding tissue:blood partition coefficients. This parameter is a key determinant of the elimination rate of TCDD in exposed organisms. The tissue:blood partition coefficients of TCDD, in turn, are determined by the relative solubility of TCDD in tissue and blood components (including neutral lipids, phospholipids, and water).

Column 1 in Table 3-1 presents the tissue:blood partition coefficients for TCDD (Emond et al., 2005, 197317; Wang et al., 1997, 104657). Column 3 of this table lists the physical volume of each tissue, scaled to a person weighing 60 kg. The last column shows the implications of the tissue volumes and tissue:blood partition coefficients for the effective volumes of distribution for each tissue and for the body as a whole. It can be seen that, purely on the basis of solubility space, the fat should be expected to contain about 94% of the TCDD in the body, and that the body as a whole behaves as if it is about 1,200 liters in terms of blood-equivalents (i.e., approximately 22−fold larger than its physical volume).

Maruyama et al. (2002, 198448) have published another set of tissue/blood partition coefficients for TCDD and other dioxin congeners based in part on observations of tissue concentrations measured in autopsy specimens from eight Japanese people without known unusual exposures to TCDD. Their estimates of TCDD partition coefficients seem to be rather large and variable, with a fat:blood value of 247 ± 78 (standard deviation [SD]), a liver:blood value of 9.8 ± 5.7 and a muscle:blood value of 18 ± 10.6. Depending on time of autopsy, tissue samples may not be an accurate source of information on observed, in vivo partition coefficients because weight loss is likely to occur pre and post mortem. In particular, a decline in fat stores volume could lead to an increased concentration of dioxin in fat in autopsy specimens relative to what would be observed in vivo.

The calculations shown in Table 3-1 do not include the additional amount that will be bound to induced proteins in the liver. That induction and binding will tend to increase the contribution of the liver on the effective volume of distribution (Birnbaum, 1986, 548749).

It is also of interest to point out some basic implications of the data in Table 3-1 for the expected rates of perfusion-mediated transfer of TCDD between blood and each of the organ/tissues. The rate of loss from a tissue (occurring primarily via blood flow) and the corresponding half-life can be calculated using the following equations:

$$\text{Rate constant for loss (hour}^{-1}) = \frac{\text{Blood flow (liters / hour)}}{\text{Tissue volume (liters)} \times \text{Tissue / Blood Partition Coefficent}} \quad \text{(Eq. 3-1)}$$

$$t_{1/2} \text{ for tissue perfusion loss} = \frac{\ln(2)}{\text{Rate constant for loss}}$$

$$= \frac{\ln(2) \times \text{Tissue volume (liters)} \times \text{Tissue/Blood Partition Coefficent}}{\text{Blood flow (liters/hour)}} \quad \text{(Eq. 3-2)}$$

Because TCDD is highly lipophilic, its concentration in the aqueous portion of the blood is very small, and TCDD tends to partition from blood components into cellular membranes and tissues, probably in large part via diffusion. As a result, full equilibrium concentrations of TCDD are not attained by the end of the transit time through organs from the arterial to venous blood. For organs in which this occurs, diffusion coefficients or "permeability factors" have been estimated to assess the fractional attainment of equilibrium concentration that occurs by the time the blood leaving each organ reaches the venous circulation. Table 3-2 presents the permeability factors and implications for perfusion half-lives for TCDD, per Emond et al. (2005, 197317; 2006, 197316).

Despite the high lipid bioconcentration potential of TCDD, the adipose tissue does not always have the highest concentration (Abraham et al., 1988, 199510; Geyer et al., 1986, 064899; Poiger and Schlatter, 1986, 197336). Further, the ratios of tissue:tissue concentrations of TCDD and related compounds (e.g., the liver:adipose ratio) may not remain constant during nonsteady-state conditions. TCDD concentrations have been observed to decrease more rapidly in the liver than in adipose tissue. For example, Abraham et al. (1988, 199510) found that the liver:adipose tissue concentration ratio in female Wistar rats exposed to a subcutaneous TCDD dose of 300 ng/kg decreased from 10.3 at 1 day postexposure to 0.5 at 91 days postexposure. It should be noted that even at a ratio of 0.5, the amount of TCDD in the liver is greater than that based on lipid content of the tissue alone, consistent with the presence of hepatic TCDD binding proteins. The liver/adipose tissue ratio also was dose-dependent, such that the liver TCDD burden increased from ~11% of the administered dose at low doses (i.e., 1−10 ng/kg) to ~37% of the dose at an exposure level of 300 ng/kg. The increase in TCDD levels in liver, accompanied by a decrease in concentration in the adipose tissue, is a particular behavior to be considered in

high dose to low dose extrapolations. This behavior is essentially a result of dose-dependent hepatic processes, as described below.

3.3.2.3. *Metabolism and Protein Binding*

The metabolism of TCDD is slow, particularly in humans, and it is thought to be mediated by the CYP1A2 enzyme that is inducible by TCDD (Olson et al., 1994, 198008; Ramsey et al., 1982, 548750; Weber et al., 1997, 548753; Wendling et al., 1990, 548751). The low rate of metabolism in combination with sequestration appear to account for the retention of TCDD in liver, and these processes collectively contribute to the long half-life for elimination of TCDD from the body.

Dynamic changes in TCDD binding in liver and partitioning to fat have been studied extensively in rats and mice (Diliberto et al., 1995, 197309; 2001, 197238). Figure 3-1 shows observations by Diliberto et al. (1995, 197309) of the ratio of liver concentrations to adipose tissue concentrations for mice given doses spread over a 100−fold range and studied at four different times following exposure. It can be seen that even for the lowest dose studied the liver:fat concentration ratio is higher than would be expected based on the lipid contents of the tissues (i.e., 0.06:1, corresponding to the ratio of human liver:blood and fat:blood partition coefficients; see Table 3-1). Moreover, the relative concentration in the liver consistently rises with dose, with the steepest rise observed during the first two weeks after dosing. If the distribution of TCDD were governed solely by passive partitioning into fat, there should be no such change in relative concentrations with dose. However, data presented in Figure 3-1 illustrate that at longer time points, the ratio of TCDD in the liver to TCDD in fat decreases, indicating that a redistribution of the chemical occurs as time goes on for each applied dose. The redistribution of TCDD tissue levels from liver to fat with increasing time suggests that binding of the chemical in the liver (including via induction of CYP1A2) is an important kinetic consideration at early exposure points with relatively high applied doses.

Experiments with CYP1A2 "knock-out" mice (i.e., congenic strains differing in only a single gene that is "knocked out" in one of the strains) indicate that the inducible binding of TCDD is attributable to CYP1A2 (Diliberto et al., 1997, 548755; 1999, 143713). As noted previously, this enzyme is believed to make an important contribution to metabolism of TCDD. Given the critical role of CYP1A2 induction in the kinetics of TCDD, dose-and time-dependent

induction of this protein in rats has been examined and modeled (Emond et al., 2004, 197315; Emond et al., 2006, 197316; Santostefano et al., 1998, 200001; Wang et al., 1997, 104657). Accordingly, the amount of CYP1A2 in the liver can be computed as the time-integrated product of inducible production and a simple first-order loss process (Wang et al., 1997, 104657):

$$\frac{dCYP_{2A1}}{dt} = S(t)K_0 - K_2C_{A2t} \qquad \text{(Eq. 3-3)}$$

where CYP_{2A1} is the concentration of the enzyme, K_2 is the rate constant for the first order loss, C_{A2t} is the concentration of CYP1A2 in the liver, K_0 is the basal rate of production of CYP1A2 in the liver, and $S(t)$ is a multiplicative stimulation factor for CYP1A2 production in the form of a Hill-type function:

$$S(t) = 1 + \frac{In_{A2}(C_{Ah\text{-}TCDD})^h}{(\mathrm{IC}_{\mathrm{A2}})^{\mathrm{h}} + (C_{Ah\text{-}TCDD})^h} \qquad \text{(Eq. 3-4)}$$

where IC_{A2} corresponds to the concentration of the aryl hydrocarbon (Ah)-TCDD complex at which half of the maximum fold stimulation of CYP2A production is reached, and h, the Hill exponent, determines the curvature of the stimulation in relation to concentration of the Ah-TCDD complex at relatively low doses. A value of 0.6 as the Hill exponent has been used by Wang et al. (1997, 104657; 2000, 198738) and Emond et al. (2004, 197315; 2005, 197317; 2006, 197316), indicative of a negative cooperation, i.e., the curve is convex-upward (supralinear), depicting a faster increase in the low-dose region compared to a straight line. Additional parameters in this expression include In_{A2}, the maximum fold increase in the CYP1A2 synthesis rate over the basal rate that can occur at high levels of TCDD, and ($C_{Ah\text{-}TCDD}$), the concentration of TCDD bound to the aryl hydrocarbon receptor (AhR). This concentration in turn depends on the concentration of TCDD in the liver (C_{Lif}), the concentration of the AhR (Ah_{Li}) in liver, and the dissociation constant for the Ah-TCDD receptor complex, K_{DAh}:

$$C_{Ah\text{-}TCDD} = \frac{Ah_{Li} \times C_{Lif}}{K_{DAh} + C_{Lif}} \qquad \text{(Eq. 3-5)}$$

3.3.2.4. *Elimination*

Elimination half-lives (i.e., the time taken for the concentration to be reduced to one-half of its initial level) of TCDD range from 11 days in the hamster to 2,120 days in humans (U.S. EPA, 2003, 537122). Hepatic metabolism and binding processes, fecal excretion, and accumulation in adipose tissue collectively determine the dose-dependent elimination half-lives in various species. Aylward et al. (2005, 197114) depicted the relationship between the elimination rate versus initial level of lipid-corrected TCDD in serum for 36 people (see Figure 3-2). Even though this analysis was done using the initial TCDD level, rather than the geometric mean or midpoint level in the decline for each person, it indicated a concentration-dependency of the half-life and elimination of TCDD in exposed individuals.

3.3.2.5. *Interspecies Differences and Similarities*

Among the pharmacokinetic determinants of TCDD, some are known to vary markedly between species whereas others are not characterized sufficiently in this regard. Overall, the qualitative determinants of the body burden and elimination half-lives appear to be similar across species. Based on empirical observations for TCDD as well as with other PCDFs, Carrier et al. (1995, 197618; 1995, 543780) argued that in rats, monkeys, and humans, the dose-dependent changes in the fraction contained in liver and adipose tissue follow a similar pattern across species. The authors suggested that the half-saturation body burden is around 100 ng/kg and the plateau of liver dose (as fraction of body burden) appears to occur around 1,000 ng/kg. Literature also indicates that AhR is conserved phylogenetically (Fujii-Kuriyama et al., 1995, 543727; Harper et al., 2002, 198124; Nebert et al., 1991, 543728) and is present in mammalian species, including experimental animals and humans (Lorenzen and Okey, 1991, 198397; Manchester et al., 1987, 198054; Okey et al., 1994, 548759; Roberts et al., 1985, 198706; Roberts et al., 1986, 198780). These qualitative similarities in pharmacokinetic determinants and outcome support the use of animal data to infer general patterns of the pharmacokinetic behavior of TCDD in humans. However, quantitative differences in determinants, including physiological, physicochemical, and biochemical, need to be taken into account. Even though species-specific physiological parameters can be obtained from the literature, key data on species-specific biochemical parameters (particularly binding constants, maximal capacity, induction rates, and other parameters) are not available for humans at this time. However, these

can be inferred by using a pharmacokinetic model fit to in vivo data on the rate of TCDD elimination from specific compartments in humans (Aylward et al., 2005, 197014; Carrier et al., 1995, 197618; Carrier et al., 1995, 543780; Emond et al., 2004, 197315; Emond et al., 2005, 197317; Emond et al., 2006, 197316).

3.3.3. PK of TCDD in Humans: Interindividual Variability

TCDD pharmacokinetics and tissue doses vary across the human population as a function of the interindividual variability of the key kinetic determinants. Because the NAS comments focused on health effects associated with chronic, lifetime exposure, the key kinetic determinants for such exposures include clearance, binding, and temporal changes in volume of distribution. When considering the interindividual variability in pharmacokinetics and dose metrics of TCDD, it is important to recognize that the elevated lipid-corrected serum concentrations in highly exposed persons are associated with greater elimination rates, probably due to greater degrees of induction of CYP1A2 in the liver and possibly other related metabolic enzymes (Abraham et al., 2002, 197034; Aylward et al., 2005, 197014; Emond et al., 2006, 197316; Grassman et al., 2000, 548762).

The interindividual variability in fat content is a critical parameter in pharmacokinetic models given the characteristics of TCDD (see Section 3.3.2). Both metabolic elimination and elimination via the GI tract depend on the fraction of TCDD in the body that is available outside of adipose tissue. As body fat content rises, a smaller portion of the total body TCDD will be contained in the relatively available fraction outside of the adipose tissue. Because elimination of TCDD by both metabolism and fecal excretion depends on the small proportion of TCDD that exists outside of fat tissue, people with larger proportions of body fat—including many older people—will tend to require longer times to reduce TCDD levels by a given proportion than leaner people (Emond et al., 2006, 197316; Rohde et al., 1999, 548764; Van der Molen et al., 1998, 548765; Van der Molen, et al., 1996, 548768).

The sections that follow highlight key aspects of interindividual variability in TCDD pharmacokinetics, with an emphasis on the available data related to elimination half-lives and volume of distribution.

3.3.3.1. *Life Stage and Gender*

The influence of the variability of fat content in human population on the distribution and clearance of TCDD has been evaluated by several investigators. There are data showing an inverse dependency of TCDD elimination rate on percent body fat. Figure 3-3 shows this relationship in a study in which TCDD elimination via feces was measured in six people in relation to their body fat content (Rohde et al., 1999, 548764). Observations of TCDD elimination rates in a small number of men and women in the Seveso cohort (Aylward et al., 2005, 197114) provide a modest opportunity to compare TCDD elimination rates with actual human data. Based on the partition coefficients reported by Emond et al. (2006, 197316), the elimination rates for the men in the sampled group are expected to be greater than the elimination rates in the women. Taking into consideration calculations similar to those shown in Table 3-2, and fat proportions inferred from body mass indices using the equations of Lean et al. (1996, 548770), the Seveso men studied are expected to have an overall average of about 3.92% of their TCDD body burden outside of fat, whereas the women are expected to have an average of only 2.36% outside of fat. On this basis, the TCDD elimination rates in the men are expected to be 3.92/2.36 = 1.66 times faster than the elimination rates in the women. By comparison, Michalek et al. (2002, 199579) reported observed elimination rates in men and women that result in a slightly lower ratio:

$$\frac{\text{men: } 0.111\ \text{year}^{-1} \pm 0.010\ \text{(std.error)}}{\text{women: } 0.071\ \text{year}^{-1} \pm 0.010\ \text{(std.error)}} = 1.56 \qquad \text{(Eq. 3-6)}$$

The central estimates for the elimination rates correspond to half lives of 6.5 and 9.6 years for men and women, respectively.

A further point of comparison can be derived using the observed body mass index (BMI)[12] and TCDD elimination rate of each of the male Ranch Hand military veterans, whose TCDD elimination rates were observed between 9 and 33 years after their time in Vietnam. The average BMI over that time was 29.44 (based on 287 measurements for the 97 veterans, tabulated in three periods by Michalek et al., 2002, 199579), and their average age was about

[12]The body mass index, or BMI, is calculated as the body weight in kilograms divided by the square of the height in meters.

44.5 for the measurements. Based on these data, the corresponding average estimated percent body fat is 29.7% using the Lean et al. (1996, 548770) formula for men. The observed average TCDD elimination rate constant for these men for the period was 0.092 $year^{-1}$ ± 0.004 (standard error), corresponding to a half life of 7.5 years. This half life is slightly longer than the central estimate of the half life of 6.2 years (i.e., ln(2)/0.111) for the smaller group of Seveso males with their slightly smaller estimated percent body fat. Figure 3-4 shows a simple plot of these data and a fitted unweighted regression line characterizing the relationship between estimated fat content and TCDD elimination rates. Variation in metabolic enzyme activities and other routes of loss is also likely to be important, but there is little human quantitative information available on these issues.

More recently, Kerger et al. (2006, 198651) estimated the slope of the relationship between half-life and age to be 0.12 years (95% confidence interval, 0.10–0.14), which corresponds to the rate of increase in TCDD half-life for each year of age. The authors speculated that although age explained most of the variance in the individual half-life trends, it was also correlated with TCDD concentration, BMI, and body fat mass. The regression model developed by these authors discriminated between the high and low TCDD exposures or concentrations. Thus, after accounting for the TCDD (concentration × age) term's effect on the slope of age, the final model for TCDD concentration ≤700 ppt was

$$t_{1/2} = 0.35 + 0.12 \times Age \qquad \text{(Eq. 3-7)}$$

For TCDD concentration >700 ppt, the final model was:

$$t_{1/2} = 0.35 + 0.088 \times Age \qquad \text{(Eq. 3-8)}$$

where $t_{1/2}$ is the half-life and Age is the age at time of subsequent sampling. Pharmacokinetic information relevant to specific age groups is presented in the sections that follow.

3.3.3.1.1. *Prenatal period.*

Data to estimate TCDD elimination rates for fetuses are not available. Levels of TCDD in fetal tissues for rats were experimentally estimated at different gestational periods and utilized in a developmental model by Emond et al. (2004, 197315). There is information on body

composition that is relevant to prediction of TCDD dose to fetus. These data, summarized as part of the radiation dosimetry model of the International Commission on Radiological Protection, are consistent with the idea that early fetuses are nearly all water and less than 1% lipid, and lipid levels rise toward parity with protein near the time of normal delivery.

Bell et al. (2007, 197050) reported that the disposition of TCDD into the fetus shows dose dependency, with a greater proportion of the dose reaching the fetus at lower doses of TCDD. Further, both CYP1A1 and CYP1A2 are highly inducible (~103−fold) in fetal liver, whereas CYP1A2 shows much lower induction (10−fold) in maternal liver. It has been speculated that this is due to the lower basal levels of CYP1A2 in fetal liver, as compared to maternal liver (Bell et al., 2007, 197050). The greater relative disposition to the fetus at low doses may be the result of higher bioavilalibty due to less hepatic sequestration and elimination in the mother.

3.3.3.1.2. *Infancy and childhood.*

Hattis et al. (2003, 548773) describe the general pattern of change of body fat content with age in children. Central tendency values for percent body fat begin at about 12% at birth and rise steeply to reach about 26% near the middle of the first year of life. Fat content then falls to reach a minimum of approximately 15% at 5−8 years of age, followed by a sex-dependent "adiposity rebound" that takes females to about 26% body fat while the males remain near 16−17% on average by age 20. The interindividual variability distributions about these central values are complex, as some children experience the "adiposity rebound" earlier than others, and this creates patterns that are not simply interpretable as unimodal normal distributions. Hattis et al. (2003, 548773) did find it possible to fit distributions of body fat content inferred from NHANES skin fold measures to mixtures of two normal distributions for children between age 5 and 18.

At least two groups of authors have published PBPK modeling results indicating generally more rapid clearance of TCDD in children than in adults, a trend that is consistent with the generally lower fat content of children (Kreuzer et al., 1997, 198088; Leung et al., 2006, 548779; Van der Molen et al., 2000, 548777). The rapid expansion of the adipose tissue compartment can contribute, in part, to the reduced apparent half-life in children (Clewell et al.,

2004, 056269). This reduction may also be due to varying rates of metabolism and/or fecal lipid excretion (Abraham et al., 1996, 548782; Kerger et al., 2007, 548784).

Furthermore, very young children have different modes and quantities of exposure compared to adults. Lakind et al. (2000, 198094) characterize distributions of milk intake for nursing infants to characterize distributions of TCDD exposure. This is also a corresponding route of loss of TCDD stores for lactating women, as described in Section 3.3.3.2 below.

3.3.3.1.3. *Adulthood and old age.*

The fraction of fat in relation to body weight in adulthood and old age can be computed as a function of the BMI and age (e.g., Lean et al., 1996, 548770):

$$\%\,Body\,Fat\,(\text{males}) = 1.33 \times BMI + 0.236 \times Age - 20.2 \qquad \text{(Eq. 3-9)}$$

$$\%\,Body\,Fat\,(\text{females}) = 1.21 \times BMI + 0.262 \times Age - 6.7 \qquad \text{(Eq. 3-10)}$$

The above equations are the result of analysis of data based on underwater weighing of 63 men and 84 women (age range 16.8–65.4). The salient observation with respect to TCDD for these data is that age and BMI-dependent variability in fat content have implications for the variability in TCDD elimination rates and internal dose among adults.

3.3.3.2. *Physiological States: Pregnancy and Lactation*

Data on body fat content in pregnant women at various stages of gestation (Pipe et al., 1979, 548786) have potential implications for TCDD elimination rates during pregnancy, even though the relationship between these parameters has not been formally analyzed.

Lactation is viewed as an additional route of elimination for some chemicals such as TCDD. According to a recent study, a breast-feeding woman expels through lactation an estimated 8.76 kg fat per year [q_f (kg/day), 0.8 kg milk/day with an average 3% lipid], and the partition coefficient between blood lipid and milk fat (K_{BM}) for TCDD is 0.92 (Milbrath et al., 2009, 198044; Wittsiepe et al., 2007, 548736). The estimated rate of elimination of TCDD due to breast-feeding (k_{bfed}) can then be computed as follows (Milbrath et al., 2009, 198044):

$$k_{bfed} = \frac{q_f \times \Delta t_{bfed}}{K_{BM} \times \frac{pbf_i}{100} \times BW_i} \qquad \text{(Eq. 3-11)}$$

where

Δt_{bfed} (unitless) = the fraction of the year during which the woman was actively breast-feeding;

pbf_i = woman's percent body fat; and

BW = woman's body weight in kg.

Assuming no interaction between breast-feeding and other half-life determinants Milbrath et al. (2009, 198044), the authors predicted a half-life of 4.3 years for TCDD in a 30-year-old, nonsmoking woman with 30% body fat if she did not breast-feed that year, and a half-life of 1.8 years if she breast-fed for 6 months.

3.3.3.3. *Lifestyle and Habits*

One of the factors related to lifestyle and habits that could influence TCDD kinetics is smoking. Smoking has been reported to enhance the elimination of dioxin and dioxin-like compounds (Ferriby et al., 2007, 548789; Flesch-Janys et al., 1996, 197351). Milbrath et al. (2009, 198044) accounted for interindividual variation in body composition as well as smoking habits in an empirical model. The predicted half-life (years) for an individual *i* as a function of age, smoking status, and percent body fat *i* was as follows

$$t_{1/2}(age, smoke, pbf)_i = [\beta_{(0age)} + \beta_{(age)} \times age_i] \times SF_i \times \frac{pbf_i}{pbf_{ref(age_i)}} \qquad \text{(Eq. 3-12)}$$

where

$\beta_{(0age)}$ = intercept constant derived from regressed data;

$\beta_{(age)}$ = slope constant derived from regressed data;

age_i = specific age *i* (years);

pbf_i = individual percent body fat;

$pbf_{ref(age_i)}$ = reference percent body fat; and

SF_i = the unitless, multiplicative smoking factor.

3.3.3.4. *Genetic Traits and Polymorphism*

One particular genetic locus that is potentially related to TCDD pharmacokinetics and tissue dose is the gene for the AhR. Eight candidate AhR polymorphisms have been identified to date (Connor and Aylward, 2006, 197632; Harper et al., 2002, 198124). Given the role of AhR in regulating the induction of CYP1 isozymes (Baron et al., 1998, 548791; Connor and Aylward, 2006, 197632; Toide et al., 2003, 548792), the polymorphism might lead to interindividual differences in metabolic clearance, the significance of which would depend upon the dose, fat content, and exposure scenario. In this regard, it should be noted that the inducibility of aromatic hydrocarbon hydroxylase in human tissues has been reported to be highly variable, up to 100−fold (Connor and Aylward, 2006, 197632; Smart and Daly, 2000, 548794; Wong et al., 1986, 548795).

Finally, the scientific literature contains values of K_d (the dissociation constant of the TCDD−AhR complex) ranging from about 1 to much higher values (corresponding to lower binding affinity) (reviewed in Connor and Aylward, 2006, 197632). This provides suggestive evidence for a heterogeneous human AhR, with functionally important polymorphisms (Micka et al., 1997, 548797; Roberts et al., 1986, 198780), even though some of the range may be attributed to experimental procedural differences and to other factors (Connor and Aylward, 2006, 197632; Harper et al., 2002, 198124; Lorenzen and Okey, 1991, 198397; Manchester et al., 1987, 198054).

The various pharmacokinetic processes and determinants (see Sections 3.3.2 and 3.3.3), individually or together, might influence the dose metrics of relevance to the dose-response modeling of TCDD.

3.3.4. Dose Metrics and Pharmacokinetic Models for TCDD

3.3.4.1. *Dose Metrics for Dose-Response Modeling*

The **dose metric** related to a toxicologic endpoint can range from the maximal concentration, the area under a time-course curve (AUC), or the time-averaged concentration of

the toxic moiety in the body, blood, or target tissue, to an appropriate measure of the resulting interactions in the target tissue (e.g., receptor occupancy or functional biomarkers related to specific effects). A single dose metric, however, is unlikely to be sufficient for all endpoints and exposure durations. Further, the ideal dose metric chosen on the basis of the mode of action (MOA) may not be the dose metric for which model predictions can be obtained with a high level of confidence. Consideration of these issues is critical to the selection of the dose metrics of relevance to dose-response modeling of TCDD.

Figure 3-5 lists a range of alternative dose metrics for TCDD in terms of their relevance based on considerations of pharmacokinetic mechanisms and MOA. The **administered dose** or daily intake (ng/kg-day) is the least relevant dose metric for dose-response modeling of TCDD. This dose adjusts only for body weight differences between species. The administered dose**,** when used with an uncertainty factor for kinetics (or kinetic adjustment factor, such as $BW^{3/4}$) and an uncertainty factor for dynamics, can also account for allometrically-predicted pharmacokinetic (clearance) and pharmacodynamic differences between species in deriving the human equivalent dose (HED). In effect, the use of kinetic and dynamic adjustment or uncertainty factors facilitates the computation of HED. Such a calculation of HED is associated with the steady-state blood concentration of parent chemical in rats by accounting for species differences in metabolic clearance. This is generally done by relating to body surface area or metabolic rates, with no corresponding temporal changes in the volume of distribution (see, for example, Krishnan and Andersen, 1991, 548799). Such calculations of HED for TCDD may not be appropriate given that (1) steady-state was not attained in all critical toxicological studies chosen for the assessment, (2) the clearance is mainly due to enzyme(s) and processes whose levels/rates do not necessarily vary across species or life stages as a function of body surface differences, and (3) there is a likelihood of change in volume of distribution over time. Furthermore, the use of administered dose does not explicitly account for the dose-dependent elimination of TCDD from tissues as demonstrated in multiple studies (reviewed in Sections 3.3.2 and 3.3.4). The use of administered dose in TCDD dose-response modeling is unlikely to facilitate the characterization of the true relationship between the response and the relevant measures of internal dose that are influenced by dose-dependent elimination and binding processes. Additionally, the use of administered dose to extrapolate across species or life stages

would not effectively take into account the differences in fat content or the demonstrated dose-dependent and species-dependent differences in elimination half-life of TCDD.

Dose metrics for TCDD may include absorbed dose, body burden, serum or whole blood concentration, tissue concentration, and possibly functional-related metrics of relevance to the MOA (e.g., receptor occupancy, change in protein levels). These measures can be calculated as a current (terminal), average (over a defined period), or integral quantity. The applicability of the integral measures, such as the AUC (i.e., the area under the curve of a plot of blood or plasma concentration vs. time), traditionally used for analyzing chronic toxicity data, is questionable in the case of TCDD. This is because of differences in lifespan and uncertainties regarding the appropriateness of the duration to be specified for averaging the AUC in experimental animals and humans for certain critical effects (NAS, 2006, 198441).

Among the alternative dose metrics, the **absorbed dose** accounts for differences in body weight as well as species-specific differences in bioavailability. Thus, the **absorbed dose** is equivalent to **body burden**. **Body burden**, or more appropriately the body concentration, represents the amount of TCDD per kg body weight. TCDD body burdens, like other dose measures, can be determined as the peak, the average over the period of the bioassays, or the level at the end of the experiments. Thus, the terminal or average body burdens can be obtained either using data or pharmacokinetic models and used in dose-response modeling. The body burden is a measure of TCDD dose that reflects the net impact of bioavailability, uptake, distribution, and elimination processes in the organism. It is essentially a function of the volume of distribution and clearance processes, and as such it does take into account the temporal changes in volume of distribution as well as the concentration-dependent clearance. These are phenomena that are critical to the understanding of TCDD dose to the target. However, the body burden may not accurately reflect the tissue dose (NAS, 2006, 198441), and as such does not allow for analysis of species-specific differences in target organ sensitivity to TCDD. In essence, the body burden represents only an "overall average" of TCDD concentration in the body, without regard to the differential partitioning and accumulation in specific tissues, including the target tissue(s).

Serum (or blood) concentration of TCDD is a dose metric that reflects both the body burden and the dose to target tissues. Serum or blood concentration, at steady-state, would be reflective of the impact of clearance processes, and expected to be directly proportional to the

tissue concentrations of TCDD (NAS, 2006, 198441). This dose metric for lipophilic chemicals such as TCDD is often expressed as a lipid-normalized value, to adjust for varying serum lipid content (e.g.; DeKoning and Karmaus, 2000, 548801; Niskar et al., 2009, 548802) (Patterson et al., 2009), particularly in human biomonitoring studies, thus of relevance to dose-response modeling; however, the serum lipid-normalized concentrations of TCDD are not routinely collected and reported in animal toxicologic studies. Serum lipid-adjusted of TCDD concentration is calculated as the ratio of serum TCDD content over serum lipid content per unit volume. Altenratively, TCDD serum lipid-normalized calculation can be estimated by using the formula TL = (2.27 × TC) +TG + 62.3 mg/dL where the total lipid (TL) content of each sample is estimated from its total cholesterol (TC) and triglyceride (TG) (Patterson et al., 2009). The lipid-adjusted serum concentration, however, would be reflective of the lipid-adjusted concentration of TCDD in other organs (reviewed in Aylward et al., 2008, 197068) depending upon the extent of steady-state attained and the similarity of lipid composition across tissues in each species. In essence, the serum lipid-normalized measure is representative of the amount of TCDD per specified volume of total lipids, whereas the whole blood measure will be reflective of the ensemble of free, lipid-bound and protein-bound TCDD in plasma and erythrocytes, which may be species-specific. Even though these dose metrics are thought to be more closely and directly related to the tissue concentrations associated with an effect, a less direct association might occur at increasing doses when nonlinear processes dominate the kinetics and distribution of TCDD into organs such as the liver.

Tissue concentration of TCDD, as free, bound, or total TCDD, is a more relevant pharmacokinetic measure of dose, given that it provides a measure of exposure of the target cells to the chemical. In this regard, the CYP1A2-bound fraction may be considered as a relevant dose metric for certain toxic effects; however, the available data contain mixed results regarding the mechanistic linkage of this dose metric to toxicity and carcinogenicity (reviewed in Budinsky et al., 2006, 594248). In such cases, the use of alternative dose metrics (e.g., bound concentration as well as the serum concentration) in dose-response modeling could be considered. Other function-related biomarkers and dose metrics could facilitate the additional consideration of pharmacodynamic aspects reflecting tissue- and species-specific sensitivity. These metrics represent the most relevant measures of tissue exposure and sensitivity to TCDD.

Empirical time-course data on the alternative dose metrics of TCDD associated with epidemiologic and experimental (animal) studies are not available, requiring the use of pharmacokinetic models to obtain estimates of these dose metrics. These models may be simple, based on first order kinetics (see Section 3.3.4.2), or more complex based on physiochemical, biochemical, and physiological parameters for simulating uptake, distribution (including sequestration to proteins), and clearance of TCDD (see Section 3.3.4.3). Receptor occupancy and functional biomarkers as dose metrics for TCDD require a clear understanding of mode of action of TCDD and availability of relvant data. In the absence of such information, these possible dose metrics can not be utilized at the present time.

3.3.4.2. *First-Order Kinetic Modeling*

Figure 3-6 illustrates the process of estimating a human-equivalent TCDD oral exposure from an experimental animal-administered dose, based on the assumption that body burden is the effective dose metric for TK equivalence across species. The primary assumption is that the time-weighted average (TWA) TCDD body burden over some critical time period is the proximate toxicokinetically-effective dose eliciting a toxicologic effect.[13] The process consists of estimating the effective average body burden in the experimental animal over some time t_A (generally the experimental duration) using a TK model, then "back-calculating" a daily human exposure level that would result in that average body burden over some time t_H (the human equivalent to t_A).

The following closed-form equation is the general formula used to calculate a TCDD terminal body burden in an experimental animal or human at time (t).

$$BB(t) = BB(0) + \frac{d(1 - e^{-kt})\,fa}{k} \qquad \text{(Eq. 3-13)}$$

where

$BB(t)$ = the body burden at time t (ng/kg);

$BB(0)$ = the initial body burden (ng/kg);

d = the daily dose (ng/kg-day);

k = the whole-body elimination rate (days^{-1});

[13]The conversion depicted in Figure 3-6 does not account for toxicodynamic differences between species.

t = the time at which the body burden is determined (days); and

fa = the fraction of oral dose absorbed (unitless).

For the experimental animal, *BB(t)* is $BB_A(t) = BB_A(0)e^{-k_A t_A} + \frac{d_A(1-e^{-k_A t_A})fa_A}{k_A}$, and for humans, this parameter is $BB_H(t) = BB_H(0)e^{-k_H t_H} + \frac{d_H(1-e^{-k_H t_H})fa_H}{k_H}$.

Setting $BB_H(t) = BB_A(t)$ obtains the following expression:

$$BB_H(0)e^{-k_H t_H} + \frac{d_H(1-e^{-k_H t_H})fa_H}{k_H} = BB_A(0)e^{-k_A t_A} + \frac{d_A(1-e^{-k_A t_A})fa_A}{k_A} \quad \text{(Eq. 3-14)}$$

Rearranging yields the general solution for d_H.

$$d_H = d_A \frac{k_H}{k_A}\frac{fa_A}{fa_H}\frac{(1-e^{-k_A t_A})}{(1-e^{-k_H t_H})} + BB_A(0)e^{-k_A t_A} - BB_H(0)e^{-k_H t_H} \quad \text{(Eq. 3-15)}$$

Assuming that initial body burdens are very small compared to BB(t) and that the fraction of TCDD absorbed is the same for humans and experimental animals, and using the relationship $k = \frac{\ln(2)}{t_{1/2}}$, where $t_{½}$ is the whole-body half-life, a simplified solution for d_H is obtained.

$$d_H = d_A \frac{t_{1/2A}}{t_{1/2H}}\frac{(1-e^{-k_A t_A})}{(1-e^{-k_H t_H})} \quad \text{(Eq. 3-16)}$$

The term $1-e^{-kt}$ is the daily fraction eliminated. Therefore, d_H can be seen to be the average daily administered dose to the experimental animal times the ratio of the animal:human half-life times the ratio of the animal:human daily fraction eliminated over the respective times, t_A and t_H. For both species at (theoretical) steady state ($t \rightarrow \infty$; daily fraction eliminated $\rightarrow$ 1), the latter ratio approaches unity, reducing the animal:human conversion factor to the ratio of the

half-lives. The latter approach was used in the 2003 Reassessment for conversion of animal cancer slope factors to the human equivalent, where only lifetime exposures are relevant.[14]

However, for less-than-lifetime exposures eliciting noncancer effects, specific values for t_A and t_H must be considered. Furthermore, Eq. 3-16 computes d_H on the basis of *terminal* body burdens at times t_A and t_H. The more representative metric for toxicokinetic equivalence based on average body burden over the respective time periods is given in Eq. 3-17.

$$BB(t) = BB(0)\frac{1}{t}\int_0^t e^{-k\tau}d\tau + d\frac{fa}{k}\frac{1}{t}\int_0^t (1-e^{-k\tau})d\tau = BB(0)\frac{\left(1-e^{-kt}\right)}{kt} + d\frac{fa}{k}\left[1-\frac{\left(1-e^{-kt}\right)}{kt}\right] \quad \text{(Eq. 3-17)}$$

On the basis of average body burden as given in Eq. 3-17, is transformed again assuming minimal initial body burden (BB(0) ~ 0), as follows:

$$d_H = d_A\frac{t_{1/2A}}{t_{1/2H}}\frac{\left[1-\frac{\left(1-e^{-k_A t_A}\right)}{k_A t_A}\right]}{\left[1-\frac{t_{H0}}{t_H}-\frac{\left(e^{-k_H t_{H0}}-e^{-k_H t_H}\right)}{k_H t_H}\right]} \quad \text{(Eq. 3-18)}$$

where t_{H0} is the initial human exposure time.

The value of t_A is the duration of the experimental exposure period. For some gestational exposures, if a critical exposure window is defined, t_A will be the duration of the critical exposure window. The value of t_H is the human-equivalent duration corresponding to t_A. However, for t_A less than lifetime (less than 2 years in rodents) and no defined susceptible life stage, t_H cannot begin at 0 (because typically animal experiments do not begin at age 0), but must end at 25,550 days (70 years) to include the terminal (pseudo) steady-state level, at which the $BB_H(t)$: d_H ratio is highest. Otherwise, starting t_H at 0 would not be protective for less-than-lifetime effects that could be manifest at any age in humans; the average is determined from the terminal end of the human exposure period because the daily exposure achieving the target blood concentration is smaller than for the same exposure period beginning at birth (i.e., d_H would be

[14]No conversions to human-equivalent exposures were attempted for other effects in the 2003 Reassessment.

higher for earlier exposure periods) and is health protective for effects occurring after shorter-term exposure.[15] Figure 3-7 depicts the relationship of daily dose to TWA body burden graphically for several exposure duration scenarios. For shorter durations occurring later in life, the average body burden over the exposure period does not differ substantially from the steady-state value. Even for half-lifetime exposures, the deviation of the average from steady state is minimal. Only for lifetime exposures does the difference become more marked, but only by about 15%. Note that in the 2003 Reassessment, a constant value of 3,000 was used for $BB_H(t):d_H$, based on the relationship of continuous exposure to theoretical steady-state body burden (t = lifetime, $t_{½}$ = 2,593 days); this approach, while conservative, does not account for exposure scenarios of different durations and does not strictly reflect the average body burden dose metric.

The simulation in Figure 3-7 is based on a unit daily exposure to humans, such that the target body burden represents $BB_H(t_H):d_H$ as a general scalar for calculating d_H from any given d_A. Table 3-3 shows the resulting TK conversion factors for the rodent species and strains comprising the bulk of the experimental animals in TCDD studies. Monkey and mink values are not shown in this table because, for the former, only chronic exposures were evaluated and, for the latter, no TCDD half-life information is available. Monkey (Rhesus) half-life estimates range from about 200−500 days. A representative value of 365 days is used for this TCDD assessment. The d_A to d_H conversion factor for the chronic monkey exposures (3.5−4 years) in TCDD studies is 9.2−9.7 ($BB_A:d_A$ = 279−263).

Application of first order kinetics for the risk assessment of TCDD can only be used to estimate total body burdens or back-calculate administered dose from experimental data. Body burden calculations using first order kinetics is based on the assumption of a first order decrease in the levels of administered dose as function of time. In that sense, any loss of TCDD from the body is described by using a rate constant that is not specific to any biological process. This constant is usually estimated from estimates of half-life of TCDD. Assuming a constant half-life value for the clearance for long-term or chronic TCDD exposure is not biologically supported given the observed data indicating early influence of CYP1A2 induction and binding to TCDD and later redistribution of TCDD to fat tissue. Abraham et al. (1988, 199510) found that the liver:adipose tissue concentration ratio in female Wistar rats exposed to a subcutaneous TCDD

[15]See the following section (3.3.4.3) for a more detailed discussion of this concept.

dose of 300 ng/kg decreased from 10.3 at 1 day postexposure to 0.5 at 91 days postexposure. Consequently, using half-life estimates based on observed steady-state levels of TCDD will not account for the possibility of accelerated dose-dependent clearance of the chemical at the early stages and thus would result in estimation of lower administered levels of the chemical. The dynamic change in half-life due to dose-dependent elimination at the early stages of TCDD exposure and its later redistribution to fat tissues for steady-state levels is better described using biologically-based models, such as the PBPK models and concentration- and age-dependent elimination (CADM) models (Aylward et al., 2005, 197014; Carrier et al., 1995, 197618; Carrier et al., 1995, 543780; Emond et al., 2004, 197315; Emond et al., 2005, 197317; Emond et al., 2006, 197316). Additionally, these models provide estimates for other dose metrics (e.g., serum or tissue levels) that are more biologically relevant to response than administered dose or total body burden (see Section 3.3.4.3).

3.3.4.3. *Biologically-Based Kinetic Models*

The development and evolution of biologically-based kinetic models for TCDD have been reviewed by EPA (2003, 537122) and Reddy et al. (2005, 594251). The initial PBPK model of Leung et al. (1988, 198815) was developed with the consideration of TCDD binding to CYP1A2 in the liver. The next level of PBPK models by Andersen et al. (1993, 196991) and Wang et al. (1997, 104657) used diffusion-limited uptake and described protein induction by interaction of DNA binding sites. The models of Kohn et al. (1993, 198601) and Andersen et al. (1997, 197172) further incorporated extensive hepatic biochemistry and described zonal induction of CYP by TCDD. TCDD PBPK models have evolved to include detailed descriptions of gastrointestinal uptake, lipoprotein transport, and mobilization of fat, as well as biochemical interactions of relevance to organ-level effects (Kohn et al., 1996, 022626; Roth et al., 1994, 198063). Subsequently, developed PBPK models either used constant hepatic clearance rate (Maruyama et al., 2002, 198448; Wang et al., 1997, 104657; Wang et al., 2000, 198738) or implemented varying elimination rates as an empirical function of body composition or dose (Andersen et al., 1993, 196991; Andersen et al., 1997, 197172; Kohn et al., 1996, 022626; Van der Molen et al., 1998, 548765; Van der Molen et al., 2000, 548777). The more recent pharmacokinetic models explicitly characterize the concentration-dependent elimination of TCDD (Aylward et al., 2005, 197014; Carrier et al., 1995, 197618; Carrier et al., 1995, 543780;

Emond et al., 2004, 197315; Emond et al., 2005, 197317; Emond et al., 2006, 197316). The biologically-based pharmacokinetic models describing the concentration-dependent elimination (i.e., the pharmacokinetic models of Aylward et al. (2005, 197014) and Emond et al. (2005, 197317; 2006, 197316) are relevant for application to simulate the TCDD dose metrics in humans and animals exposed via the oral route. The rationale for considering the application of Aylward et al. (2005, 197014) and Emond et al. (2004, 197315; 2005, 197317; 2006, 197316) models for estimating dose metrics for possible application to TCDD risk assessment is based on the following considerations.

- Both models represent research results from the more recent peer-reviewed publications.
- Both models are relatively simple and less parameterized than earlier kinetic models for TCDD. The Aylward et al. (2005, 197014) model is based on two-time scale TCDD kinetics described by Carrier et al. (1995, 197618), and the Emond et al. (2004, 197315; 2005, 197317; 2006, 197316) PBPK models are reduced versions of earlier complex PBPK models. Although simple, both the Aylward et al. (2005, 197014) and Emond et al. (2004, 197315; 2005, 197317; 2006, 197316) models are still inclusive of important kinetic determinants of TCDD disposition.
- Both models are uniquely formulated with dose-dependent hepatic elimination consistent with the physiological interpretations commonly accepted by the scientific community.
- Both models and extrapolated human versions were tested against human data collected in a variety of human exposure scenarios (Aylward et al., 2005, 197014; Emond et al., 2005, 197317).
- Both models are capable of deriving one or more of the candidate dose-metrics that are of interest to EPA's dose-response assessment of TCDD.

3.3.4.3.1. *CADM model.*

3.3.4.3.1.1. ***Model structure.***

The pharmacokinetic model of Aylward et al. (2005, 197014), referred to as the CADM model in this report, is based on an earlier model developed by Carrier et al. (1995, 197618; 1995, 543780) that describes the dose-dependent elimination and half-lives of polychlorinated dibenzo-*p*-dioxins and furans. This model describes the TCDD levels in blood (body), liver, and adipose tissue. Blood itself is not characterized physically as a separate compartment within the model, and the distribution of TCDD to tissues other than adipose tissue and liver (usually less than 4%) is not accounted for by the model. The original structure of the Carrier et al. (1995,

197618; 1995, 543780) model was modified by Aylward et al. (2005, 197014) to include TCDD elimination through partitioning from circulating lipids across the lumen of the large intestine into the fecal content (see Figure 3-8). The most recent version of the Carrier model (Aylward et al., 2005, 197014; 2008, 197068) includes fecal excretion of TCDD from two routes: (1) elimination from circulating blood lipid through partitioning into the intestinal lumen; and (2) elimination of unabsorbed TCDD from dietary intake.

A basic assumption of this model is that metabolic elimination of TCDD is a function of its current concentration in the liver. The current concentration of TCDD in the liver increases with increasing body burden in a nonlinear fashion as a result of the induction of (and binding of TCDD to) specific proteins (i.e., CYP1A2). Consequently, the fraction of TCDD body burden contained in the liver increases nonlinearly (with a corresponding decrease in the fraction contained in adipose tissues) with increasing body burden of TCDD (Aylward et al., 2005, 197114; Carrier et al., 1995, 197618).

Of particular note is that the adipose tissue compartment of the model is considered to represent the lipid contained throughout the body. It then assumes that the concentrations of TCDD in lipids of plasma and various organs is essentially equivalent to that of adipose tissue, and as such these concentrations are included in the adipose compartment of the model. Even though this approximation is fairly reasonable given the available data, there is some concern that the adipose compartment of this model also includes the lipid content of the liver to some unknown extent. Removal of lipid volume from the liver would mathematically alter total hepatic concentration and therefore would affect the estimated levels of the chemical available for binding to proteins.

Distribution in the body is modeled to occur between hepatic and adipose/lipid compartments, with the fraction of body burden in liver increasing according to a function that parallels the induction of the binding protein CYP1A2. Elimination is modeled to occur through hepatic metabolism (represented as a first-order process with rate constant K that decreases with age) and through lipid-based partitioning of unmetabolized TCDD across the intestinal lumen into the gut, which is also modeled as a first-order process. As the body burden increases, the amount of TCDD in the liver increases nonlinearly, resulting in an increased overall elimination rate.

3.3.4.3.1.2. ***Mathematical representation.***

The CADM model describes the distribution to tissues (including liver and adipose tissue) based on exchange from blood at time intervals of one month. The model is based on quasi-steady-state-approximation, and thus it is also based on the consideration that the intertissue processes reach their equilibrium values "quasi-instantaneously." In this regard, absorption and internal distribution reflective of kinetics at the cellular level (e.g., diffusion, receptor binding, and enzyme induction) likely occur on a relatively fast time scale (a few hours to a few days). However, the overall body concentration (i.e., body burden) varies slowly with time such that it remains virtually unchanged during short time intervals.

The CADM model does not differentiate between binding to AhR and CYP1A2, and it lacks explicit descriptions of CYP1A2 induction, a key determinant of TCDD kinetics. However, the empirical equation in the CADM model is based on five parameters (i.e., f_{min}, f_{max}, K, W_a, and W_l; see Tables 3-4 and 3-5) that allow the successful description of the behavior of TCDD in liver and adipose tissue (i.e., TCDD half-lives in each compartment increase with decreasing body burden). This observation implies that the model adequately accounts for the ensemble of the processes. Essentially, the CADM model describes the rate of change in tissue concentrations of TCDD as a function of total body burden such that the global elimination rate decreases with decreasing body burden or administered dose.

3.3.4.3.1.3. ***Parameter estimation.***

The CADM model is characterized by its simplicity and fewer parameters compared to physiologically-based models. Reflecting this simplicity, hepatic extraction is computed with a unified empirical equation that accounts for all relevant processes (i.e., protein induction and binding).

The key parameters (f_{min}, f_{max}, K, and k_e) were all obtained by fitting to species-specific pharmacokinetic data. The physiological parameters (such as tissue weights) used in the model are within ranges documented in the literature. The fat content is described to vary as a function of age, sex, and BMI. However, the BMI of the model is not allowed to change during an individual simulation (which can range from 20 years to 70+ years) when in reality the percentage of fat in humans changes over time. None of the TCDD-specific parameters were estimated a priori or independent of the data set simulated by the model.

3.3.4.3.1.4. *Model performance and degree of evaluation.*

The CADM model was not evaluated for its capabilities in predicting data sets not used in its parameterization. In other words, one or more of the key input parameters (f_{hmin}, f_{hmax}, k_e, K) was or were obtained essentially by fitting to the species-specific pharmacokinetic data, such that there was no "external" validation data set to which the model was applied. Despite the lack of emphasis on the "external" validation aspect, the authors (Aylward et al., 2005, 197114); (Carrier et al., 1995, 197618; Carrier et al., 1995, 543780) have demonstrated the ability of the model to describe multiple data sets covering a range of doses and species.

The visual comparison of the simulated data to experimental values suggests that the model could, to an approximate degree, correctly reproduce the whole set of data (e.g., pharmacokinetic [PK] profile over a range of dose and time) and not just part of the PK curve, essentially with the use of a single set of equations and parameters.

The pharmacokinetic data sets for TCDD that were used to calibrate/evaluate the CADM model by Aylward et al. (2005, 197114; Carrier et al., 1995, 197618; Carrier et al., 1995, 543780) included the following:

- Adipose tissue and liver concentrations of TCDD following a single oral dose of 1 µg/kg in monkeys (McNulty et al., 1982, 543782);
- Percent dose retained in liver for a total dose of 14 ng in hamsters (Van den Berg et al., 1986, 543781);
- Elimination kinetics of TCDD in female Wistar rats following a single subcutaneous dose of 300 ng/kg (data from Abraham et al., 1988, 199510);
- Liver and adipose tissue concentrations (terminal measurements) in Sprague–Dawley rats given 1, 10 or 100 ng TCDD/kg bw during 2 years (Kociba et al., 1978, 001818); and
- Serum lipid concentrations of TCDD over a period of several years in 54 adults (29 men and 25 women) from Seveso and in three Austrian patients (Aylward et al., 2005, 197114).

For illustration purposes, Figure 3-9 shows model simulations of rat data from Carrier et al. (1995, 197618). Figure 3-2 (see Section 3.3.2.4) depicts the human data that were used by the authors to support the concentration-dependent elimination concept; the model was parameterized to fit approximately to these data (Aylward et al., 2005, 197114).

The authors did not report any specialized analyses that quantitatively evaluated the uncertainty, sensitivity, and/or variability of CADM model parameters and structure.

3.3.4.3.1.5. ***Confidence in CADM model predictions of dose metrics.***

A qualitative level of confidence associated with the predictability and reliability of absorbed dose and body burden for oral exposures in humans (as well as several animal species) by this model can be ranked as high (see Table 3-6). This model, however, does not account for the differential solubility of TCDD in serum lipids and adipose tissue lipids, nor does it account for the diffusion-limited uptake by adipose tissue. Due to these limitations, the confidence associated with the predictions of the serum lipid concentration of TCDD is considered medium, particularly when it is not documented that steady-state is reached during the critical toxicologic studies and human exposures. Furthermore, the CADM model does not facilitate the computation of TCDD concentrations in specific internal organs (other than liver and adipose tissue). The reliability of this model for simulating the liver concentration (free, bound, or total) of TCDD at low doses is considered to be low. This low confidence level is a result of the uncertainty associated with the key parameter f_{hmin}. This parameter needs to be re-calibrated for each study/species/population to effectively represent the free fraction of TCDD in liver and the amount of TCDD contained in the hepatic lipids and bound to the liver proteins (whose levels might be reflective of background exposures of various sources; see Carrier et al., 1995, 197618). The uncertainty related to the numerical value of this parameter in animals and humans—particularly at very low exposures—raises concern regarding the use of this model to predict TCDD concentration (free, bound, or total) in liver as the dose metric for dose-response modeling. Although the use of the parameter f_{hmax} permits the prediction of the dose to liver at high doses, it does not specifically facilitate the simulation of the amount bound to the protein or level of induction in liver. Because the CADM model is not capable of simulating enzyme induction based on biologically-relevant parameters, its reliability for predicting the concentration of TCDD bound specifically to the AhR is not known. Finally, due to the lack of parameterization or verification with kinetic data in pregnant, lactating, or developing animals or humans, the CADM model is unlikely to be reliable in the current form for use in *predicting* potential dose metrics in these subpopulations or study groups that might form the basis of points of departure (PODs) for the assessment.

3.3.4.3.2. *PBPK model.*

3.3.4.3.2.1. ***Model structure.***

Emond et al. (2004, 197315; 2006, 197316) simplified the eight-compartment rat model of Wang et al. (1997, 104657) to a four-compartmental model (liver, fat, rest of body and placenta with fetal transfer) (Emond et al., 2004, 197315), and later to a three-compartment adult model (liver, fat, rest of the body) (Emond et al., 2006, 197316) (see Figures 3-10 and 3-11). Their rationale for simplification of the model was based on evaluating, critiquing, and improving all earlier PBPK models by Wang et al. (1997, 104657). In general, the main reason for the simplification was that extrapolation of a PBPK model to humans with these many (i.e., eight compartments) compartments would be problematic due to the limited availability of relevant human data for validation (Emond et al., 2004, 197315). One major difference from earlier models, repeatedly emphasized by Emond et al. (2005, 197317; 2006, 197316), was their description (included in their simplified PBPK models) of the dose-dependent, inducible elimination of TCDD. The rationale for including TCDD binding and induction of CYP1A2 into the model was earlier described by Santostefano et al. (1998, 200001).

The most recent version of the rat and human PBPK models developed by Emond et al. (2006, 197316) describes the organism as a set of three compartments corresponding to real physical locations—liver, fat, and rest of the body—interconnected by systemic circulation (see Figure 3-10). The liver compartment includes descriptions of CYP1A2 induction, which is critical for simulating TCDD sequestration in liver and dose-dependent elimination of TCDD. In this model, the oral absorption of TCDD from the GI tract accounts for both the lymphatic (70%) and portal (30%) systems.

The biological relationship between TCDD "sequestration" by liver protein and its "elimination" by the liver is not entirely clear. TCDD is metabolized slowly by unidentified enzymes. CYP1A2 is known to metabolize TCDD based on studies in CYP1A2 KO mice (Diliberto et al., 1997, 548755; 1999, 143713), in which the metabolic profile is different compared to wild-type mice. However, since several metabolites appear in the feces of CYP1A2 knock out mice, it is assumed that there are other enzymes involved in TCDD metabolism. TCDD binds to the AhR and induces not only CYP1A2, but also CYP1A1, CYP1B1, and several UGTs and transporters (Gasiewicz et al., 2008, 473406). Both hydroxylated and glucuronidated hydroxyl metabolites are found in the feces of animals treated with TCDD (Hakk et al., 2009,

594256). Because the exact enzymes involved with TCDD are unknown and yet the metabolism is induced by TCDD, an assumption of increased the elimination rate of TCDD in proportion to the induction of CYP1A2 is made. In the PBPK model, CYP1A2 is needed because TCDD binds to rat, mouse, and human CYP1A2 (Diliberto et al., 1999, 143713; Staskal et al., 2005, 198276). Thus CYP1A2 induction is necessary to describe TCDD pharmacokinetics due to TCDD binding. Hence, CYP1A2 can be used as a marker of Ah-receptor induction of "TCDD metabolizing enzymes." Other models use AhR occupancy as a marker of induction of "TCDD metabolizing enzymes" (Andersen et al., 1997, 197172; Kohn et al., 2001, 198767).

Figure 3-11 depicts the structure of the rat developmental-exposure PBPK model (Emond et al., 2004, 197315). This model was developed to describe the relationship between maternal TCDD exposure and fetal TCDD concentration during critical windows of susceptibility in the rat. In formulating this PBPK model, Emond et al. (2004, 197315) reduced the original 8-compartment model for TCDD in adult rats by Wang et al. (1997, 104657) to a 4-compartment (i.e., liver, fat, placenta, and rest of the body) model for maternal rat. Activation of the placental compartment and a separate fetal compartment occurs during gestation (Emond et al., 2004, 197315).

3.3.4.3.2.2. ***Mathematical representation.***

The key equations of the PBPK model of Emond et al. (2004, 197315) are reproduced in Text Boxes 3-1 and 3-2, whereas those from Emond et al. (2005, 197317; 2006, 197316) are listed in Table 3-7. The rate of change of TCDD in the various tissue compartments is modeled on the basis of diffusion limitation considerations. Accordingly, mass balance equations are used to compute the rate of change in the tissue (i.e., intracellular compartment) and tissue blood (i.e., extracellular compartment). The membrane transfer of TCDD is computed using a permeation coefficient-surface area cross product (PA) for each tissue. Metabolism and binding of TCDD to the AhR and inducible hepatic protein (CYP1A2) are described in the liver. The total mass in the liver was then apportioned between free dioxin (C_{lf}) and bound forms of TCDD (see Figure 3-12). The dose- and time-dependent induction of hepatic CYP1A2 in the liver is described per Wang et al. (1997, 104657) and Santostefano et al. (1998, 200001). Accordingly, the amount of CYP1A2 in the liver was computed as the time-integrated product of inducible production and a simple first-order loss process (Wang et al., 1997, 104657):

$$\frac{dCYP_{1A2}}{dt} = S(t)K_0 - K_2C_{A2t} \quad \text{(Eq. 3-19)}$$

In this expression, CYP_{1A2} is the concentration of the enzyme (nmol/g), K_2 is the rate constant for the first order loss (hour^{-1}), C_{A2t} is the concentration of CYP1A2 in the liver (nmol/g), K_0 is the basal rate of production of CYP1A2 in the liver (nmol/g.hr), and $S(t)$ (unitless) is a multiplicative stimulation factor for CYP1A2 production in the form of a Hill-type function (see Section 3.3.2.3):

$$S(t) = 1 + \frac{In_{A2}(C_{Ah\text{-}TCDD})^h}{(IC_{A2})^h + (C_{Ah\text{-}TCDD})^h} \quad \text{(Eq. 3-20)}$$

where, $S(t)$ is the stimulation function, In_{A2} is the maximum fold of CYP1A2 synthesis rate over the basal rate, $C_{Ah\text{-}TCDD}$ is the concentration of AhR occupied by TCDD, and IC_{A2} is the Michaelis-Menten constant of CYP1A2 induction (nM). The dose-dependent or variable elimination of TCDD was described using the relationship:

$$KBILE\ LI = \left[\frac{CYP1A2_{induced} - CYP1A2_{basal}}{CYP1A2_{basal}}\right] \times Kelv \quad \text{(Eq. 3-21)}$$

where $CYP1A2_{induced}$ is the concentration of induced CYP1A2 (nmol/mL), $CYP1A2_{basal}$ is the basal concentration of CYP1A2 (nmol/mL), and $Kelv$ is the interspecies constant adjustment for the elimination rate (hour^{-1}).

There are various ways of formulating the dose-dependent elimination as a function of the level of CYP1A2, and the above equation (used by the authors) can be viewed as one means of describing this behavior quantitatively. The numerator in the equation above will always be greater than zero when there is TCDD in the system (including TCDD derived from either background exposures or defined external sources). Consequently, the rate of elimination will correspond to a nonzero value for situations involving TCDD exposures. Furthermore, the numerator in Eq. 3-21 should more appropriately be $CYP1A2_{induced}$ rather than [$CYP1A2_{induced} - CYP1A2_{Basal}$] to avoid the problem of lower levels of induction at low doses resulting in a lower

than basal rate of synthesis of *CYP*1A2. The above equation, however, does not describe changes in elimination rate in direct proportionality with the CYP1A2 levels; also, the *Kelv* value by itself does not reflect a scalable basal metabolic rate. Rather, these two terms collectively describe the outcome related to the TCDD elimination processes, based on fitting to observations in rats (Santostefano et al., 1998, 200001). The impact of CYP1A2 induction and sequestration on binding and elimination of TCDD is simulated using the Emond et al. (2004, 197315) model.

The gestational model consisted of a fetal compartment, and the transfer of TCDD between the placental and fetal compartments was described as a diffusion-limited (rather than a perfusion-limited) process (see Text Boxes 3-1 and 3-2).[16]

Text Box 3-1.

Variation of Body Weight with Age: $$BW_{Time}(g) = BW initial \times \left(\frac{0.41 \times Time}{1402.5 + Time} \right)$$

Cardiac Output: $$Qc(mL/h) = Qcc \times 60 \left(\frac{BWmother}{1,000} \right)^{0.75}$$

A factor of 60 corresponds to the conversion of minutes to hours, and 1,000 is the conversion of body weight from g to kg.

Blood Compartment:

$$Cb(nmol/mL) = \frac{((Qf \times Cfb) + (Qre \times Creb) + (Qli \times Clib) + (Qpla \times Cplab) + Lymph)) - (Cb \times Clru)}{Qc}$$

[16]Diffusion limited, sometimes also known as "membrane limited," means a chemical's movement from one side of the membrane to the other is limited by the membrane. Thus, the membrane, in this case, is a limiting factor for uptake. Perfusion limited, also known as "flow limited" indicates that a chemical is so rapidly taken up (e.g., by the tissue from the blood) that the flow rate is the only limiting factor.

Text Box 3-2.

Placenta Tissue Compartment

(a) Tissue-blood subcompartment

$$\frac{dAplab}{dt}(nmol/h) = Qpla(Ca - Cplab) + PApla(Cplab - Cplafree)$$

$$Cplab = \frac{Aplab}{Wplab}$$

(b) Tissue cellular matrices

$$\frac{dApla}{dt}(nmol/h) = PApla(Cplab - Cplafree) - \frac{dApla\_fet}{dt} + \frac{dAfet\_pla}{dt}$$

$$Cpla(nmol/mL) = \frac{Apla}{Wpla}$$

Free TCDD Concentration in Placenta

$$Cplafree(nmol/mL) = Clpla - \left[(Cplafree \times Ppla + \left(\frac{Plab\max \times Cplafree}{Kdpla + Cplafree}\right)\right]$$

Dioxin Transfer from Placenta to Fetuses

$$\frac{dAPla\_fet}{dt}(nmol/h) = Cl_{Pla\_fet} \times Cpla$$

Dioxin Transfer from Fetuses to Placenta

$$\frac{dAfet\_Pla}{dt}(nmol/h) = Cl_{Pla\_fet} \times CfetV$$

Fetal Dioxin Concentration (Fetuses 5 = Per Litter)

$$\frac{dAfet}{dt}(nmol/h) = \frac{dAPla\_fet}{dt} - \frac{dAfet\_Pla}{dt}$$

$$Cfet(nmol/h) = \frac{Afet}{Wfet}$$

$$CfetV(nmol/mL) = \frac{Cfet}{Pfet}$$

1

2

3.3.4.3.2.3. *Parameter estimation.*

Table 3-8 lists the numerical values of the adult rat and human PBPK models of Emond et al. (2005, 197317; 2006, 197316). The values for key input parameters of the rat gestational model are summarized in Table 3-8 as well as Figure 3-13.

The parameters for the rat model were obtained primarily from Wang et al. (1997, 104657) except that the value of affinity constant for CYP1A2 was changed from 0.03 to 0.04 nmol/mL to get better fit to experimental data (Emond et al., 2004, 197315) and the variable elimination parameter (*Kelv*) was obtained by optimization of model fit to kinetic data from Santostefano et al. (1998, 200001) and (Emond et al., 2005, 197317; Emond et al., 2006, 197316; Wang et al., 1997, 104657). Wang et al. (1997, 104657) used measured tissue weights whereas the tissue blood flows and tissue blood weights were obtained from International Life Sciences Institute (ILSI, 1994, 046436). The partition coefficients (which were similar to those of Leung et al., 1988, 198815; 1990, 192833), the permeability x area (PA) value for tissues, the dissociation constant for binding to CYP1A2 (IC_{A2}) and the Hill coefficient (*h*) were estimated using a two-stage process of fitting to dose-response and time-course data on TCDD tissue distribution (Wang et al., 1997, 104657). In the initial stage, the experimental data of arterial blood concentrations were used as input to the individual compartment to estimate the parameters; then, with the values obtained during stage one as initial estimates, those unknown parameters were re-estimated by solving the entire model at once using an optimization route (Wang et al., 1997, 104657). The receptor concentrations and dissociation constant of TCDD bound to AhR were obtained by fitting the model to TCDD tissue concentration combining with enzyme data reported by Santostefano et al. (1998, 200001) whereas the basal CYP1A2 in liver was based on literature data (Wang et al., 1997, 104657).

The parameters for the human PBPK model were primarily based on the rat model (Emond et al., 2005, 197317; Emond et al., 2006, 197316; Wang et al., 1997, 104657). Specifically, the blood fraction in the tissues, the tissue:blood partition coefficients, tissue permeability coefficient, the binding affinity of TCDD to AhR and CYP, and the maximum binding capacity in the liver for AhR were all set equal to the values used in the rat model. The species-specific *Kelv* was estimated by fitting to human data (Emond et al., 2005, 197317).

For the gestational rat model, the parameters describing the growth of the placental and fetal compartments as well as temporal change in blood flow during gestation were incorporated

based on existing data. Exponential equations for the growing compartments were used (see Figure 3-13), except for adipose tissue for which a linear increment based on literature data was specified. While physiological parameters for the pregnant rat were obtained from the literature, all other input parameters were set equal to that of nonpregnant rat (obtained from Wang et al., 1997, 104657), see Tables 3-7 and 3-8. The current version of the rat gestational model contains parameters for variable elimination from Emond et al. (2006, 197316; Table 3-8), and still provides essentially the same predictions as the original publication (Emond et al., 2004, 197315).

3.3.4.3.2.4. ***Model performance and degree of evaluation.***

The PBPK model of Emond et al. (2004, 197315; 2005, 197317; 2006, 197316) had parameters estimated by fitting to kinetic data, such that the resulting model consistently reproduced the kinetic data. The same model structure with a single set of species-specific parameters could reproduce the kinetics of TCDD following various doses and exposure scenarios not only in the rat but also in humans. The simulations of the PBPK model of Emond et al. (2006, 197316) have been compared with two sets of previously published rat data: blood pharmacokinetics following a single dose of 10 μg/kg (the dose corresponding to the mean effective dose for induction of CYP1A2) (Santostefano et al., 1998, 200001) (see Figure 3-14); and hepatic TCDD concentrations during chronic exposure to 50, 100, 500, or 1,750 ng/kg (Walker et al., 1999, 198615) (see Figure 3-15). It is relevant to note that the PBPK model of Emond et al. (2004, 197315; 2006, 197316) is essentially a reduced version of the Wang et al. (1997, 104657) model, and it therefore provides simulations of liver and fat concentrations of TCDD that deviated by not more than 10−15% of those of Wang et al. (1997, 104657). The nongestational model of Emond et al. (2004, 197315) simulated the kinetic data in liver, fat, blood and rest of body of female Sprague-Dawley rats given a single dose of 10 μg TCDD/kg (data from Santostefano et al., 1996, 594258) and in liver and fat of male Wistar rats treated with a loading dose of 25 ng/kg followed by a weekly maintenance dose of 5 ng TCDD/kg by gavage (data from Krowke et al., 1989, 198808).

The gestational rat PBPK model simulated the following PK data sets (Emond et al., 2004, 197315):

- TCDD concentration in blood, fat, liver, placenta, and fetus of female Long–Evans rats given 1, 10, or 30 ng/kg, 5 days/week, for 13 weeks prior to mating followed by daily exposure through parturition (Hurst et al., 2000, 198806);
- TCDD concentration in tissues (liver, fat), blood, placenta and fetus determined on gestation day (GD) 16 and GD 21 following a single dose of 0.05, 0.8, or 1 µg/kg given on GD 15 to pregnant Long Evans rat (Hurst et al., 2000, 199045);
- Maternal and fetal tissue concentrations on GD 9, GD 16 and GD 21 after a single dose of 1.15 µg TCDD/kg given to Long–Evans rats on GD 9 or GD 15 (Hurst et al., 1998, 134516); and
- Fetal TCDD concentrations determined on GD 19 and GD 21 in rats exposed to 5.6 µg TCDD/kg on GD 18 (Li et al., 2006, 199059).

Furthermore, the scaled rat model was shown to be capable of simulating human data from the Austrian and Seveso subjects (see Figures 3-16 and 3-17). In this regard, it is useful to note that the computational version of the PBPK model of Emond et al. (2005, 197317; 2006, 197316) also contained the necessary equation to transform the model output of blood concentration into serum lipid adjusted concentration of TCDD.

The human model of Emond et al. (2005, 197317; Emond model) has advantages for improving the TCDD dosimetry used in existing human epidemiological studies because the model predicts the redistribution of TCDD within the body (to stores in fat and liver) based on physiological principles. However, because the dose-dependency of metabolic elimination in the Emond model was not calibrated to human data, it is important to review the predictions of this model using a database of human observations that is as extensive as possible and a spread of internal TCDD concentrations that is as wide as possible. Thus, presented below is a juxtaposition of modeled elimination rates from the Emond model with observations for two highly exposed Austrian patients (severe intoxication of "unknown origin" (Geusau et al., 2001, 197444)) and nine of 10 Ranch Hand veterans[17] used for the original "validation" comparisons presented in the Emond et al. (2005, 197317).

Figure 3-18 shows the time course of the declines in TCDD serum concentrations in two highly-exposed Austrian subjects compared with the Emond model results. The comparison in Figures 3-17 and 3-18 indicates that the Emond model adequately describes the rate of TCDD

[17]In preliminary comparisons, the simulation run for the 10th Ranch Hand veteran appeared anomalous and was therefore excluded from this summary.

elimination for the more highly exposed Austrian patients, but predicts a somewhat faster rate of decline than that observed for the less heavily exposed patient.

Figure 3-19 shows the results of combining the simulated and observed rates of loss for a group of Austrian and Ranch Hand subjects evaluated by Emond et al. (2005, 197317), counting only one data point per person. The X-axis in this figure is the TCDD serum concentration at the midpoint of the observations for each subject. The error bars in the figure represent ±1 standard error. The results of this figure illustrate two points: (1) the Emond model simulation (open squares) are generally very close to the actual data (solid circles) for the nine Ranch hands (clustered toward lower left corner) and one of the the two Austrian patients (upper right corner); and (2) both the Emond model simulation results and the actual data show a linear trend and linear regression lines were plotted, respectively, as shown in Figure 3-19.

Table 3-9 presents the results of regression analyses of the observed rates of decline in relation to the estimated TCDD serum levels at the midpoint of the observations for each subject in the Ranch Hand study (see Figure 3-19). These results indicate that some appreciable dose dependency of TCDD elimination is unequivocally supported. However, the central estimate of the slope of the relationship between the log of the TCDD elimination rate and the log of the TCDD level is only about 75% of that expected under the Emond et al. PBPK model (i.e., $0.092 \div 0.123 = 0.748$).

Overall, the conclusion from the above analysis is that the Emond model is reasonable to use, but the model might be improved by (1) include the two nondose-dependent pathways of elimination documented in the Geusau papers (GI elimination via the feces and loss via the sloughing of skin cells), and (2) reducing the extent of loss via the dose-dependent metabolism pathway from the liver (Geusau et al., 2002, 594259; Harrad et al., 2003, 197324) so that overall loss rates for the average elimination rates from the Ranch Hand veterans is maintained.

A sensitivity analysis of inputs used to estimate inducible elimination rate for a single oral dose of 0.001 to 10 μg/kg in the rat indicated that the number of key parameters ranged from seven at the low dose region to 12 at the high dose (see Figure 3-20)(Emond et al., 2006, 197316). The sensitive parameters identified included the oral absorption parameters (KABS), volumes of liver and adipose tissue (WLIO, WFO), adipose tissue:blood partition coefficient (PF), and the basal CYP1A2 level (CYP1A2 1A2). At high doses, the most sensitive parameters

also included those related to the maximal induction of CYP1A2 and AhR binding capacity (see Figure 3-20) (Emond et al., 2006, 197316).

The gestational rat model described in Emond et al. (2004, 197315), upon reparameterization, could simulate the kinetics of TCDD in mice. The initial changes to the rat model parameters included: rest of the body:blood partition coefficient (PRE), basal concentration (CYP1A2_1A2), delay in induction time (CYP1A2_1TAU) and adipose tissue permeability coefficient (PAFF), in accordance with Wang et al. (2000, 198738) (see Table 3-8). Subsequently, four parameters (adipose tissue:blood partition coefficient, CYP1A2 affinity parameter, GI tract elimination transit constant ($hour^{-1}$) and the interspecies metabolic parameter *Kelv* ($hour^{-1}$) were re-estimated based on visually fit of model simulations to the PK data from Diliberto et al. (2001, 197238), following an oral dose 150 ng TCDD/kg/day, 5 days/week for 17 weeks (see Table 3-7). The resulting mouse model is capable of reproducing the kinetics of TCDD in the adult (see Figures 3-21 through 3-27), as well as, to a very limited extent, the kinetics during gestation (see Figure 3-28).

3.3.4.3.2.5. ***Confidence in PBPK model predictions of dose metrics.***

The PBPK model facilitates prediction of absorbed dose, body burden, and blood concentration of TCDD for oral exposures in adult humans and rats (adult and developing) with high confidence (see Table 3-10). The model output of blood concentration can be normalized to lipid content representative of the study group (species, sex, age, lifestage, and diet). However, the PBPK model of Emond et al. (2004, 197315; 2005, 197317; 2006, 197316) does not simulate plasma and erythrocyte TCDD concentrations separately, and it predicts tissue concentrations on the basis of tissue:whole blood partition coefficients and not on the basis of serum lipid-normalized values.

The reliability of this model for simulating the liver concentration of TCDD in rats is considered to be high but it is considered to be medium for humans. Although empirical data on bound or free concentrations were not used to evaluate model performance in humans, the biological phenomena (consistent with available data) related to the hepatic sequestration, enzyme induction, and dose-dependent elimination are described in the model. This is one of the situations where PBPK models are uniquely useful; that is, they permit the prediction of system behavior based on understanding of the mechanistic determinants, even though the required data

cannot be directly obtained in the system (e.g., bound concentrations in the liver of exposed humans). For these dose measures (i.e., bound concentration and total liver concentration), the level of confidence can be further improved or diminished by the outcome of sensitivity analysis. In this regard, the results of a focused sensitivity analysis indicate that the most sensitive parameters of the human model are among the most uncertain (i.e., those parameters for which estimates were not obtained in humans) with respect to prediction of liver TCDD concentration, contrary to the animal model (see Section 3.3.6).

With respect to the mouse model, however, the level of confidence is low to medium, given that it has not been verified extensively with blood, body burden, or tissue concentration time-course or dose-response data. However, the mouse PBPK model, based on the rat model that has been evaluated with several PK data sets, has been shown to reproduce well the limited mouse liver kinetic data (see Figures 3-21 through 3-28; Boverhoff et al., 2005, 594260). The same model structure has been used for simulating kinetics of TCDD in humans successfully. Overall, the adult mouse model, given its biological basis combined with its ability to simulate TCDD kinetics in multiple species, is considered to exhibit a medium level of confidence for simulating dose metrics for use in high to low dose extrapolation and interspecies (mouse to human) extrapolation. Even though similar considerations are applicable to gestational model in mice, the confidence level is considered to be low since very limited comparison with empirical data has been conducted (see Figure 3-28). Despite the uncertainty in these predictions, the scaled rat gestational model, given its biological and mechanistic basis, might be of use in predicting dose metrics in these groups that might form the basis of PODs in certain key studies.

3.3.4.4. *Applicability of PK Models to Derive Dose Metrics for Dose-Response Modeling of TCDD: Confidence and Limitations*

Both the CADM and PBPK models describe the kinetics of TCDD following oral exposure to adult animals and humans by accounting for the key processes affecting kinetics, including hepatic sequestration phenomena, induction, and nonlinearity in elimination, and distribution in adipose tissue and liver. Both models can be used for estimating body burdens and serum lipid adjusted concentrations of TCDD. However, there are several differences between these two models. The PBPK model calculates the free and bound concentrations of TCDD in the intracellular subcompartment of tissues. The total or receptor-bound

concentrations in liver are unambiguous and more easily interpretable with the PBPK model than with the CADM model. In addition, the PBPK model computes bound and total concentrations as a function of the free concentration in the intracellular compartment of the tissue. By contrast, the CADM model simulates the total concentration based on empirical consideration of hepatic processes. Consequently, the amount of TCDD bound to AhR or CYP1A2 cannot be simulated with the CADM model. The CADM model computes only the total TCDD concentration in liver, and describes TCDD elimination through partitioning from circulating lipids across the lumen of the large intestine into the feces, while the PBPK model accounts for this process empirically within its hepatic elimination constant. Elimination of TCDD via skin, a minor process, is not described by either model. Thus, dose-response modeling based on body burden of TCDD in adult animals and humans can be conducted with either of the models, provided the duration of the experiment is at least one month, due to limitations in the CADM model. As shown in Figure 3-29, the predicted slope and body burden over a large dose range are quite comparable (generally within a factor of two).

Results of simulations of serum lipid concentrations or liver concentrations vary for the two models to a larger extent (up to a factor of 7), particularly for simulations of short duration. These differences reflect two characteristics of the PBPK model: first, quasi-steady-state is not assumed in the PBPK model; second, the serum lipid composition used in the model is not the same as the adipose tissue lipids. The CADM model does not account for differential solubility of TCDD in serum lipids and adipose tissue lipids, nor does it account for the diffusion-limited uptake by adipose tissue. Therefore, the PBPK model would appear to be superior to the CADM model with respect to the ability to simulate serum lipid and tissue concentrations during exposures that do not lead to the onset of steady-state condition in the exposed organism.

The CADM model is simple and based on fewer parameters than the PBPK model. Because the CADM model is constructed by fitting to data, its performance is likely to be reliable for the range of exposure doses, species, and life stages from which the parameter estimates were obtained. On the other hand, the PBPK model structure and parameters are biologically-based and can be adopted for each species and life stage. Accordingly, the PBPK model has been adopted to simulate the kinetics of TCDD in the fetus and in pregnant rats, as well as in adult humans and rats (Emond et al., 2004, 197315; Emond et al., 2005, 197317; Emond et al., 2006, 197316). The time step for calculation and dosing in the CADM model

corresponds to 1 month. This requirement represents a constraint in terms of the use of this model to simulate a variety of dosing protocols used in animal toxicity studies. This requirement, however, is not a constraint with the PBPK models. So, simulating the body burden and serum lipid concentrations for a longer duration of exposure, either model would appear to be useful; but the PBPK model would be the tool of choice for simulating alternative dose metrics of TCDD (e.g., blood concentration, total tissue concentration, bound concentration) for various exposure scenarios (including single dose studies), routes and life stages in the species of relevance, to TCDD dose-response assessment, particularly, mice, rats, and humans.

Two minor modifications, to enhance the biological basis, were made to the PBPK model of Emond et al. (2006, 197316), before its use in the computation of dose metrics for TCDD. The first one involved the recalculation of the volume of the rest of the body as follows:

$$WRE0 = (0.91 - (WLIB0 \times WLI0 + WFB0 \times WF0 + WLI0 + WF0)/(1 + WREB0)) \quad (3\text{-}22)$$

where

$WRE0$ = weight of cellular component of rest of body compartment (as fraction of body weight);

$WLI0$ = weight of cellular component of liver compartment (as fraction of body weight);

$WF0$ = weight of cellular component of fat compartment (as fraction of body weight);

$WREB0$ = weight of the tissue blood component of the rest of body compartment (as fraction of body weight);

$WLIB0$ = weight of the tissue blood component of the liver compartment (as fraction of body weight); and

$WFB0$ = weight of the tissue blood component of the fat compartment (as fraction of body weight).

In the original code, the weight of the rest of body compartment was calculated as the difference between 91% of body weight and the sum total of the fractional volumes of blood, liver tissue (intracellular component), and adipose tissue (intracellular component). The blood compartment in the PBPK model is not explicitly characterized with a volume; as a result, the

total volume of the compartments is less than 91%. The recalculations shown above were used to address this problem. Given the very low affinity of TCDD for blood and rest of the body, reparameterizing the model resulted in less than a 1% change in output compared to the published version of the PBPK model for chronic exposure scenarios (Emond et al., 2006, 197316).

The second minor modification related to the calculation of the rate of TCDD excreted via urine. The original model code computed the rate of excretion by multiplying the urinary clearance parameter with the concentration in the rest of the body compartment. Instead, the code was modified to use the blood concentration in this equation. This resulted in the re-estimation of the urinary clearance value in the rat and human models but it did not result in any significant change in the fit and performance of the original model.

The revised parameter estimates of the rat, mouse, and human models are captured in Table 3-8 with a footnote.

3.3.4.5. *Recommended Dose Metrics for Key Studies*

The selection of dose metrics for the dose-response modeling of key studies is largely the result of (1) the relevance of a dose metric on the basis of current knowledge of TCDD's mechanism of action for critical endpoints and (2) the feasibility and reliability of obtaining the dose metric with available PK models. Secondarily, the goodness-of-fit of the dose-response models (which reflects the relationship of the selected internal dose measures to the response) can be used to inform selection of the most appropriate dose metric for use in deriving TCDD toxicity values.

Body burden—even though this metric is based on mechanistic considerations—is a somewhat distant measure of dose with respect to target tissue dose, and this metric represents the "overall" average concentration of TCDD in the body. However, a benefit of body burden is that this metric represents a dose measure for which the available PK models can provide highly certain estimates. Thus, the overall confidence associated with the use of body burden in TCDD assessment is categorized as medium.

The confidence in the ability of PK models to simulate blood concentration as a dose metric is high, given that the models have been shown to consistently reproduce whole blood (or serum lipid-normalized) TCDD concentration profiles in both humans and rats. Considering the

facts that the PBPK models simulate whole blood rather than the serum lipid-normalized concentrations of TCDD and that the study-specific values of serum lipid content are not known with certainty, it is preferable to rely on TCDD blood concentrations as the dose metric. The blood concentrations, if intended, can be normalized on the basis of appropriate total lipid levels. However, based on mechanistic considerations, the confidence in their use would be somewhat lower for hepatic effects. This conclusion reflects the concern regarding the inconsistent relationship between the two variables with increasing dose levels and the fraction of steady-state attained at the time of observation. For other systemic effects related to tissue concentrations, the confidence in the use of TCDD serum or blood concentration is high, particularly for chronic exposures, given the absence of data on organ-specific nonlinear mechanisms. In general, the tissue concentration typically cannot be calculated as a reliable dose metric with either the CADM or the Emond models. One exception is the use of the Emond PBPK models to estimate levels in liver, a metric that is relevant based on MOA considerations. However, it is noted that the hepatic TCDD level encompasses free and bound TCDD and it is a highly complex entity for dose metric considerations. Finally, the AhR-bound concentration may be evaluated for receptor-mediated effects. This dose metic can be obtained by PBPK models, although uncertainties associated with lack of data for this dose metric renders it to be of low confidence (see Table 3-10), The alternative dose metrics for dose-response modeling of TCDD selected on the basis of MOA and PK modeling considerations are summarized in Tables 3-11 and 3-12.

These measures of internal dose can be obtained as peak, average, integral (AUC), or terminal values. For chronic exposures in rodents (ca. 2 years), the terminal and average values would be fairly comparable under steady-state conditions. For less-than lifetime exposures, however, the terminal and average values will differ, and therefore an overall average or integrated value (AUC) would be more appropriate. Similarly, for developmental exposures, these alternative dose metrics can be obtained with reference to the known or hypothesized exposure window of susceptibility.

3.3.5. Uncertainty in Dose Estimates

3.3.5.1. *Sources of Uncertainty in Dose Metric Predictions*

3.3.5.1.1. *Limitations of available PK data.*

3.3.5.1.1.1. ***Animal data.***

The available animal data relate to blood, liver, and adipose tissue concentrations for certain exposure doses and scenarios. Although these data are informative regarding the dose- and time-dependency of TCDD kinetics for the range covered by the specific studies (see Section 3.3.2), they do not provide the peak, average, terminal, or lipid-normalized values of dose metrics associated with the key studies selected for this assessment. The limited available animal PK data are useful, however, in the evaluation of the pharmacokinetic models (see Section 3.3.4).

3.3.5.1.1.2. ***Human data.***

The human data on potential dose metrics are restricted to the serum lipid-adjusted TCDD concentrations associated with mostly uncharacterized exposures (see Sections 3.3.2 and 3.3.3). While these data are useful in estimating half-lives in exposed human individuals, they do not provide estimates of hepatic clearance or reflect target organ exposure. Some autopsy data have been used to infer the partition coefficients; however, these data were collected without quantification of the temporal nature of TCDD uptake (see Section 3.2). Despite the limitations associated with the available human data, there has been some success in using these data to infer the half-lives and elimination rates in humans using pharmacokinetic models (Aylward et al., 2005, 197014; Carrier et al., 1995, 197618; Emond et al., 2006, 197316).

3.3.5.1.2. *Uncertainties associated with model specification.*

Uncertainty associated with model specification should be viewed as a function of the specific application, such as interspecies extrapolation, intraspecies variability, or high dose to low dose extrapolation. Because the use of pharmacokinetic models in this assessment is limited to interspecies extrapolation and high dose to low dose extrapolation, it is essential to evaluate the confidence in predicted dose metrics for these specific purposes. For interspecies extrapolation, the PBPK and CADM models calculate differences in dose metric between an average adult animal and an average adult human. Both models have a biologically and

mechanistically-relevant structure along with a set of parameters with reasonable biological basis, and reproduce a variety of pharmacokinetic data on TCDD in both rodents and humans. These models possess low uncertainty with respect to body burden, blood, and TCDD/serum (lipid) concentration for the purpose of conducting rat to human extrapolation. However, for other dose metrics, such as free, total, or bound hepatic concentrations, the uncertainty is higher in the CADM model compared to the PBPK model due to model specification differences related to the mechanisms of sequestration and induction in the liver (see Section 3.3.3).

For the purpose of high dose to low dose extrapolation in experimental animals, confidence in both models is high with respect to a variety of dose metrics (see previous discussion). The high confidence results from the use of the PBPK models to reproduce a number of data sets covering a wide range of dose levels in rodents (rats, mice) including the dose ranges of most of the key toxicological studies. Given that the TCDD levels during and at the end of exposures were not measured in most of the key studies, use of the PBPK models is preferred because these models account for dose-dependent elimination, induction, and sequestration. Despite the empirical nature of the specification of these key processes in PBPK models, they essentially reproduce the dose-dependent behavior in rodents, supporting their use in deriving dose metrics for dose-response modeling of TCDD. Overall, the confidence in the use of the alternative dose metrics (identified in Table 3-10) is greater than the confidence in the use of administered dose for TCDD, for relating to the concentration within tissues to produce an effect. The administered dose does not take into account interspecies differences in the volume of distribution and clearance or the complex nonlinear processes determining the internal dose.

The PBPK model of Emond et al. (2006, 197316) could benefit from further refinement and validation, including a more explicit consideration of nondose-dependent elimination pathways. As indicated in Section 4, there is some uncertainty associated with the way the elimination of TCDD is described in the existing human PBPK model. The current model essentially treats all TCDD elimination as related to dose dependent metabolism in the liver. In this regard, the classical and more recent PK data on TCDD may be useful in further improving the confidence in their predictions. However, it is likely that there is nondose-dependent elimination of TCDD via feces and, to a lesser extent skin; juxtaposition of available elimination rate data with the PBPK model predictions suggests that the current PBPK model modestly overestimates the dose dependency of overall TCDD elimination. (The central estimate of the

slope of the relationship between the log of the TCDD elimination rate and the log of the TCDD level is only about three−fourths of that expected using the unmodified PBPK model). Emond et al. (2005, 197317) acknowledge that the model did not describe the elimination of TCDD from the blood into the intestines, but it indirectly accounted for this phenomenon with the use of the optimized elimination rate.

3.3.5.1.3. *Impact of human interindividual variability.*

The sources and extent of human variability suggested by the available data are presented in Section 3.3.3, although there is some discussion of the impact of individual differences in body fat content. The CADM model facilitates the simulation of body burden and serum lipid concentrations on the basis of BMI and tissue weights of people, and the PBPK model simulates alternative dose metrics in the fetus and in pregnant animals in addition to adult animals and humans. However, neither of these models has been parameterized for simulation of population kinetics and distribution of TCDD dose metrics. Therefore, at the present time, a quantitative evaluation of the impact of human variability on the dose metrics of TCDD is not feasible, and dose metric-based replacement of the default interindividual factor has not been attempted.

3.3.5.2. *Qualitative Discussion of Uncertainty in Dose Metrics*

The usefulness of the CADM and PBPK models for conducting dose-response modeling (rodent bioassays), interspecies (rodent to human) and intraspecies (high-dose to low–dose) extrapolations is determined by their reliability in predicting the desired dose metrics. The confidence in the model predictions of dose metrics is dictated by the extent to which the model has been verified with empirical data relevant to the dose metric, supplemented by sensitivity and uncertainty analyses. Analysis of sensitivity or uncertainty has not been conducted with the CADM model. For the PBPK model, Emond et al. (2006, 197316) published the initial results from sensitivity analyses of acute exposure modeling (see Section 3.3.3). One of the objectives of a sensitivity analysis that is of highest relevance to this assessment is the identification of the most critical model parameters with respect to the model output (i.e., dose metric).

If the model simulations have only been compared to entities that do not correspond to the moiety representing the dose metric, or if the comparisons have only been done for some but not all relevant dose levels, routes, and species, then the reliability in the predictions of dose

metric can be an issue. The extent to which model results are uncertain will depend largely upon the extent to which the dose metric is measurable (e.g., serum concentrations of TCDD) or inferred (e.g., AhR-bound TCDD concentration).

With respect to TCDD body burden, whole-liver and blood concentration predictions in the rat model, which are well-calibrated with measured data, uncertainty is relatively low. Therefore the need for sensitivity and uncertainty analysis is less critical and confidence in these dose metrics is high. For those dose metrics that are not directly measurable or are less easily verified by available calibration methods, such as free-liver and AhR-bound concentrations, sensitivity and uncertainty analyses are crucial for assessing the reliability of model predictions and confidence is low. For the human model, calibration is largely dependent on blood (LASC) TCDD meaurements, which are much less extensive than for the rat model. Because the blood measurements are reported as LASC, uncertainty and variability in serum:blood and fat:serum ratios also come into play when evaluating the adequacy of the whole-blood TCDD metric. Furthermore, the human data are mostly representative of much higher exposures than the environmental exposures of interest to the EPA. Because of these additional uncertainties only medium confidence can be held in the human model whole-blood TCDD concentration predictions at higher exposures (observed effect range) and low-to-medium confidence at lower exposures (background exposure range).

Sensitivity analysis for the Emond rat PBPK model predictions of liver TCDD concentration indicated that hepatic CYP1A2 concentration is the most sensitive parameter (Emond et al., 2006, 197316). For the Emond human PBPK model, the absorption parameters, basal concentration of CYP1A2, and adipose tissue:blood partition coefficients were identified as highly-sensitive parameters.

Confidence in the Emond rat and human PBPK models at high exposures is medium for the purpose of rat-to-human extrapolation based on blood concentrations, given that the key human model parameters are both sensitive and uncertain; confidence is low for lower exposures. Conversely, confidence in the use of AhR-bound TCDD is low because of the large uncertainty in the fraction of AhR-bound TCDD in the liver.

With regard to the predictability of body burden, the absorption and excretion parameters were among the sensitive parameters in the rat. Several other parameters were also identified as being sensitive in humans. Despite the sensitivity to these parameters and the uncertainty

associated with individual parameter estimates, the overall confidence in the model predictions of body burden appears to be high given the reproducibility of empirical data on tissue burdens and blood concentrations of TCDD in various experiments by both models. Similar conclusions can be drawn for blood concentration of TCDD predicted by the PBPK model, except that the assigned value of blood (serum) lipid content will have additional impact on this dose metric to the extent that the calibration data were in terms of LASC. Variability of total lipid levels and variability of the contribution of phospholipids and neutral lipids to the total lipid pool across species, lifestage and study groups is to be expected (Bernert et al., 2007, 594270; Poulin and Theil, 2001, 594269).

Both conceptual (biological) relevance and prediction uncertainty are important in the choice of dose metric for dose-response modeling and interspecies extrapolation. Conceptual relevance has to do with how "close" the metric is to the observed effect, taking into account both the target tissue and the MOA. In this context, a greater degree of confidence is held for dose metrics that are more proximate to the event (i.e., specific effect). Prediction uncertainty reflects the lack of confidence in the model predictions of dose metrics. Tables 3-13 and 3-14 provide a qualitative ranking of the importance and magnitude of each dose metric with respect to these two sources of uncertainty. Conceptual relevance is low for the use of administered dose in dose-response modeling because known (non-linear) physiological processes are ignored; conversely, conceptual uncertainty is much lower for use of internal dose metrics more proximal to the affected organs.

Table 3-13 presents a cross-walk of relevance, uncertainty and overall confidence associated with the use of various dose metrics for dose-response modeling of TCDD. As shown in Table 3-13, blood/serum levels have the highest overall confidence (medium) followed by body burden (medium to low) for application in dose-response modeling. When using the mouse PBPK model along with the human model (see Table 3-14), the contribution of the prediction uncertainty to the overall uncertainty increases due to the limited comparison of the mouse model simulations with empirical data.

3.3.6. Use of the Emond PBPK Models for Dose Extrapolation from Rodents to Humans

EPA has selected the Emond et al. (2004, 197315; 2005, 197317; 2006, 197316) PBPK models, as modified by EPA for this assessment, for establishing toxicokinetically-equivalent

exposures in rodents and humans.[18] The 2003 Reassessment (U.S. EPA, 2003, 537122) presented a strong argument for using the relevant tissue concentration as the effective dose metric. However, no models exist for estimation of all relevant tissue concentrations. Therefore, EPA has decided to use the concentration of TCDD in blood as a surrogate for tissue concentrations, assuming that tissue concentrations are proportional to blood concentrations. Furthermore, because the RfD and cancer slope factor are necessarily expressed in terms of average daily exposure, the blood concentrations are expressed as averages over the relevant period of exposure for each endpoint. Specifically, blood concentrations in the model simulations are averaged from the administration of the first dose to the administration of the last dose plus one dosing interval (time) unit in order to capture the peaks and valleys for each administered dose. That is, for daily dosing, 24 hours of TCDD elimination following the last dose is included in the average (the modeling time interval is one hour); for a weekly dosing protocol, a full week is included. In addition, because of the accumulation of TCDD in fat and the large differences in elimination kinetics between rodent species and humans, exposure duration plays a much larger role in TK extrapolation across species than for rapidly-eliminated compounds. Because of these factors, EPA is using discrete exposure scenarios that relate human and rodent exposure durations. The use of discrete exposure scenarios was introduced previously in Section 3.4.4.2 describing first-order kinetic modeling and is further described in the following paragraphs. This section concludes with a quantitative evaluation of the impact of exposure duration on the rodent-to-human TK extrapolation from both the human and rodent "ends" of the process.

Figure 3-30 shows the TCDD blood concentration-time profile for continuous exposure at 0.01 ng/kg-day, as predicted by the Emond human PBPK model, and the target TCDD concentrations corresponding to the three discrete exposure scenarios used by EPA in this document. The target concentrations are those that would be identified in the animal bioassay studies that correspond to a particular POD (no-observed-adverse-effect level, lowest-observed-adverse-effect level, or benchmark dose lower confidence bound) established for that bioassay. That is, the target concentrations represent the toxicokinetically-equivalent internal exposure to be translated into an equivalent human intake (or HED).

[18]The models will be referred to hereafter as the "Emond human PBPK model" and the "Emond rodent PBPK model," with variations when referring to individual species or components (e.g., gestational).

For the lifetime exposure scenario, the HED is "matched" to the lifetime average TCDD blood concentration from a lifetime animal bioassay result by determining the continuous daily intake that would result in that average blood concentration for humans over 70 years. A table for converting lifetime-average blood concentrations and other internal dose metrics to human intake is presented in Appendix C.4.

For the gestational exposure scenario, the effective TCDD blood concentration (usually the peak) determined for the particular POD in a particular developmental study is matched to the average TCDD blood concentration over the gestational portion of the human gestational exposure scenario. The HED is determined as the continuous daily intake, starting from birth that would result in that average blood concentration over the 9-month gestational period for a pregnancy beginning at 45 years of age. The choice of 45 years as the beginning age of pregnancy is health protective of the population in that the daily exposure achieving the target blood concentration is smaller than for earlier pregnancies. A table for converting average gestational blood concentrations and other internal dose metrics to human intake for the 45-year-old pregnancy scenario is presented in Appendix C.4. Also, a comparison of the 45-year old pregnancy scenario to one beginning at age 25 is presented in Table 3-15. Using the 25 year-old pregnancy scenario increases the HED by 30 to 60% for typical animal bioassay PODs (3 to 30 ng/kg).

For a less-than-lifetime exposure, the average TCDD blood concentration over the exposure period in the animal bioassay associated with the POD is matched to the average over the 5-year period that includes the peak concentration (58 years for an intake of 0.01 ng/kg-day). The HED is determined as the continuous daily intake that would result in the target concentration over peak 5-year period. The use of the peak is analogous to the approach in the 2003 Reassessment, where the terminal steady-state body burden played the same role. The 5-year average over the peak is taken to smooth out sharp peaks and more closely approximate a plateau. The choice of peak is health protective because humans of any age must be protected for short-term exposures, and the daily intake achieving a given TCDD blood concentration is smallest when matched to the peak exposure as opposed to an average over shorter durations. Thus, target concentrations for any exposure duration of less-than-lifetime must be averaged backwards from the end of the lifetime scenario, rather than from the beginning. The only exception would be if the short-term endpoints evaluated in the animal bioassay were associated

with a specific life stage (such as for the gestational scenario). Note that this scenario lumps all exposures from 1 day to over 1 year in rodents into the same less-than-lifetime category. Conceptually, duration-specific scenarios could be constructed by defining equivalent rodent and human exposure durations. However, for the most part, defining duration equivalents across species is a somewhat arbitrary exercise, not generally based on physiologic or toxicologic processes, but relying primarily on fraction-of-lifetime conversions. EPA defines "lifetime" exposure as 2 years and 70 years for rodents and humans, respectively. So, a half-lifetime equivalence of 1 year in rodents and 35 years in humans is defined easily. Also, considering a subchronic exposure to be 10–15% of lifetime, leads to an equivalence of 90 days in rodents and 7–10 years in humans. However, in the practical sense with respect to the Emond human PBPK model predictions, the difference in the dose-to-target-concentration ratios are not significantly different from the peak 5-year average scenario, differing by less than 5%. A table for converting less-than-lifetime average blood concentrations and other internal dose metrics to human intake is presented in Appendix C.4.

The net effect of using three different scenarios for estimating the HED from rodent exposures is that, for the same target concentration, the ratio of administered dose (to the rodent) to HED will be larger for short-term exposures than for chronic exposures. Figure 3-31 is similar to Figure 3-30, except that it shows the relationship of daily intake to a fixed target TCDD blood concentration level. Figure 3-31 shows that, for human intakes of approximately 0.01 ng/kg-day, the difference in the defined scenarios is 40% or less, with a lifetime-scenario daily intake of 0.014 ng/kg-day required to reach the same target concentration for a shorter-term exposure of 0.01 ng/kg-day. The corresponding daily intake for the gestational scenario is 0.011 ng/kg-day. Because of the nonlinearities in the Emond human PBPK model, the magnitude of the difference between the lifetime and less-than-lifetime exposure scenarios increases at lower intake levels, but not to a substantial degree.

The differential effect of short- and long-term exposures is much more accentuated at the rodent end of the exposure kinetic modeling. Analogous to the processes described in the previous section for first-order body burden (see Section 3.4.2.2), the TCDD blood concentration for single exposures is essentially the immediate absorbed fraction of the administered dose, which will be somewhat lower than the administered dose, while for chronic exposure, the TCDD blood concentration will reflect the long-term accumulation from daily exposure, which

will be very much larger than the administered dose (expressed as a daily intake). Table 3-16 shows the overall impact of TK modeling on the extrapolation of administered dose to HED, comparing the Emond PBPK and first-order body burden models. For comparison purposes, the administered dose is fixed at 1 ng/kg-day for all model runs. Large animal-to-human TK extrapolation factors (TK_{EF}) are evident for short-term mouse studies, decreasing in magnitude with increasing exposure duration. The only exception is the slightly lower extrapolation factor for the mouse 1-day exposure, which is the result of the relatively short TCDD half-life (10 days) in mice and the use of the peak TCDD blood concentration as representative of single exposures, compared to the average TCDD blood concentration over the exposure period used for multiple exposures. The TK_{EF}s are lower for rats because of the slower elimination of TCDD in rats compared to mice. Also, because of the nonlinear kinetics inherent in the Emond PBPK model, the span of the HED (13-fold for mice) across these exposure durations is greater than the span of the lipid-adjusted serum concentration (LASC; 4-fold for mice). Because of the dose-dependence of TCDD elimination in the Emond model, the TK_{EF} becomes smaller with decreasing intake. The result of this nonlinearity is that, although Table 3-16 shows much lower TK_{EF}s for the Emond PBPK model than for the first-order body burden metric, at much lower HED levels the two models give much closer predictions.

Table 3-1. Partition coefficients, tissue volumes, and volume of distribution for TCDD in humans

Tissue	Tissue/blood partition coefficient	Tissue volume (liters, for a 60 kg person)	Effective volume of distribution (Vd—liters of blood equivalent)	Percent total Vd
Blood	1	3	3	0.25
Fat	100	11.4	1.140	94.19
Liver	6	1.56	9	0.77
Rest of the body	1.5	38.64	58	4.79
	Total	**54.6***	**1.210**	**100.00**

*The total tissue volume presented here represents only 91% of body weight because some of the weight and volume of the body is occupied by bone and other structures where TCDD uptake and accumulation do not occur to a significant extent.

Source: Wang et al. (1997, 104657), Emond et al. (2005, 197317; 2006, 197316).

Table 3-2. Blood flows, permeability factors and resulting half lives (t½) for perfusion losses for humans as represented by the TCDD PBPK model of Emond et al. (2005, 197317; 2006, 197316)

Tissue	Permeability (fraction of compartment blood flow)	Rate constant for compartmental elimination ($hour^{-1}$)	$t_{½}$ (hrs)
Fat	0.12	0.0049	143
Liver	0.03	0.77	0.90
Rest of the body	0.35	3.84	0.18

Table 3-3. Toxicokinetic conversion factors for calculating human equivalent doses from rodent bioassays

Half-life (days)[a]	Mouse	Rat (Wistar)	Rat (other)	Guinea pig
	10	20	25	40
Exposure duration (days)	Conversion factor (CF)[b] $BB_A(t_A)$:d_A given in parentheses			
1	3882 (0.77)	3815 (0.79)	3802 (0.79)	3783 (0.79)
7	1107 (2.71)	1020 (2.94)	1004 (2.99)	979 (3.07)
14	681 (4.41)	587 (5.11)	569 (5.27)	543 (5.53)
28	453 (6.62)	350 (8.56)	331 (9.06)	303 (9.90)
90	307 (9.76)	186 (16.1)	163 (18.4)	130 (23.0)
180	282 (10.6)	154 (19.5)	129(23.2)	93 (32.1)
365	270 (11.1)	141 (21.3)	115(26.0)	77 (38.9)
730	226 (11.3)	115 (22.2)	93 (27.4)	60 (42.5)

[a]Half-life for humans = 2,593 days (7.1 years).
[b]$d_H = d_A$/CF; $BB_H(t_H)$:d_H = 2,185 (1−180 days), 2,202 (365 days), 2,555 (730 days).

Table 3-4. Equations used in the concentration and age-dependent model (CADM; Aylward et al., 2005, 197014)[a]

Parameter	Equation
Hepatic Concentration (ng/kg)	$C_{hepatic} = \frac{Q_{body}}{W_l} * (f_{\min} + \frac{(f_{\max} - f_{\min}) * C_{body}}{K + C_{body}}$
Fat Concentration (ng/kg)	$C_{adipose} = \frac{Q_{body}}{Wa} * (1 - (f_{\min} + \frac{(f_{\max} - f_{\min}) * C_{body}}{K + C_{body}}))$
Hepatic Elimination	$Exr\_hepatic = k_e * Q_{body} * (1 - (f_{\min} + \frac{(f_{\max} - f_{\min}) * C_{body}}{K + C_{body}}))$
Excretion via gut of Unchanged TCDD (Exsorption)	$Exr\_gut = k_a * Q_a$
Change of TCDD due to bodyweight change	$ChangeTCDD\_BW = Q_{body} * \frac{(BW(t+dt) - BW(t))}{BW(t)}$
Amount in body as a function of time	$Q_{body}(t+dt) - Q_{body}(t) = Exr\_hepatic + Exr\_gut + ChangeTCDD\_BW$
Adipose tissue growth	$W_a = \frac{1.2 * BMI + (0.23 * Age) - 10.8 * sex}{100}$
Change of hepatic elimination constant with age	$k_e = k_{e0} - k_{eslope} * Age$

[a]For abbreviations and parameter descriptions, see Table 3-5.

Table 3-5. Parameters of the Concentration and Age-Dependent Model (CADM; Aylward et al., 2005, 197014)

Parameter	Value	Units	Comments/sources
f_{hmin} [a]	0.01	unitless	Minimum body burden fraction in liver
f_{hmax} [a]	0.7	unitless	Maximum body burden fraction in liver
K [a]	100	ng/kg	Body burden at half-maximum of fraction liver
k_e	Calculated	per year	$k_e = k_{e0} - k_{e\_slope}$*(age) with enforced minimum of k_{e_min}
k_{e0}	0.85	per year	CADM-mean hepatic elimination base rate at age 0
k_{e_slope}	0.011	per year	Change in k_e per year of age
k_{e_min}	0.2	per year	Minimum hepatic elimination rate
w_a (adipose weight fraction)	Calculated	unitless	w_a = [(1.2*BMI)+0.23*Age-10.8*sex]/100
w_h (liver body weight fraction)	0.03	unitless	Assumed constant
k_a (adipose clearance factor)	0.0025	per month	Passive elimination rate from intestinal tract
Monthly dose	0.15507069	ng	per month
Estimated absorption fraction	0.97	unitless	From Moser and McLaghlan (2001, 198045)
Body weight	70	kg	Standard male weight
Sex	1	unitless	1 = male; 0 = female
Time of administration	840	months	
Initial Cbody	0.2	ng/kg	Estimated background young adults UMDES sampling
Absorbed monthly dose 1	0.150418569	ng	per month

[a]The values of fhmin, fhmax, and K were obtained by best fit of the model simulations to the experimental data with the method of least squares (Aylward et al., 2005, 197114; Carrier et al., 1995, 197618).

Table 3-6. Confidence in the CADM[a] model simulations of TCDD dose metrics

Dose metric	Level of confidence
Administered dose	N/A
Absorbed dose	H
Body burden	H
Serum lipid concentration	M
Total tissue (liver) concentration	L
Receptor occupancy (bound concentration)	N/A

[a]Concentration and age-dependent model (Aylward et al., 2005, 197014).
H = high, M = medium, L = low, NA = not applicable.

Table 3-7. Equations used in the TCDD PBPK model of Emond et al. (2006, 197316)

Aspect	Equation
Body weight growth with age	$BW_{time}(g) = BW\_T0 \times \left(\frac{0.41 \times time}{1402.5 + time}\right)$
Cardiac output	$Qc(mL/hr) = QCCAR \times 60\left(\frac{BW}{1000}\right)^{0.75}$ A factor of 60 corresponds to the conversion of minutes to hours, and 1,000 is conversion of BW from grams to kilograms.
Blood compartment	$Cb(nmol/mL) = \frac{\left[(Qf \times Cfb) + (Qre \times Creb) + (Qli \times Clib) + lymph\right]}{Qc} - \frac{(Cb \times CLURI)}{Qc}$
Tissue compartment (fat, rest of the body)	
Tissue blood subcompartment	$\frac{dAtb}{dt}(nmol/mL) = Qt(Ca - Ctb) - PAt\left(Ctb - \frac{Ct}{Pt}\right)$ $Ctb(nmol/mL) = \frac{Atb}{Wtb}$
Tissue cellular matrices	$\frac{dAt}{dt}(nmol/mL) = PAt\left(Ctb - \frac{Ct}{Pt}\right)$ $Ct(nmol/mL) = \frac{At}{Wt}$
Liver tissue compartment	
Tissue blood subcompartment	$\frac{dAlib}{dt}(nmol/mL) = Qli(Ca - Clib) - PALI(Clib - Clifree) + input_{oral}$ $Clib(nmol/mL) = \frac{Alib}{WLIB}$
Tissue cellular matrices	$\frac{dAli}{dt}(nmol/mL) = PALI(Clib - Clifree) - (KBILE\_LI \times Clifree \times WLI)$ $Cli(nmol/mL) = \frac{Ali}{Wli}$
Free TCDD concentration in liver	$Clifree(nmol/mL) = Cli - \left[Clifree \times PLI + \left(\frac{LIBMAX \times Clifree}{KDLI + Clifree}\right) + \left(\frac{CYP1A2 \times Clifree}{KDLI1A2 + Clifree}\right)\right]$
Concentration bound to AhR in hepatic tissue	$Ct_{AhRbound}(nmol/mL) = \frac{LIBMAX \times Clifree}{KDLI + Clifree}$ All other induction processes and equations have been described and presented by Wang et al. (1997, 104657).

Table 3-7. Equations used in the TCDD PBPK model of Emond et al. (2006, 197316) (continued)

Aspect	Equation
Gastrointestinal absorption and distribution of TCDD to the portal lymphatic circulation	
Amount of TCDD remaining in lumen cavity	$\frac{dLumen}{dt}(nmol/hr) = \left[\left(KST + KABS\right) \times lumen\right] + intake$ Lumen in the amount of TCDD remaining in the GI tract (nmol); intake is the rate of intake of TCDD during a subchronic exposure (nmol/hr).
Amount of TCDD eliminated in the feces	$\frac{dFeces}{dt}(nmol/hr) = KST \times lumen$
Absorption rate of TCDD to the blood via the lymphatic circulation	$\frac{dLymph}{dt}(nmol/hr) = KABS \times lumen \times 0.7$
Absorption rate of TCDD by the liver via portal circulation	$\frac{dPortal}{dt}(nmol/hr) = KABS \times lumen \times 0.3$

Note: Key parameters and abbreviations are defined in Table 3-10.

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Table 3-8. Parameters of the PBPK model for TCDD

Parameter Description	Symbol	Parameter values					
		Human nongestational[a]	Human gestational[a]	Mouse nongestational	Mouse gestational	Rat nongestational	Rat gestational
Body weight (g)	BW	Calculated	Calculated	23-28[b]	23-28	125-250[b]	85-190[b]
Cardiac output (mL/hour/kg)	QCCAR	15.36[c,d]	Calculated	275[c]	275[c]	311.4[e]	311.4[e]
Tissue (intracellular) volumes (fraction of BW)							
Liver	WLI0	Calculated	Calculated	0.0549[f]	0.0549[f]	0.036[e]	0.036[e]
Fat	WF0	Calculated	Calculated	0.069[e]	Calculated	0.069[e]	Calculated
Tissue blood volumes							
Liver (fraction of WLI0)	WLIB0	0.266[e]	0.266[e]	0.266[e]	0.266[e]	0.266[e]	0.266[e]
Fat (fraction of WF0)	WFB0	0.05[e]	0.05[e]	0.05[e]	0.05 [e]	0.05[e]	0.05[e]
Rest of body (fraction of WRE0)	WREB0	0.03[e]	0.03[e]	0.03[e]	0.03 [e]	0.03[e]	0.03[e]
Placenta tissue fraction of tissue blood weight (unitless)	WPLAB0	N/A	0.5[g]	N/A	0.5[e]	N/A	0.5[e]
Tissue blood flow (fraction of cardiac output)							
Liver	QLIF	0.26[c]	0.26[c]	0.161[f]	0.161[f]	0.183[e]	0.183[e]
Fat	QFF	0.05[c]	0.05[c]	0.07[h]	0.07[h]	0.069[e]	0.069[e]
Placenta	QPLAF	N/A	Calculated	N/A	Calculated	N/A	Calculated
Tissue permeability (fraction of tissue blood flow)							
Liver	PALIF	0.35[e]	0.35[e]	0.35[e]	0.35[e]	0.35[e]	0.35[e]
Fat	PAFF	0.12[i]	0.12[i]	0.12[i]	0.12[i]	0.091[e]	0.091[e]
Placenta diffusional permeability fraction (unitless)	PAPLAF	N/A	0.3[g]	N/A	0.03[g]	N/A	0.3[g]
Rest of body	PAREF	0.03[e]	0.03[e]	0.03[e]	0.03[e]	0.0298[e]	0.0298[e]

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Table 3-8. Parameters of the PBPK model for TCDD (continued)

Parameter Description	Symbol	Parameter values					
		Human nongestational[a]	Human gestational[a]	Mouse nongestational	Mouse gestational	Rat nongestational	Rat gestational
Partition coefficient							
Liver	PLI	6[e]	6[e]	6[e]	6[e]	6[e]	6[e]
Fetus/blood partition coefficient (unitless)	PFETUS	N/A	4[j]	N/A	4[j]	N/A	4[j]
Placenta/blood partition coefficient (unitless)	PPLA	N/A	1.5[j]	N/A	3[g]	N/A	1.5[j]
Fat	PF	100[e]	100[e]	400[i]	400[i]	100[e]	100[e]
Rest of body	PRE	1.5[e]	1.5[e]	3[k]	3[k]	1.5[e]	1.5[e]
Metabolism constants							
Urinary clearance elimination (mL/hour)	CLURI	4.17E-08[l]	4.17E-08[l]	0.09[i]	0.09[i]	0.01[j]	0.01[j]
Clearance - transfer from mother to fetus (mL/hour)	CLPLA_FET	N/A	16[e]	N/A	0.17[i]	N/A	0.17[i]
Liver (biliary elimination and metabolism; $hour^{-1}$)	KBILE_LI	Inducible	Inducible	Inducible	Inducible	Inducible	Inducible
Interspecies constant ($hour^{-1}$)	Kelv	0.0011[i]	0.0011[i]	0.4[i]	0.4[i]	0.15[e]	0.15[e]
AhR							
Affinity constant in liver (nmol/mL)	KDLI	0.1[e]	0.1[e]	0.0001[e]	0.0001[e]	0.0001[e]	0.0001[e]
Binding capacity in liver (nmol/mL)	LIBMAX	0.35[e]	0.35[e]	0.00035[e]	0.00035[e]	0.00035[e]	0.00035[e]
Placenta binding capacity (nmol/mL)	PLABMAX	N/A	0.2[j]	N/A	0.0002[j]	N/A	0.0002[j]
Affinity constant protein (AhR) in placenta (nmol/mL)	KDPLA	N/A	0.1[j]	N/A	0.0001[j]	N/A	0.0001[j]

Table 3-8. Parameters of the PBPK model for TCDD (continued)

Parameter Description	Symbol	Parameter values					
		Human nongestational[a]	Human gestational[a]	Mouse nongestational	Mouse gestational	Rat nongestational	Rat gestational
CYP1A2 induction parameters							
Dissociation constant CYP1A2 (nmol/mL)	KDLI2	40[j]	40[j]	0.02[i]	0.02[i]	0.04[j]	0.04[j]
Degradation process CYP1A2 (nmol/mL)	CYP1A2_1OUTZ	1,600[e]	1,600[e]	1.6[e]	1.6[e]	1.6[e]	1.6[e]
Dissociation constant during induction (nmol/mL)	CYP1A2_1EC50	130[e]	130[e]	0.13[e]	0.13[e]	0.13[e]	0.13[e]
Basal concentration of CYP1A2 (nmol/mL)	CYP1A2_1A2	1,600[e]	1,600[e]	1.5[k]	1.5[k]	1.6[e]	1.6[e]
First-order rate of degradation ($hour^{-1}$)	CYP1A2_1KOUT	0.1[e]	0.1[e]	0.1[e]	0.1[e]	0.1[e]	0.1[e]
Time delay before induction process (hour)	CYP1A2_1TAU	0.25[e]	0.25[e]	1.5[k]	1.5[k]	0.25[e]	0.25[e]
Maximal induction of CYP1A2 (unitless)	CYP1A2_1EMAX	9,300[i]	9,300[i]	600[e]	600[e]	600[e]	600[e]
Other constants							
Oral absorption constant ($hour^{-1}$)	KABS	0.06[i]	0.06[i]	0.48[i]	0.48[i]	0.48[e]	0.48[e]
Gastric nonabsorption constant ($hour^{-1}$)	KST	0.01[m]	0.01[m]	0.30[i]	0.30[i]	0.36[e]	0.36[e]

[a]Units for human nongestational parameters are L rather than mL and kg rather than g where applicable.
[b]Body weight varies by study (Emond et al., 2004, 197315).
[c]Krishnan and Andersen (2007).
[d]Units are L/kg/hr.
[e]Wang et al. (1997, 104657).
[f]ILSI (1994, 046436).
[g]Fixed.
[h]Leung et al. (1990, 192833).
[i]Optimized.
[j]Emond et al. (2004, 197315).
[k]Wang et al. (2000, 198738).
[l]Lawrence and Gobas (1997, 199072).
[m]Calculated to estimate 87% bioavailability of TCDD in humans (Poiger and Schlatter, 1986, 197336).

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Table 3-9. Regression analysis results for the relationship between log_{10} serum TCDD at the midpoint of observations and the log_{10} of the rate constant for decline of TCDD levels using Ranch Hand data

Item	Aspect	Value
Summary of fit	RSquare	0.894
	RsquareAdj	0.871
	Root mean square error	0.044
	Mean responses	0.130
	Observations (or sum weights)	11
Parameter estimates	Intercept	
	Estimate	−0.054
	Standard deviation	0.026
	t ratio	−2.07
	Prob>|t|	0.0679
	Log (TCDDpg/g)	
	Estimate	0.092
	Standard error	0.011
	t ratio	8.28
	Prob>|t|	<0.0001

Table 3-10. Confidence in the PBPK model simulations of TCDD dose metrics

Dose metric	Human model	Rat model	Mouse model
Administered dose	N/A	N/A	N/A
Absorbed dose	H	H	M
Body burden	H	H	M
Serum (blood)concentration	H	H	M
Total liver concentration	M/L	H	M
Receptor occupancy (bound concentration)	L	L	L

H = high, M = medium, L = low.

Table 3-11. Overall confidence associated with alternative dose metrics for cancer and noncancer dose-response modeling for TCDD using rat PBPK model

End point	Body burden	Blood or serum concentration	Liver concentration	Bound concentration in liver
Liver effects	M		H	M/L
Nonhepatic effects	M	H		M/L

H = high, M = medium, L = low.

Table 3-12. Overall confidence associated with alternative dose metrics for cancer and noncancer dose-response modeling for TCDD using mouse PBPK model

End point	Body burden	Blood or serum concentration	Liver concentration	Bound concentration in liver
Liver effects	M		M	L
Nonhepatic effects	M	M		L

H = high, M = medium, L = low.

Table 3-13. Contributors to the overall confidence in the selection and use of dose metrics in the dose-response modeling of TCDD based on rat and human PBPK models

Dose metric	Conceptual Relevance	Prediction uncertainty	Overall Confidence
Administered dose	L	NA	L
Body burden	M	M	M-L
Blood concentration	M	L	M
Liver concentration	L	M	L
Receptor (AhR) occupancy	H	H	L

H = high, M = medium, L = low, NA = not applicable, ? = if relevant to MOA of response.

Table 3-14. Contributors to the overall uncertainty in the selection and use of dose metrics in the dose-response modeling of TCDD based on mouse and human PBPK models

Dose metric	Conceptual uncertainty	Prediction uncertainty
Administered dose	H	NA
Absorbed dose	H	L
Body burden	M	M
Blood or serum concentration	M	M
Tissue concentration	L	M/H
Receptor occupancy	L(?)	H

H = high, M = medium, L = low, NA = not applicable, ? = if relevant to MOA of response.

Table 3-15. Comparison of human equivalent doses from the Emond human PBPK model for the 45-year-old and 25-year-old gestational exposure scenarios

Animal bioassay POD (ng/kg-day)	Species	TCDD blood concentration[a]	HED 45 year-old	HED 25 year-old	25-yr:45-yr ratio
3	Mouse	8.800E-02	6.79E-04	1.03E-03	1.5
	Rat	1.815E-01	1.87E-03	2.98E-03	1.6
30	Mouse	7.115E-01	1.51E-02	2.07E-02	1.4
	Rat	1.367E+00	4.22E-02	5.41E-02	1.3

[a]Determined from the Emond rodent PBPK models assuming a single exposure on GD13.

Table 3-16. Impact of toxicokinetic modeling on the extrapolation of administered dose to HED, comparing the Emond PBPK and first-order body burden models

Exposure duration (days)	1st-order BB		Emond PBPK		
	HED (ng/kg−day)	TK_{EF}	LASC (ng/kg)	HED (ng/kg−day)	TK_{EF}
Mouse					
1	2.57E-4	3,882	75.5	9.49E-4	1,054
14	1.47E-3	681	64.4	8.17E-4	1,224
90	3.25E-3	307	173	3.83E-3	261
365	3.70E-3	270	248	6.66E-3	150
730	4.43E-3	226	263	1.08E-2	93
Rat					
1	2.63E-4	3,802	110	1.87E-3	535
14	1.76E-3	569	208	5.22E-3	192
90	6.13E-3	163	599	2.81E-2	36
365	8.68E-3	115	811	4.52E-2	22
730	1.07E-2	93	853	6.47E-2	15

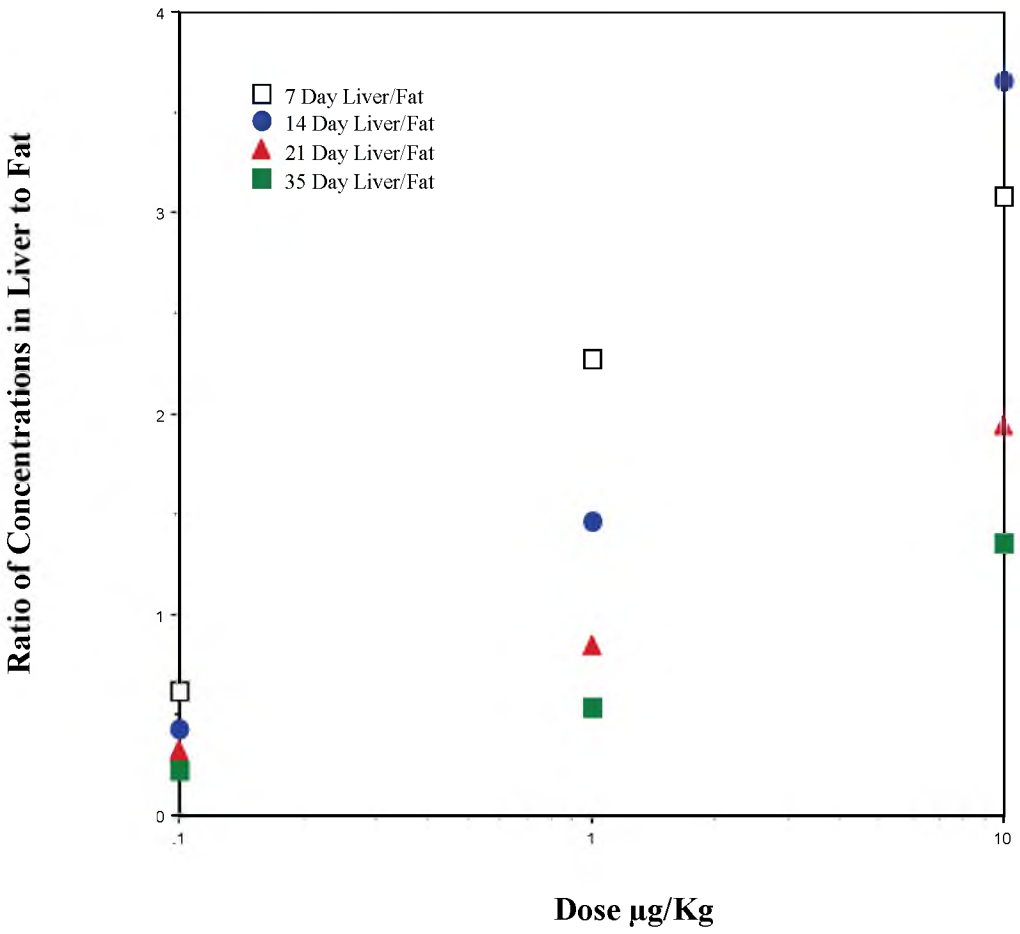


Figure 3-1. Liver/fat concentration ratios in relation to TCDD dose at various times after oral administration of TCDD to mice.

Source: Dilberto et al. (1995, 197309).

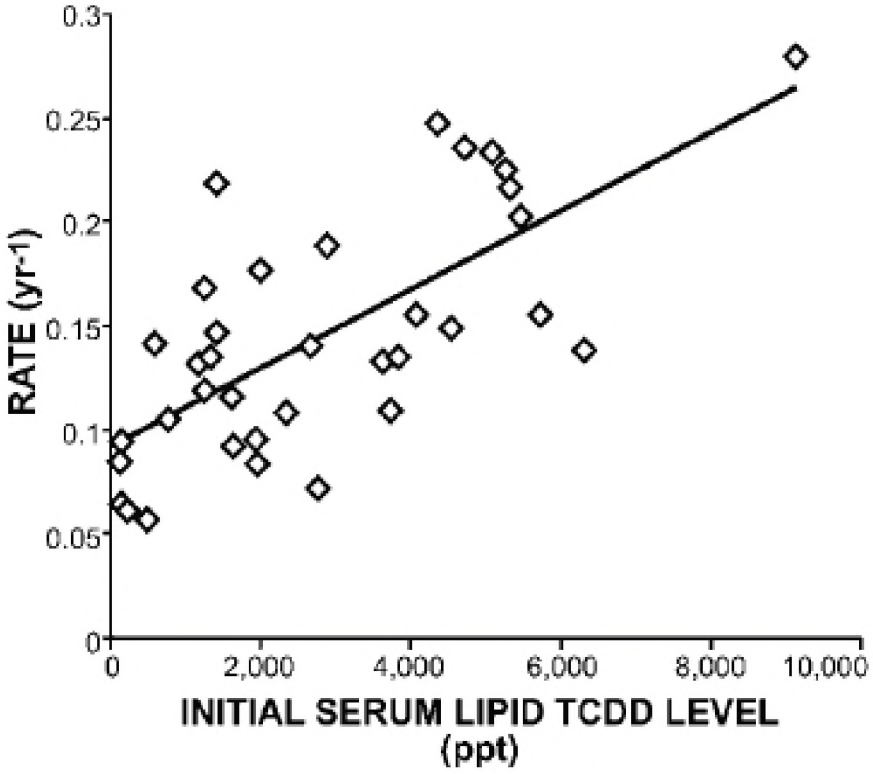


Figure 3-2. First-order elimination rate fits to 36 sets of serial TCDD sampling data from Seveso patients as function of initial serum lipid TCDD.

Source: Aylward et al. (2005, 197014).

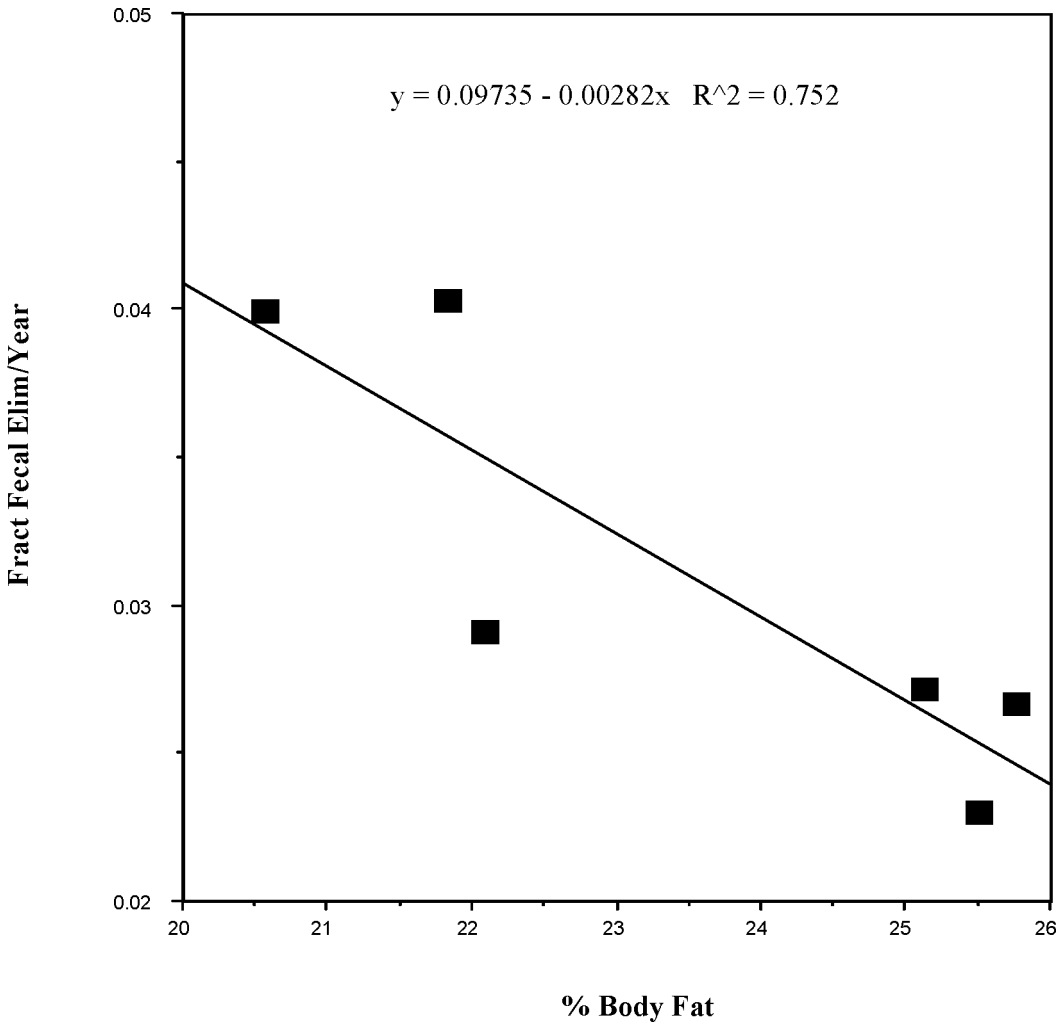


Figure 3-3. Observed relationship of fecal 2,3,7,8-TCDD clearance and estimated percent body fat.

Source: Rohde et al. (1999, 548764).

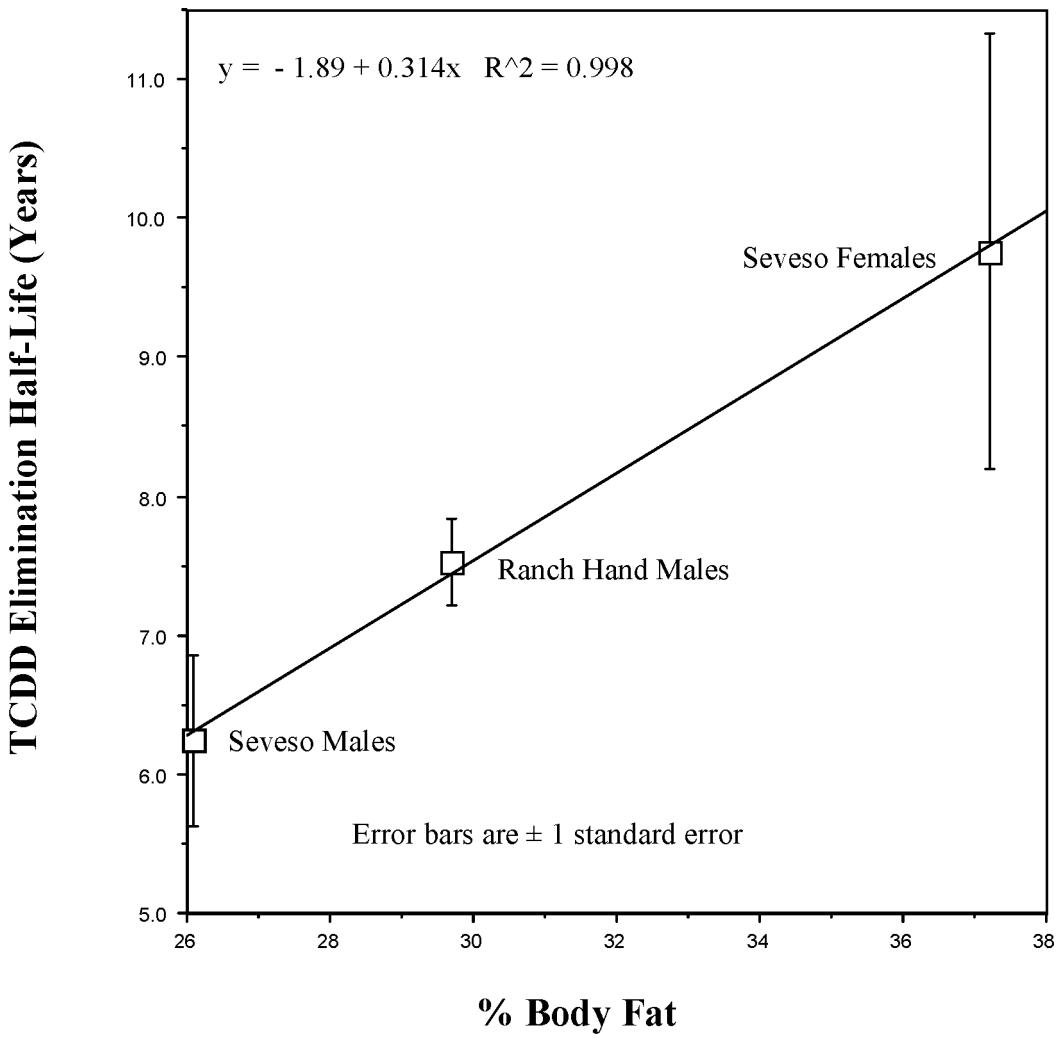


Figure 3-4. Unweighted empirical relationship between percent body fat estimated from body mass index and TCDD elimination half-life—combined Ranch Hand and Seveso observation.

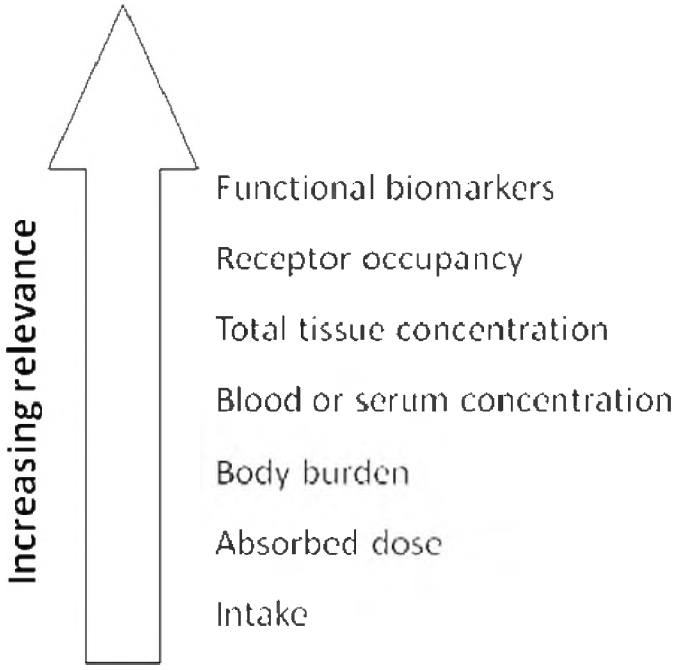


Figure 3-5. Relevance of candidate dose metrics for dose-response modeling, based on mode of action and target organ toxicity of TCDD.

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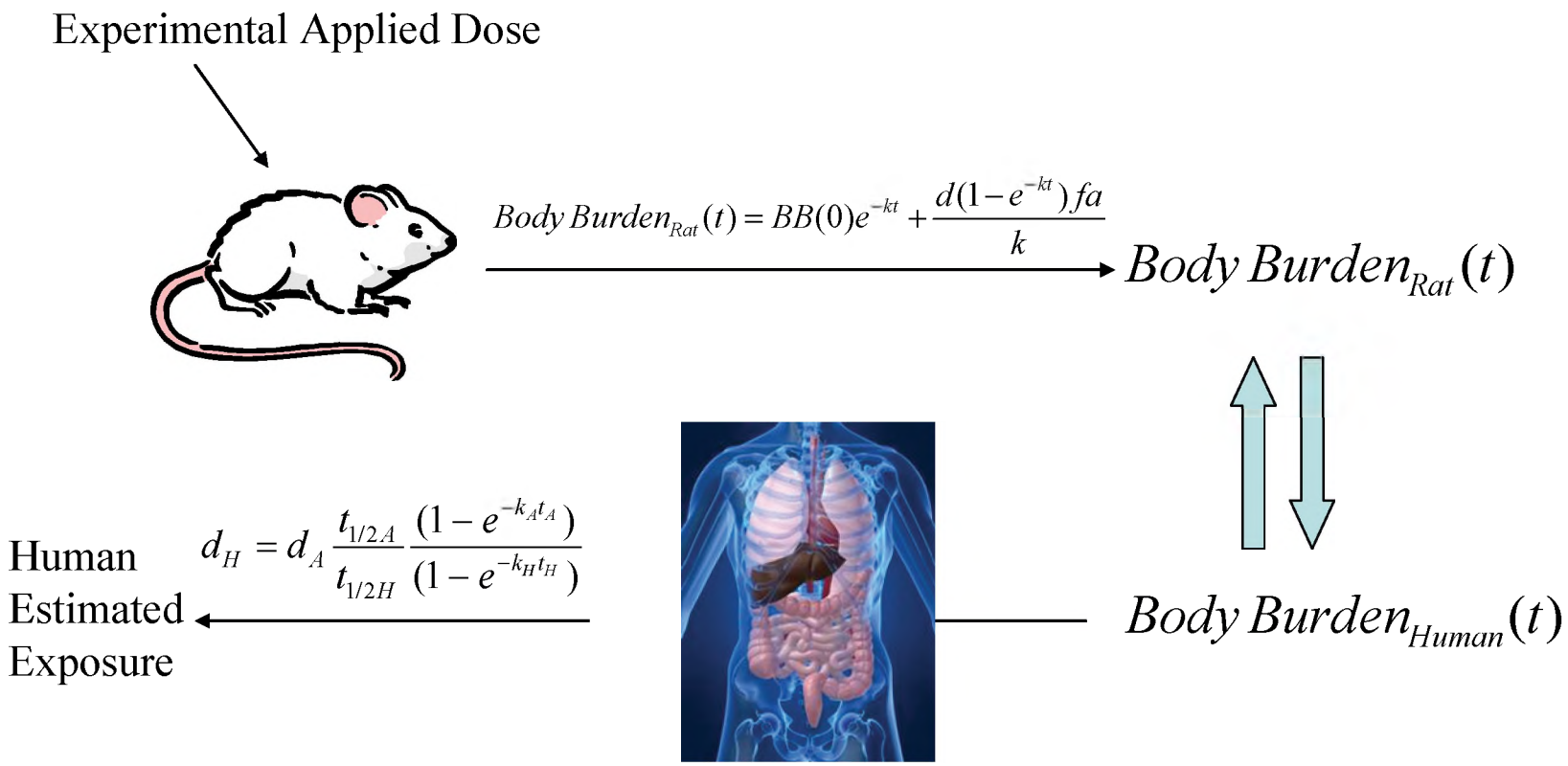


Figure 3-6. Process of estimating a human-equivalent TCDD lifetime average daily oral exposure (d_H) from an experimental animal average daily oral exposure (d_A) based on the body-burden dose metric. The arrows represent mathematical conversions based on toxicokinetic modeling. BB_A (TWA animal body burden) and BB_H (TWA human body burden) are assumed to be toxicokinetically equivalent. See text for further explanation.

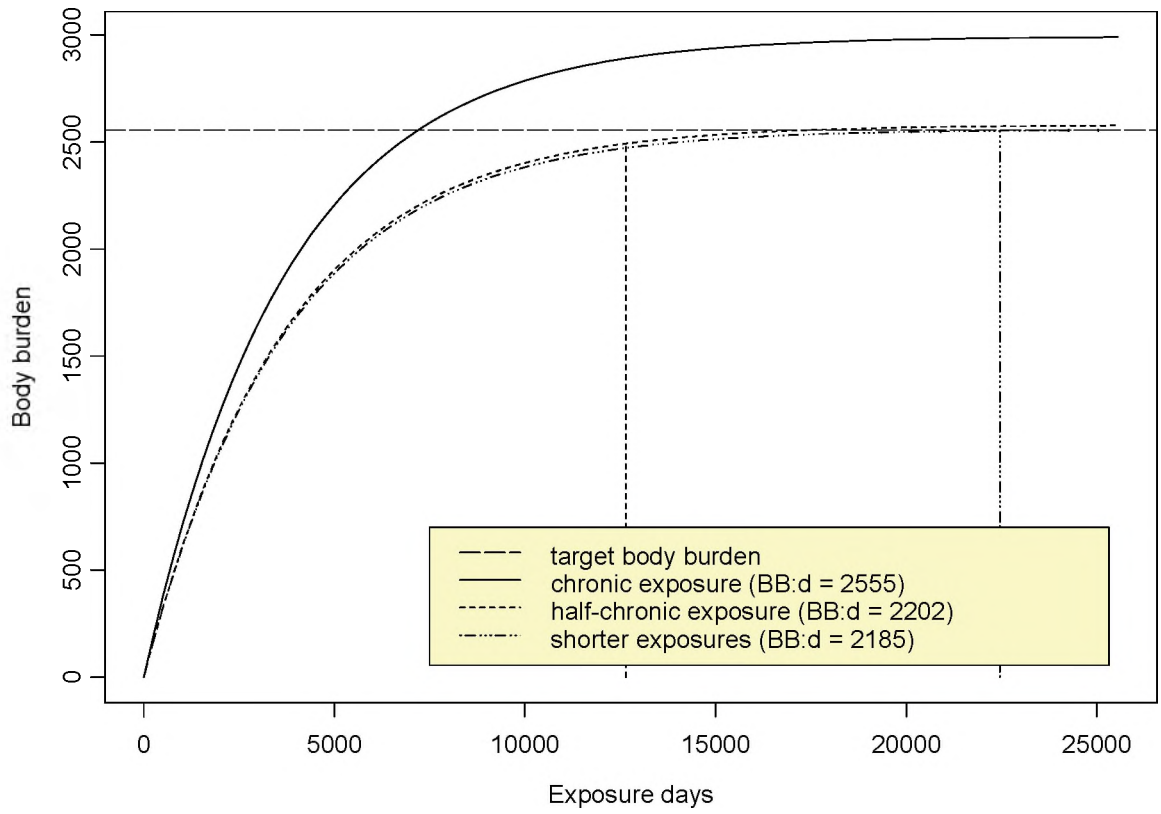


Figure 3-7. Human body burden time profiles for achieving a target body burden for different exposure duration scenarios. BB:d is $BB_H(t_H)$:d_H in Figure 3-6. The curve depicted using the solid line illustrates the increase in the human body burden over time for a hypothetical human administered a daily TCDD dose where the time-weighted average human body burden estimate over the lifetime is equal to the target body burden attained in a rodent bioassay. When compared to shorter durations (dashed lines), a higher average daily TCDD dose is required to yield a time-weighted average human body burden over a lifetime that is equal to the target body burden attained in a rodent bioassay. The half-chronic exposure scenario (depicted using a dashed line) is equivalent to a 1-year exposure in rodents. When compared to a chronic BB_H, a lower value of d_H is needed to attain the target body burden in a rodent bioassay when the time-weighted average is over the last 35 years of life; the dose to plateau ratio is also smaller (i.e., $d_{H,C} < d_{H,SC}$ to attain the target body burden in a rodent bioassay). The shorter exposure scenario is equivalent to most other shorter rodent exposure durations, from 1 day to subchronic, which are indistinguishable with respect to the BB:d ratio (subchronic shown).

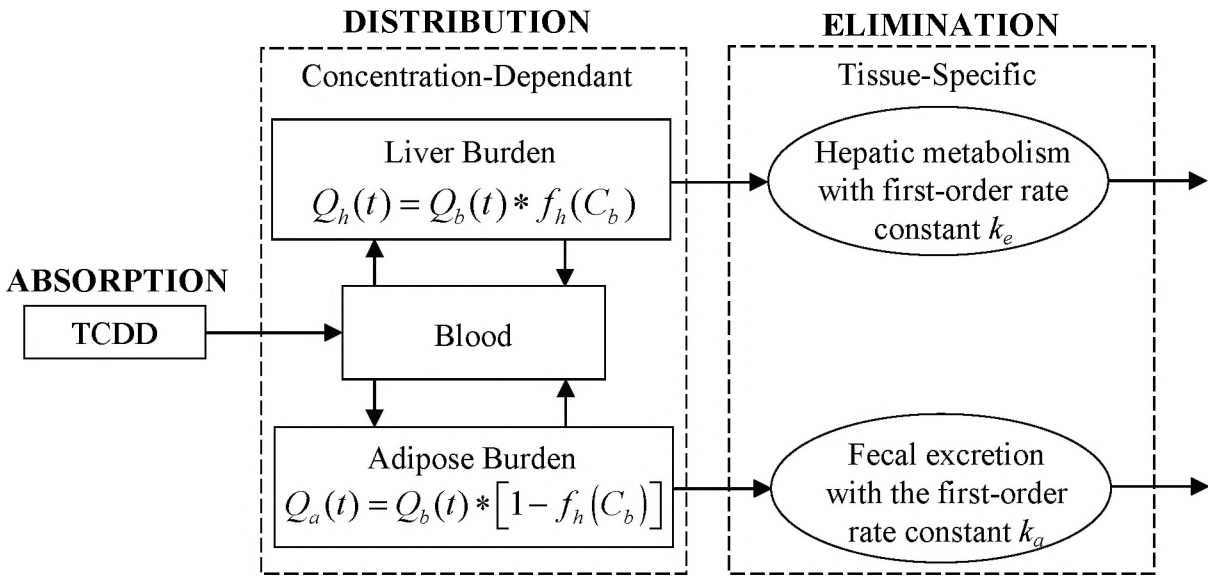


Figure 3-8. Schematic of the CADM structure.

Source: Aylward et al. (2005, 197014).

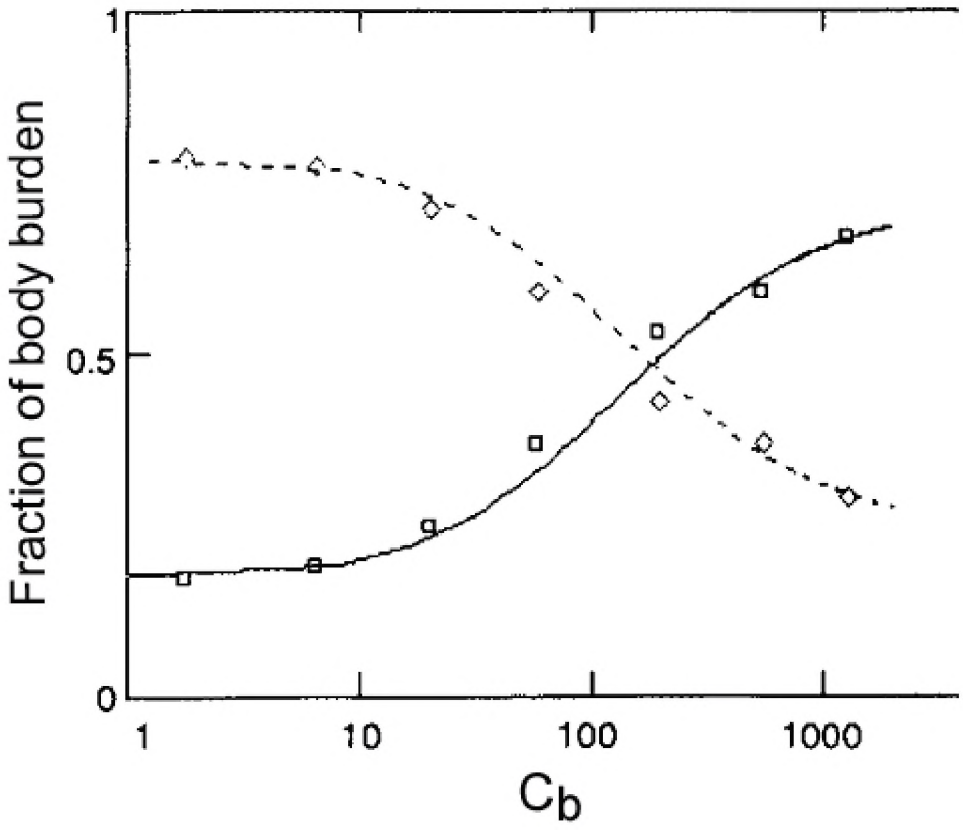


Figure 3-9. Comparison of observed and simulated fractions of the body burden contained in the liver and adipose tissues in rats. f_h, fraction contained in liver (observation) (□); $f_{h\text{-}sim}$, fraction contained in liver (simulation) (—); f_{at}, fraction contained in the adipose tissue (observation) (◊); $f_{at\text{-}sim}$, fraction contained in the adipose tissue (simulation) (---); and C_b, body concentration in ng TCDD/kg body wt.

Source: Carrier et al. (1995, 197618); data from Abraham et al. (1988, 199510) measured 7 days after dosing.

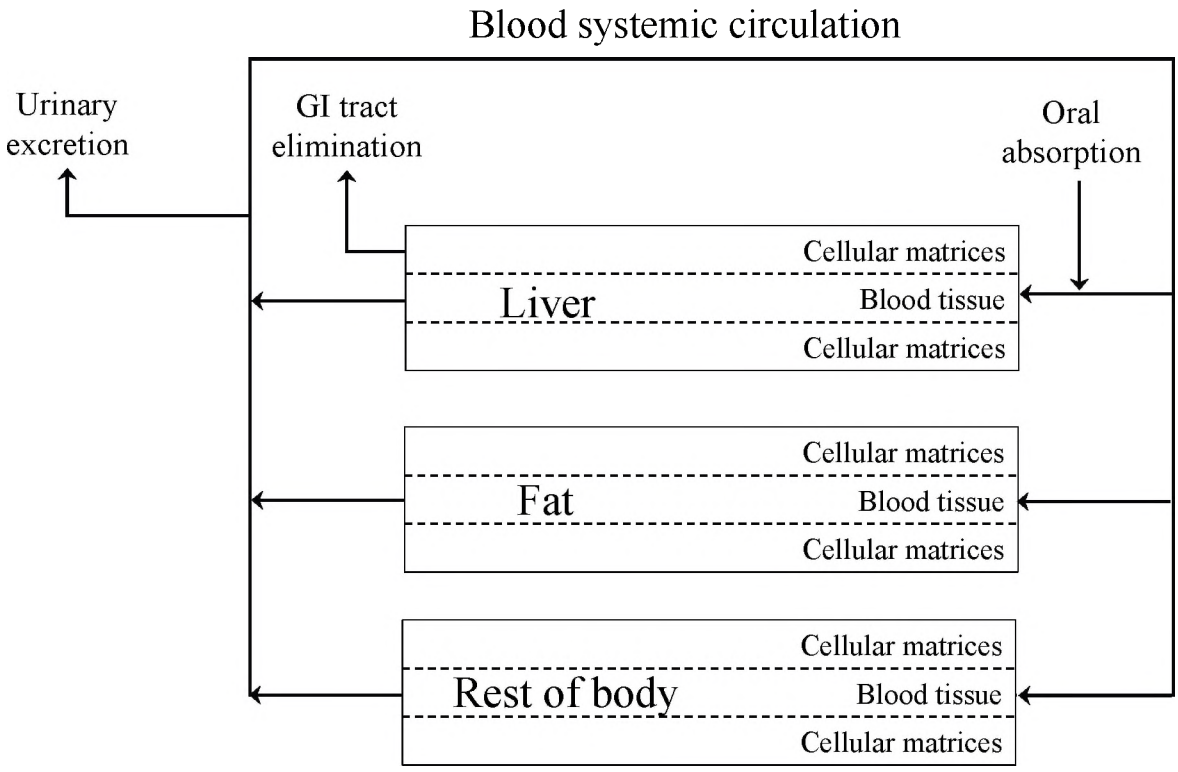


Figure 3-10. Conceptual representation of PBPK model for rat exposed to TCDD.

Source: Emond et al. (2006, 197316).

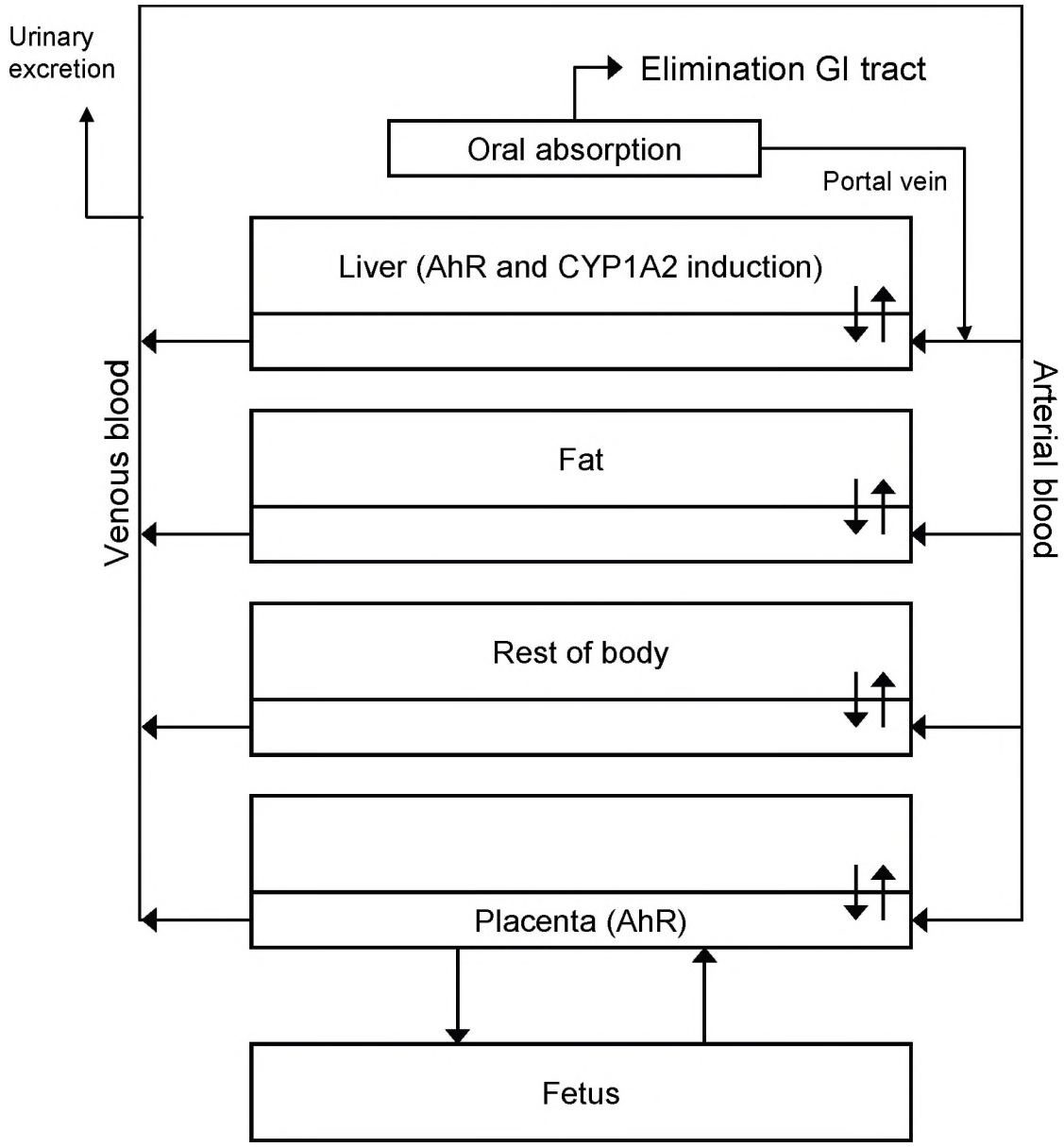


Figure 3-11. Conceptual representation of PBPK model for rat developmental exposure to TCDD.

Source: Emond et al. (2004, 197315).

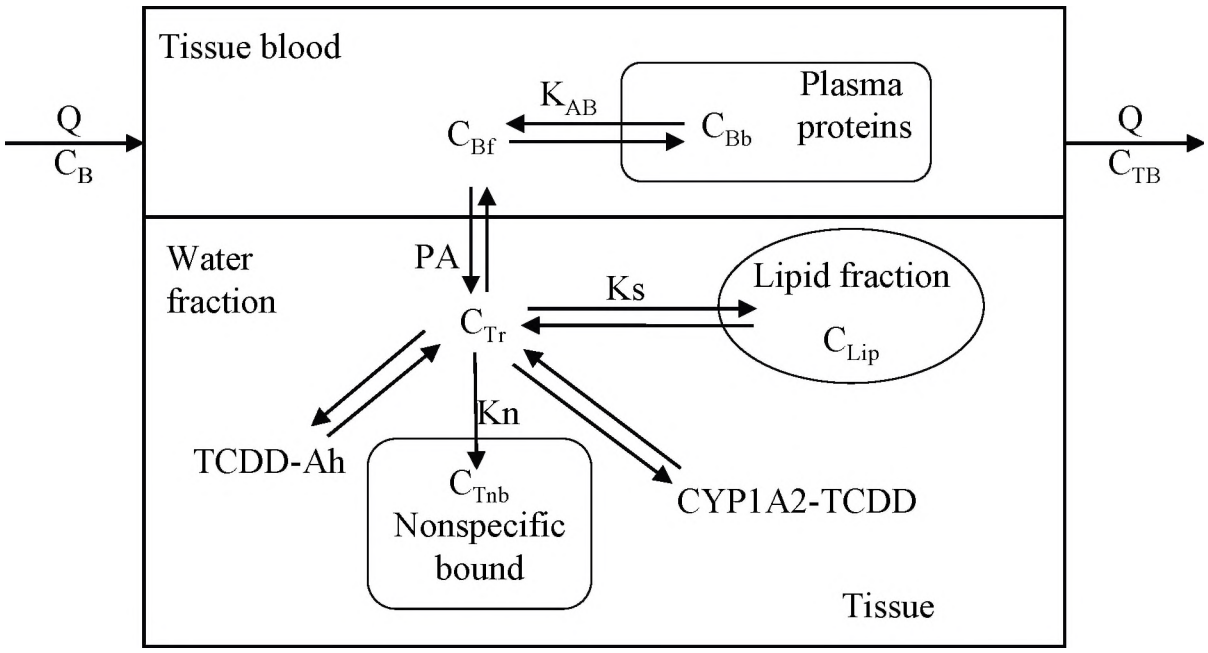


Figure 3-12. TCDD distribution in the liver tissue.

Source: Wang et al. (1997, 104657).

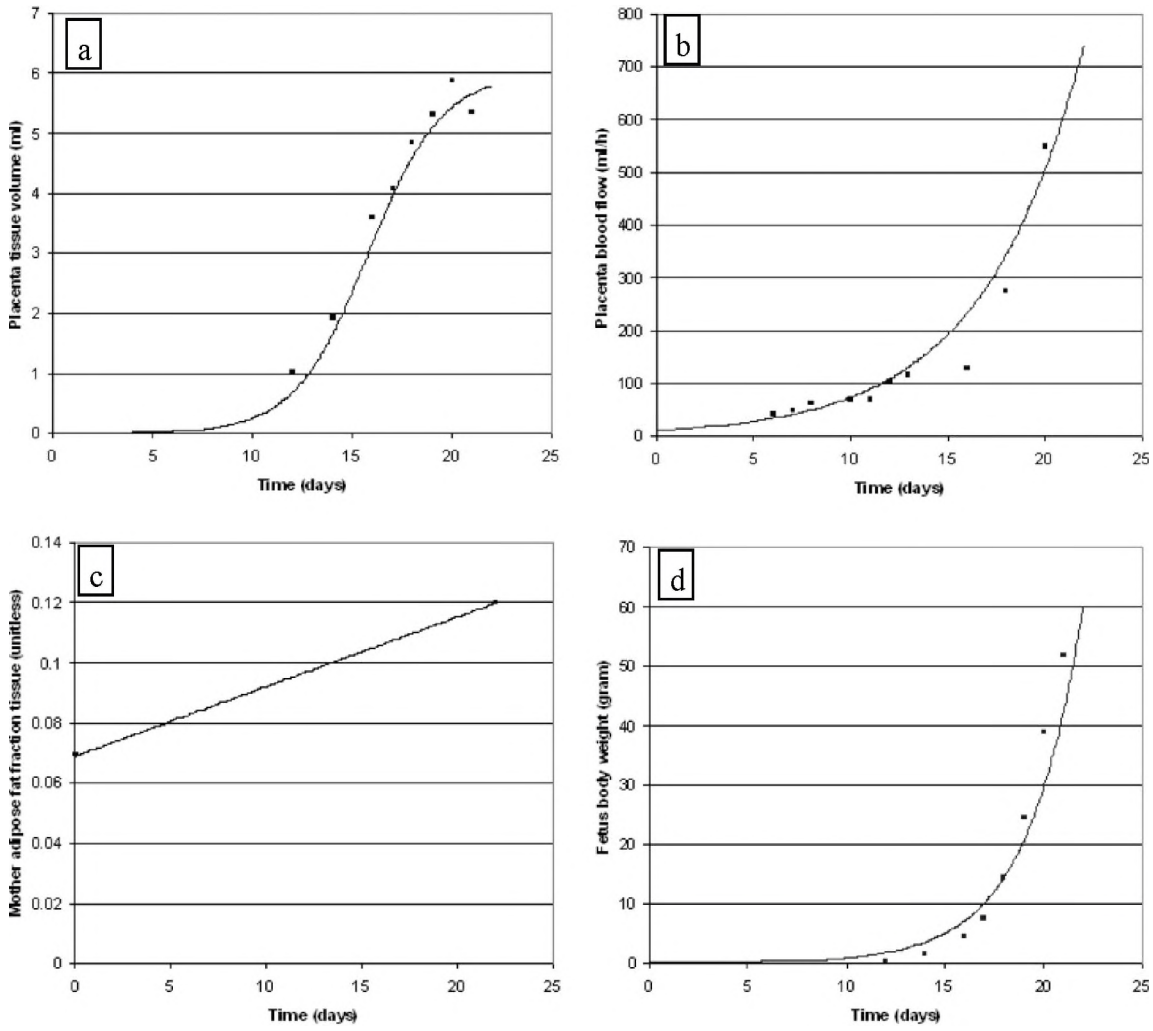


Figure 3-13. Growth rates for physiological changes occurring during gestation. (a) Placental growth during gestation (calculated for $n = 10$ placenta). Experimental data from Sikov (1970, 594274). (b) Blood flow rate in Placental compartment during gestation. Experimental data from Buelke-Sam et al. (1982, 020478; 1982, 020477). (c) Fat fraction of body weight during gestation. Experimental data came from Fisher et al. (1989, 065288), and (d) Fetal growth during gestation. Experimental data obtained from Sikov (1970, 594274).

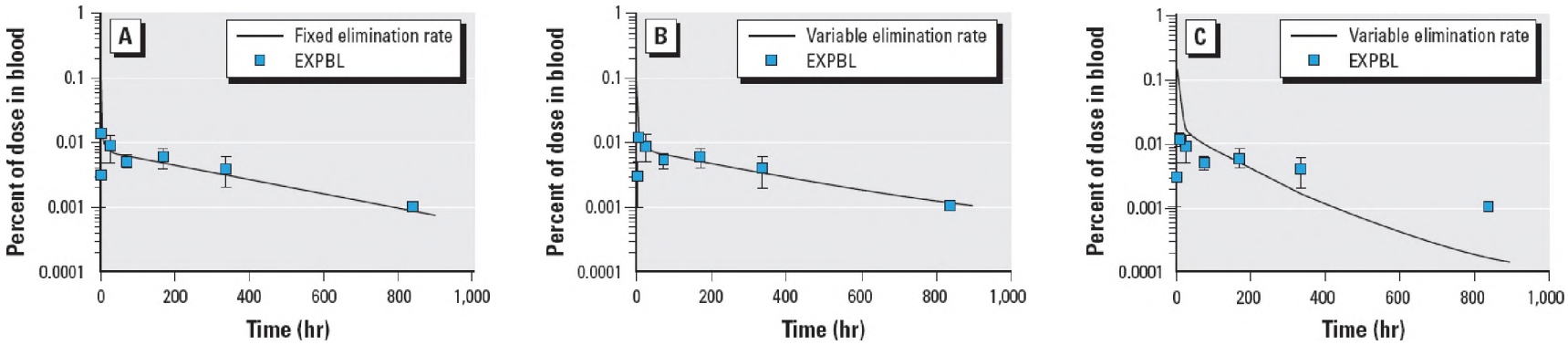


Figure 3-14. Comparisons of model predictions to experimental data using a fixed elimination rate model with hepatic sequestration (A) and an inducible elimination rate model with (B) and without (C) hepatic sequestration. EXBL, experimental blood levels. Model predictions were compared with the data of Santostefano et al. (1998, **200001**), where female rats were exposed to a single oral dose of 10 µg of TCDD/kg BW. Error bars are ± SD.

Source: Edmond et al. (2006, 197316).

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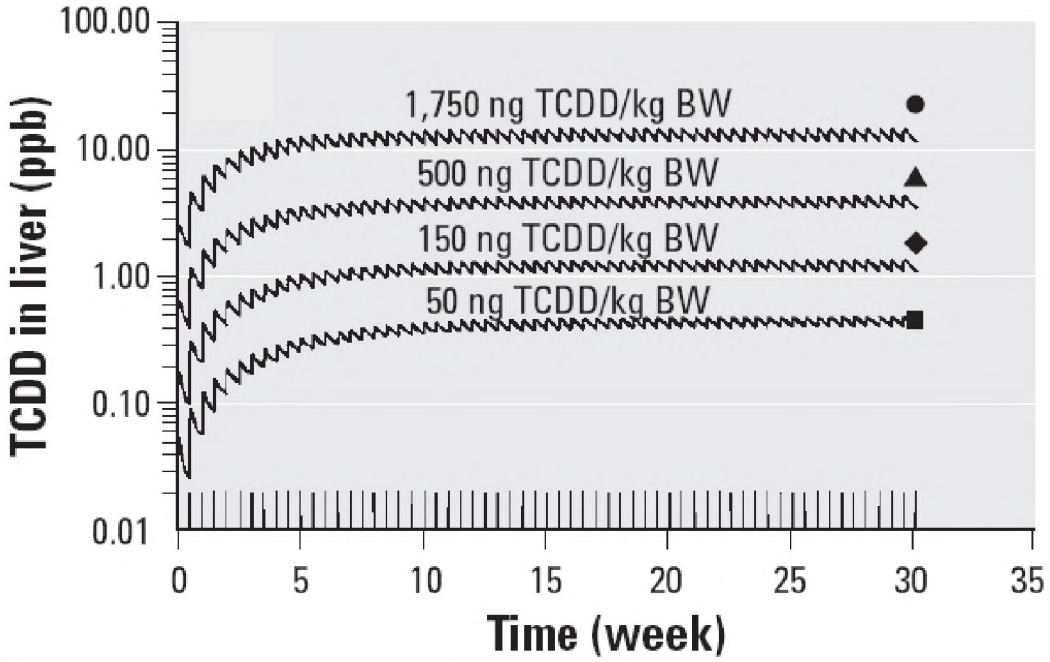


Figure 3-15. PBPK model simulation of hepatic TCDD concentration (ppb) during chronic exposure to TCDD at 50, 150, 500, 1,750 ng TCDD/BW using the inducible elimination rate model compared with the experimental data measured at the end of exposure.

Source: Emond et al. (2006, 197316).

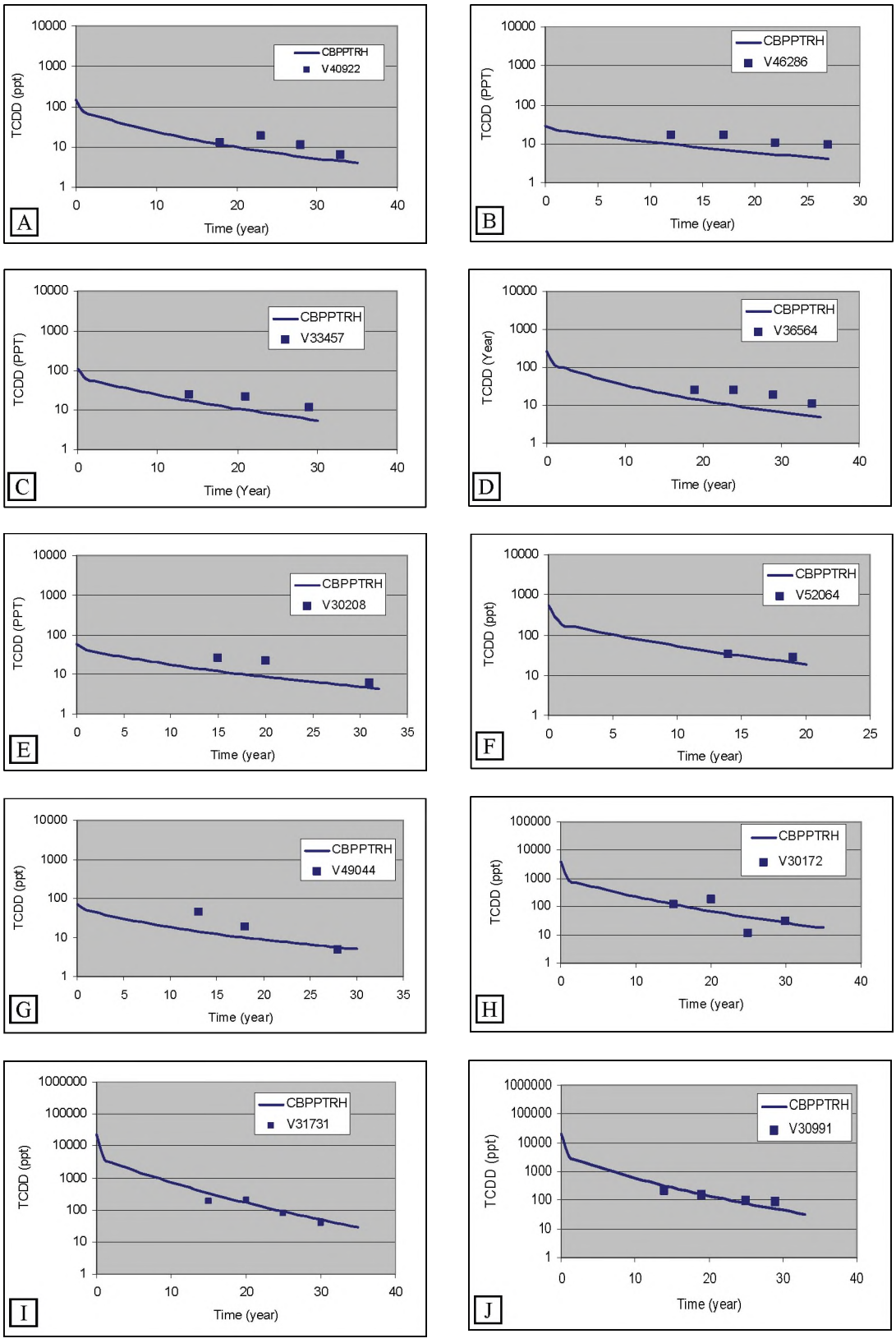


Figure 3-16. Model predictions of TCDD blood concentration in 10 veterans (A-J) from Ranch Hand Cohort.

Source: Emond et al. (2005, 197317).

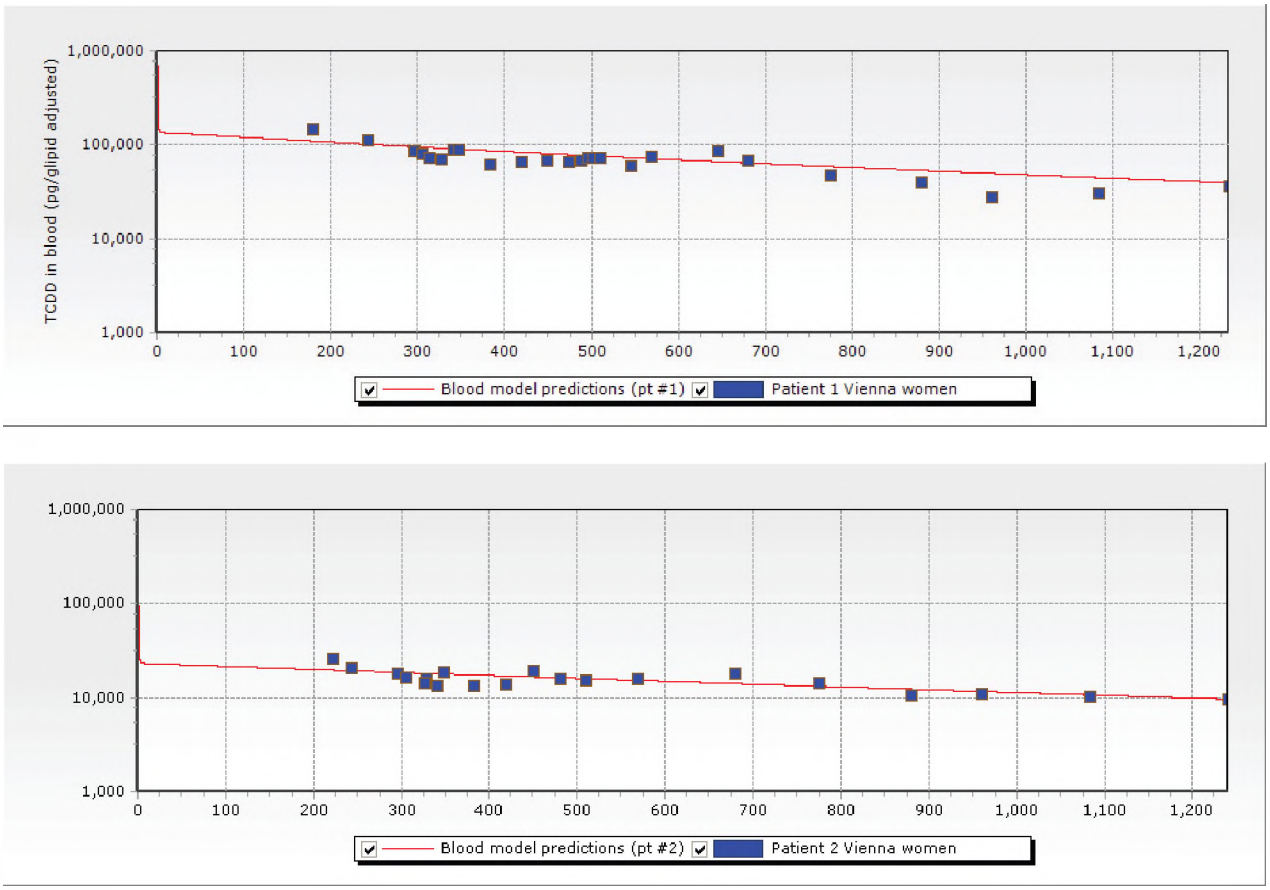


Figure 3-17. Time course of TCDD in blood (pg/g lipid adjusted) for two highly exposed Austrian women (patients 1 and 2). Symbols represent measured concentrations, and lines represent model predictions. These data were used as part of the model evaluation (Geusau et al., 2002, **594259**).

Source: Emond et al. (2005, 197317).

pg g p

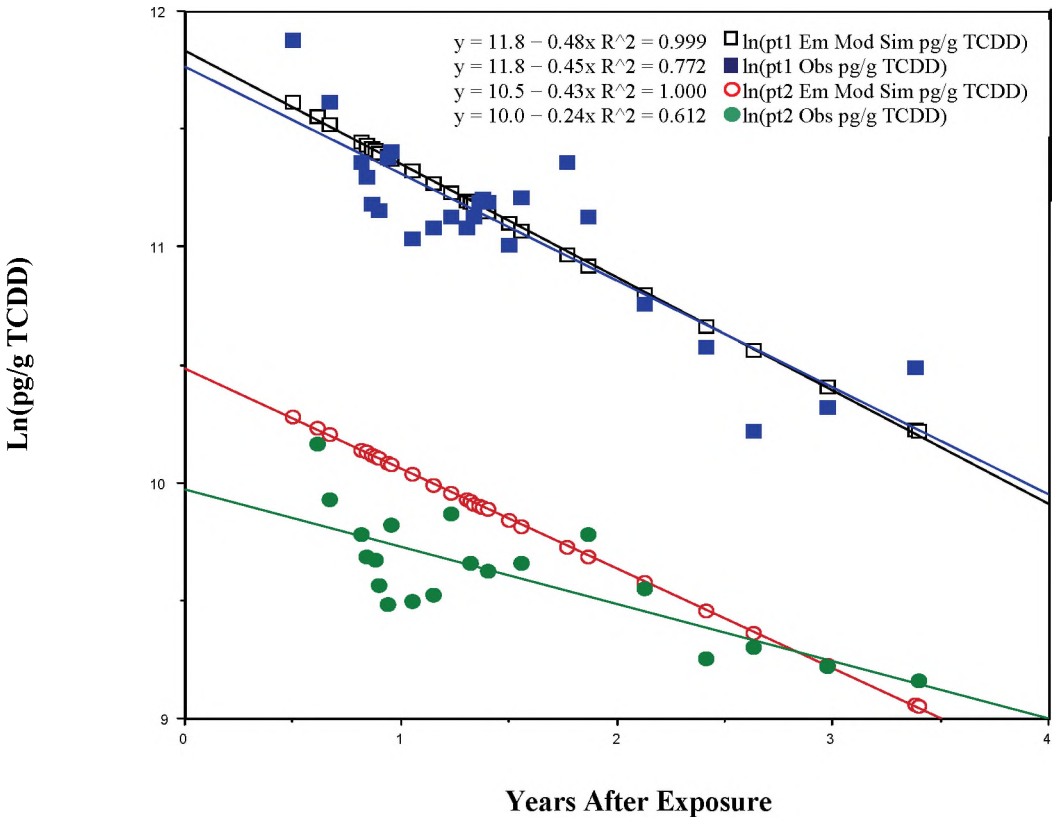


Figure 3-18. Observed vs. Emond et al. (2005, 197317) model simulated serum TCDD concentrations (pg/g lipid) over time (ln = natural log) in two Austrian women. Data from Geusau et al. (2002, 594259).

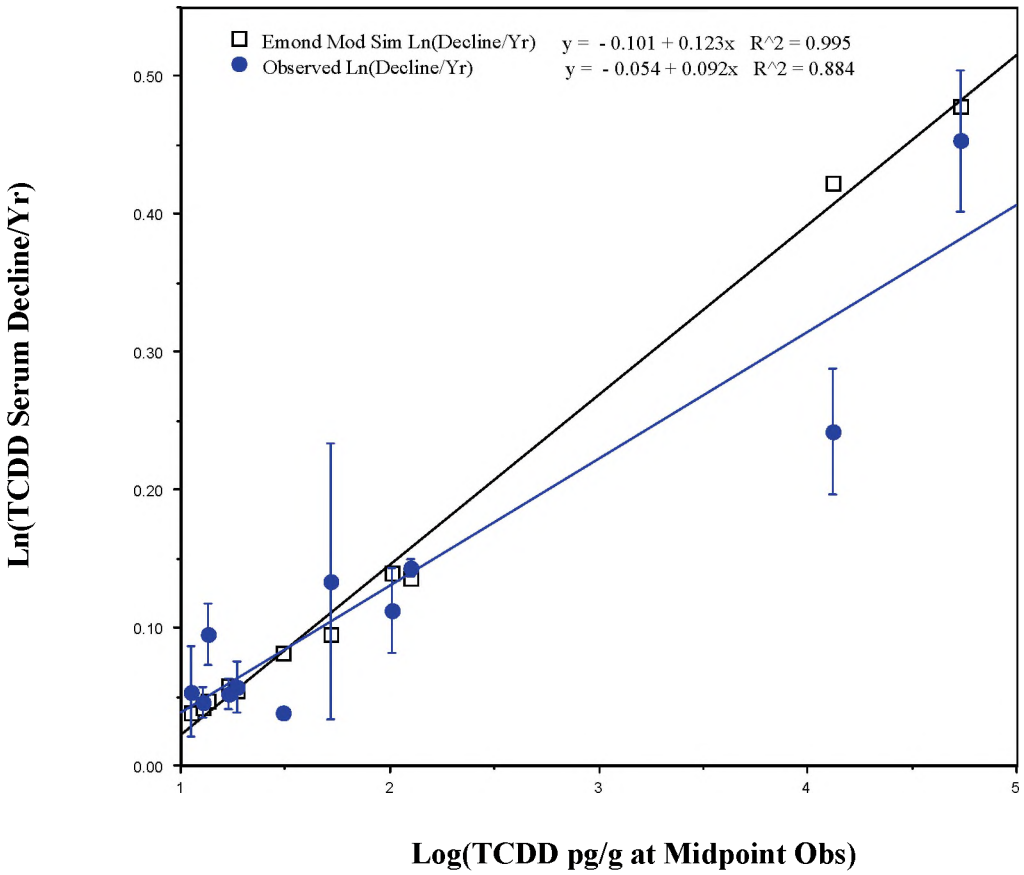


Figure 3-19. Comparison of the dose dependency of TCDD elimination in the Emond model vs. observations of nine Ranch Hand veterans and two highly exposed Austrian patients. Circles are observed data.

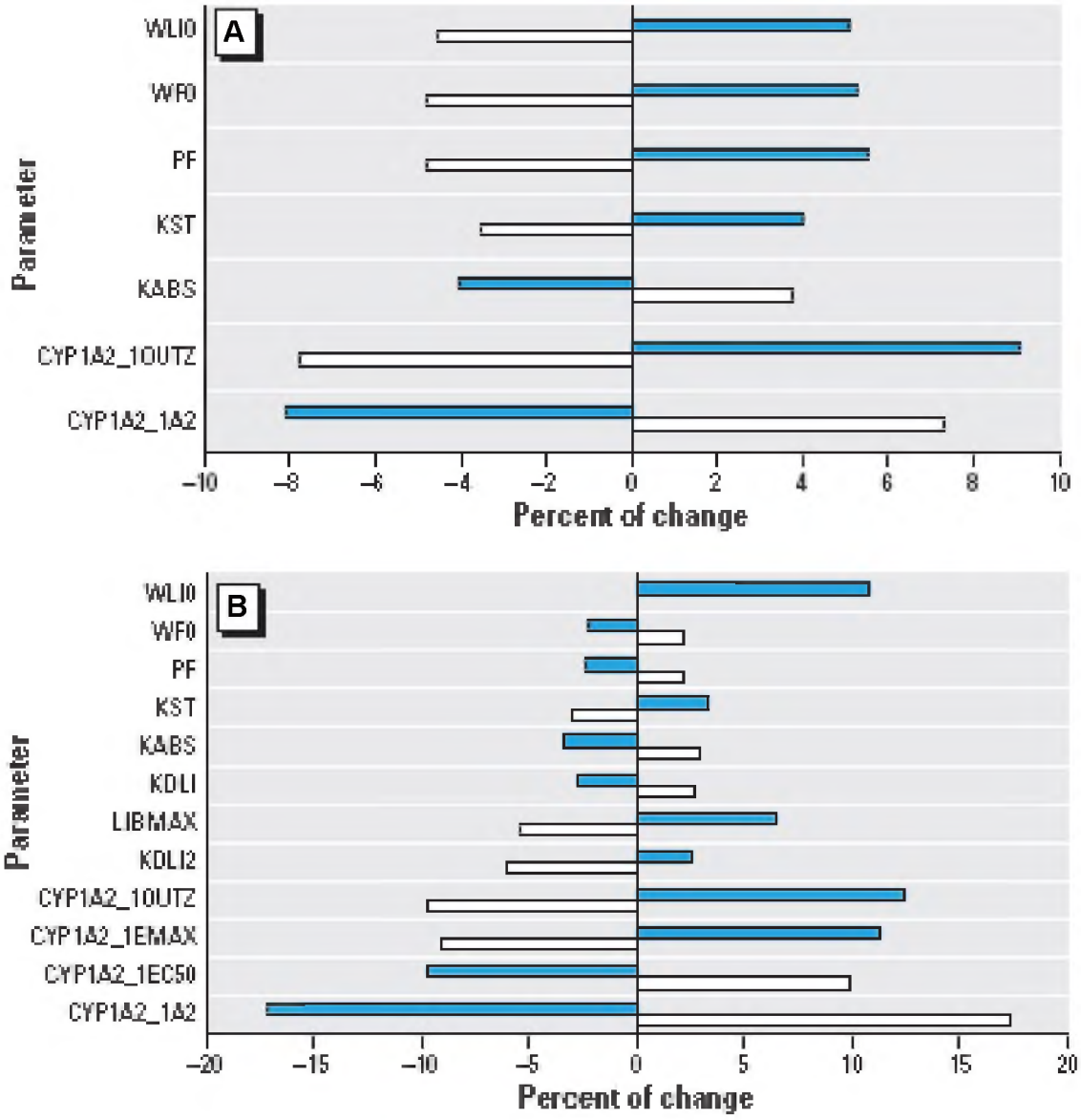
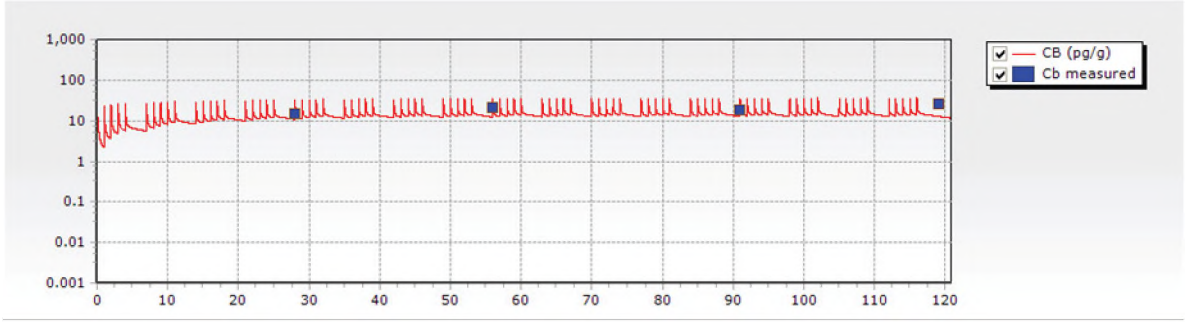


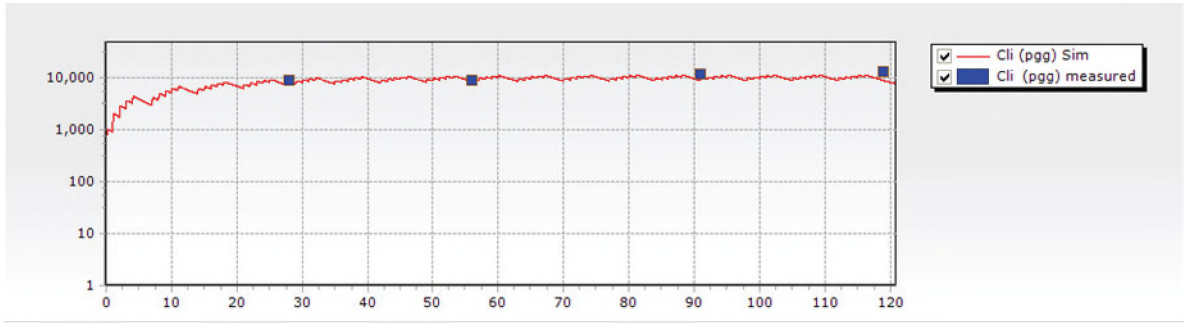
Figure 3-20. Sensitivity analysis was performed on the inducible elimination rate. The analysis was performed at 0.001 µg/kg (A) and at 10 µg/kg (B). The blue and white bars are results from −10% and +10% changes, respectively.

Source: Emond et al. (2006, 197316).

A



B



C

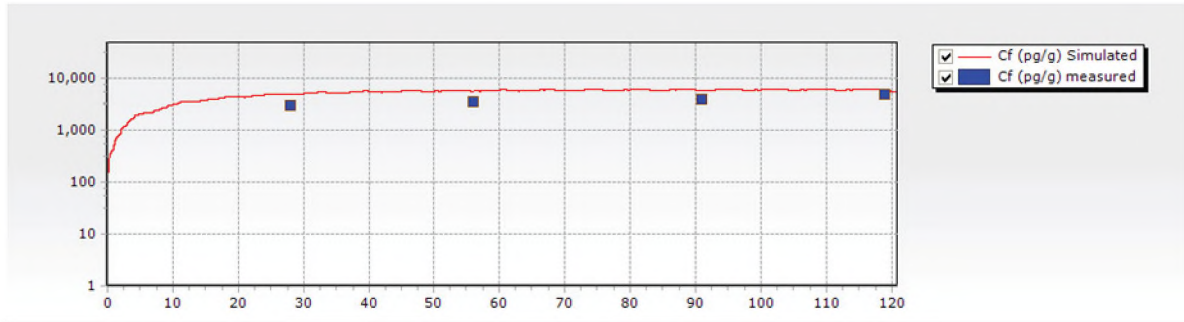


Figure 3-21. Experimental data (symbols) and model simulations (solid lines) of (A) blood, (B) liver and (C) adipose tissue concentrations of TCDD after oral exposure to 150 ng/kg-day, 5 days/week for 17 weeks in mice. Y-axis represents concentration in pg/g and X-axis represents time in days.

Source: Experimental data were obtained form Diliberto et al. (2001, 197238).

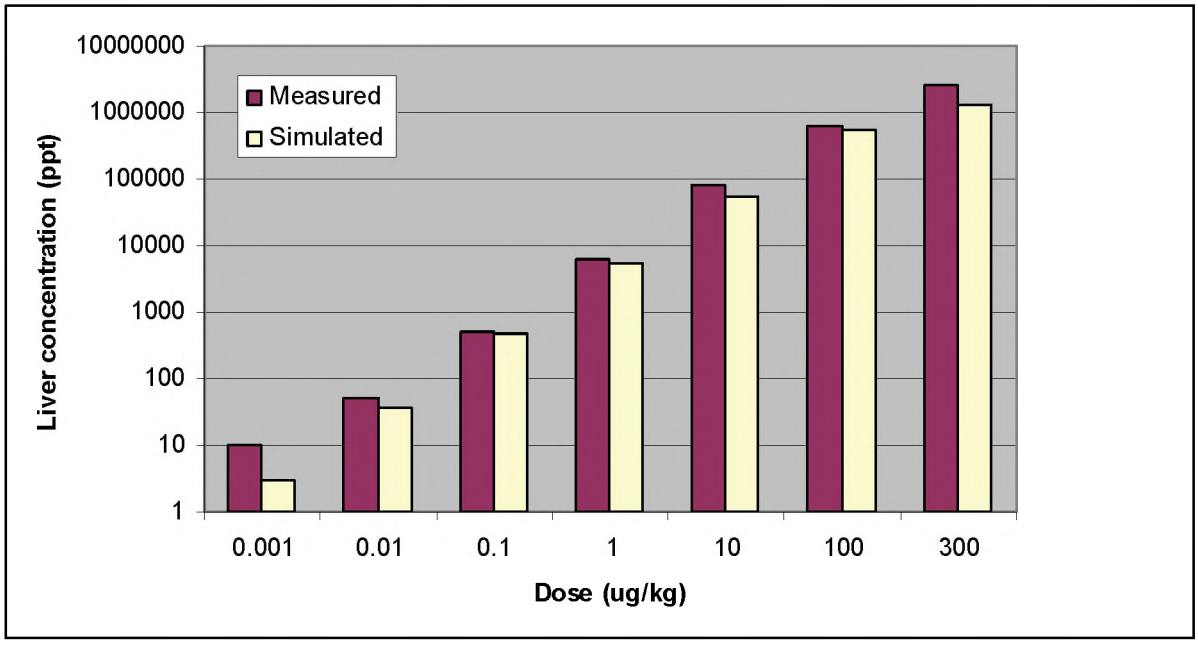


Figure 3-22Comparison of PBPK model simulations with experimental data on liver concentrations in mice administered a single oral dose of 0.001−300 µg TCDD/kg. The simulations and experimental data were obtained 24 hour post-exposure.

Source: Data obtained from Boverhoff et al. (2005, 594260).

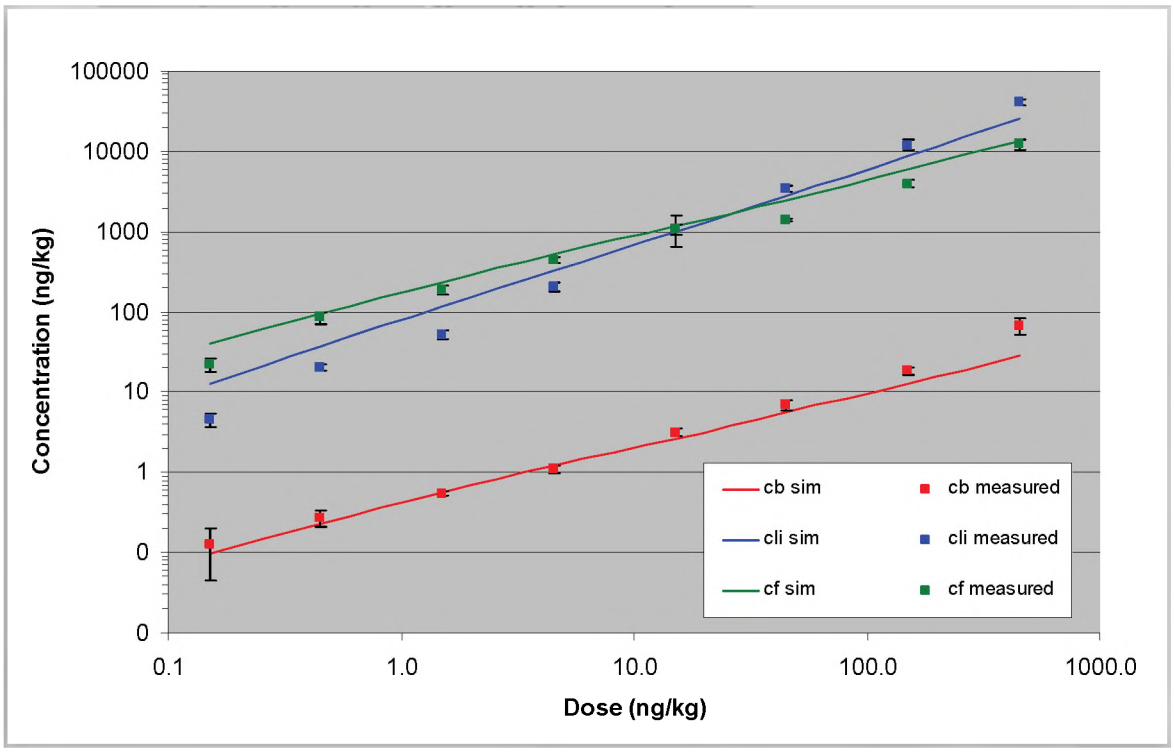
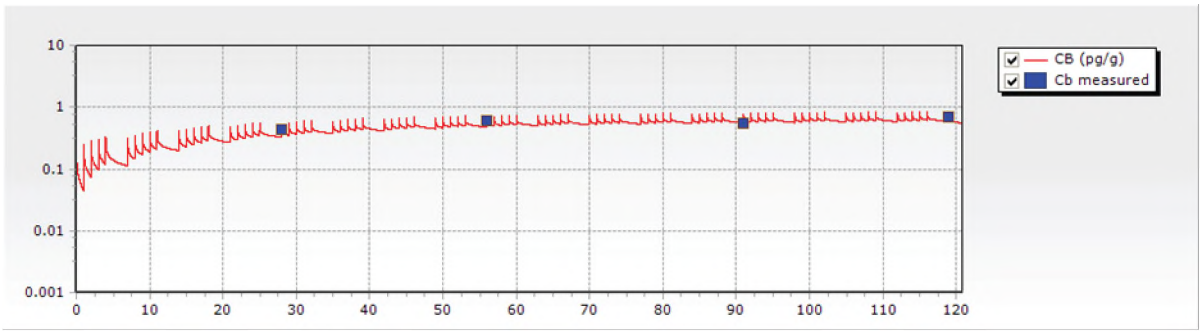


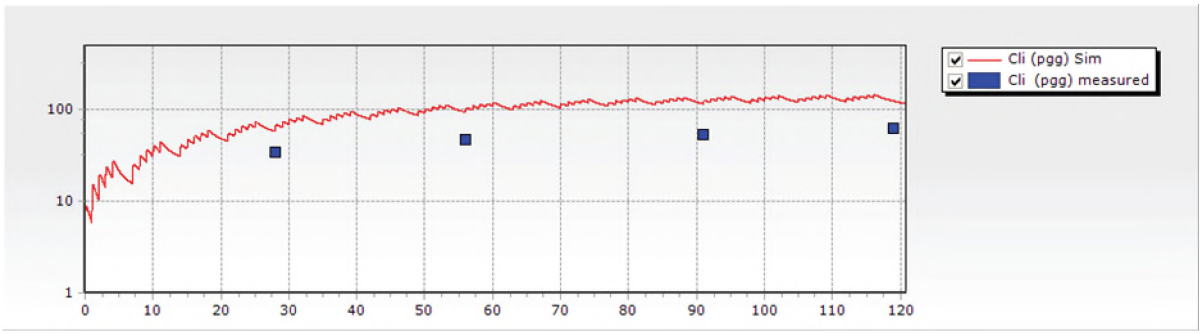
Figure 3-23. Comparison of model simulations (solid lines) with experimental data (symbols) on the effect of dose on blood (cb), liver (cli) and fat (cf) concentrations following repetitive exposure to 0.1−450 ng TCDD/kg, 5 days/week for 13 weeks in mice.

Source: Data obtained from Diliberto et al. (2001, 197238).

A



B



C

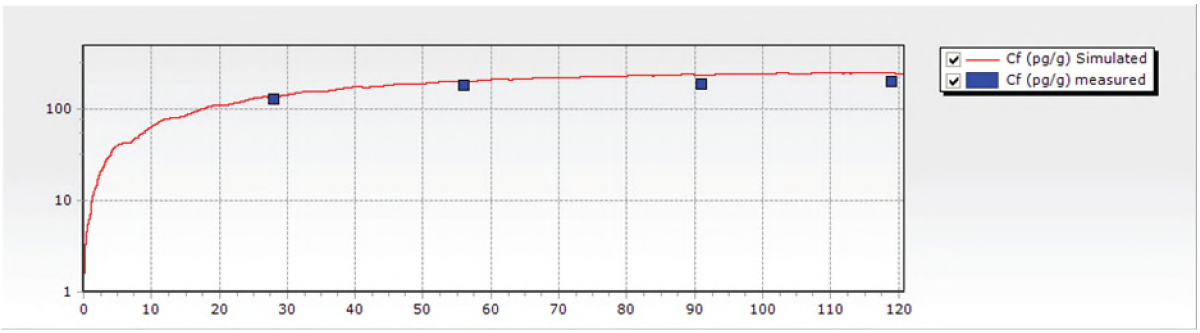


Figure 3-24. Comparison of experimental data (symbols) and model predictions (solid lines) of (A) blood, (B) liver and (C) adipose tissue concentrations of TCDD after oral exposure to 1.5 ng/kg-day, 5 days/week for 17 weeks in mice. Y-axis represents concentration in pg/g and X-axis represents time in days.

Source: Experimental data were obtained form Diliberto et al. (2001, 197238).

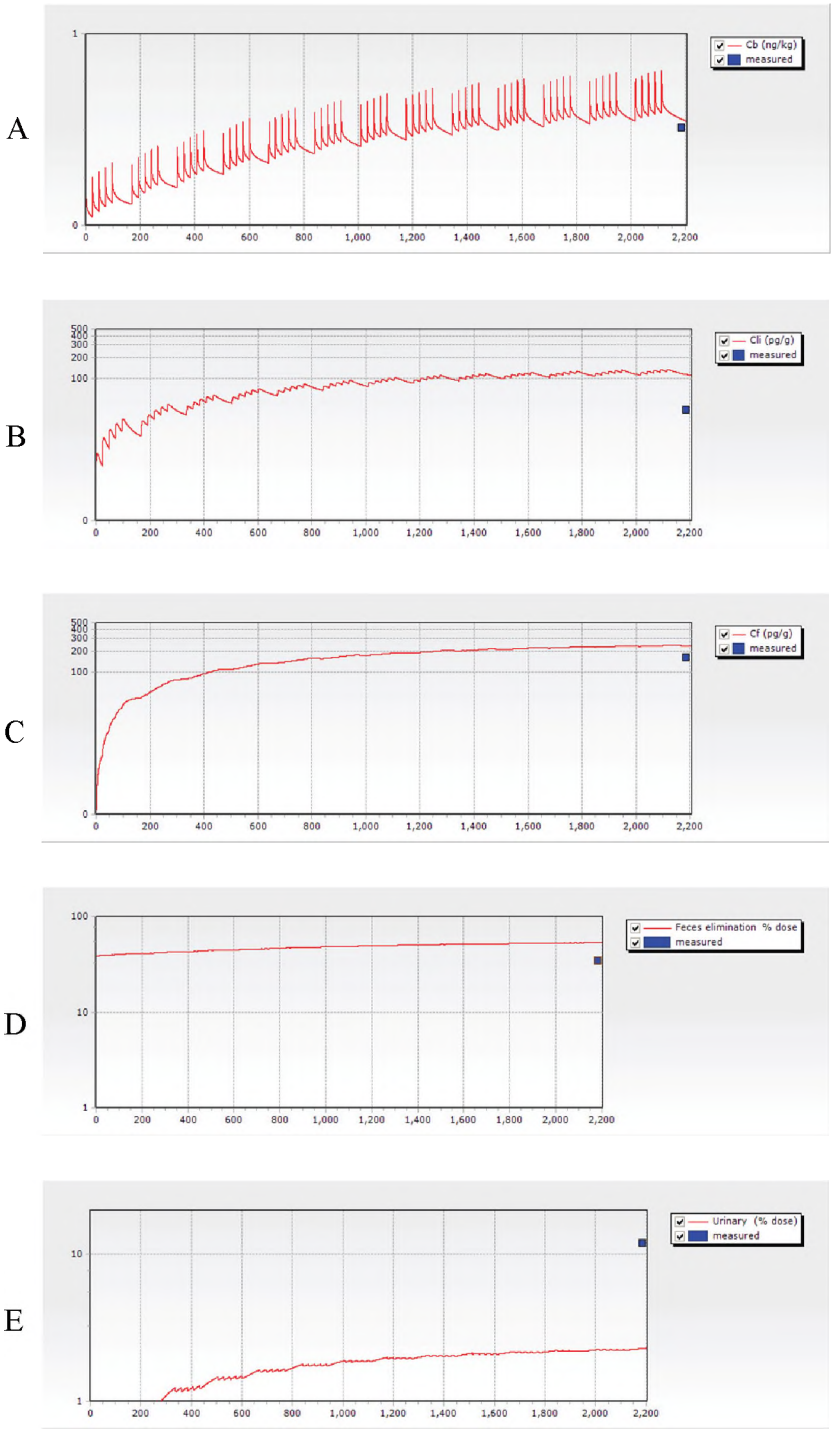


Figure 3-25. Comparison of experimental data (symbols) and model predictions (solid lines) of (A) blood concentration, (B) liver concentration, (C) adipose tissue concentration (D) feces excretion (% dose) and (E) urinary elimination (% dose) of TCDD after oral exposure to 1.5 ng/kg-day, 5 days/week for 13 weeks in mice. Y-axis represents concentration in pg/g and X-axis represents time in days.

Source: Experimental data were obtained form Diliberto et al. (2001, 197238).

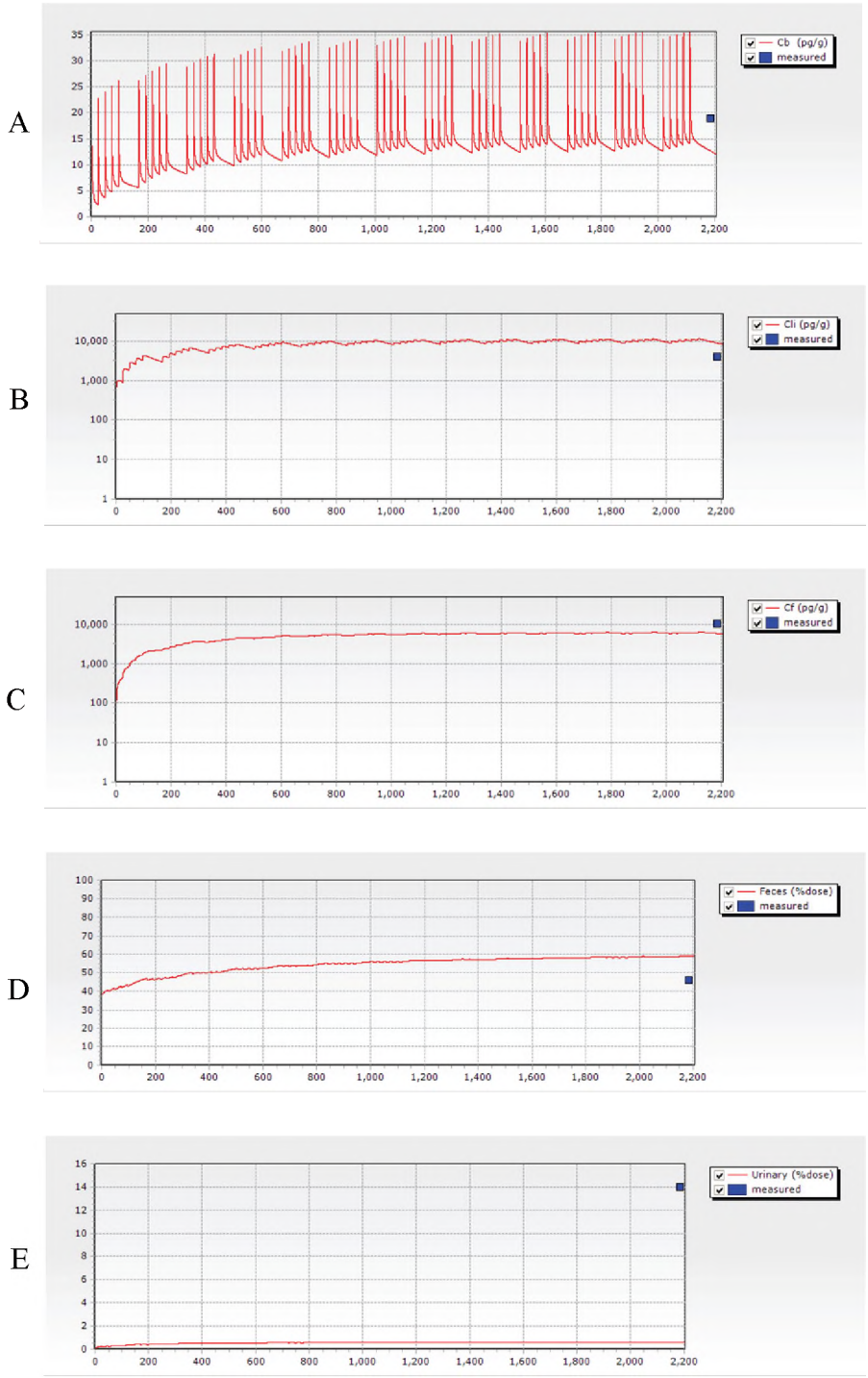


Figure 3-26. Comparison of experimental data (symbols) and model predictions (solid lines) of (A) blood concentration, (B) liver concentration, (C) adipose tissue concentration (D) feces excretion (% dose) and (E) urinary elimination (% dose) of TCDD after oral exposure to 150 ng/kg-day, 5 days/week for 13 weeks in mice. Y-axis represents concentration in pg/g and X-axis represents time in days.

Source: Experimental data were obtained form Diliberto et al. (2001, 197238).

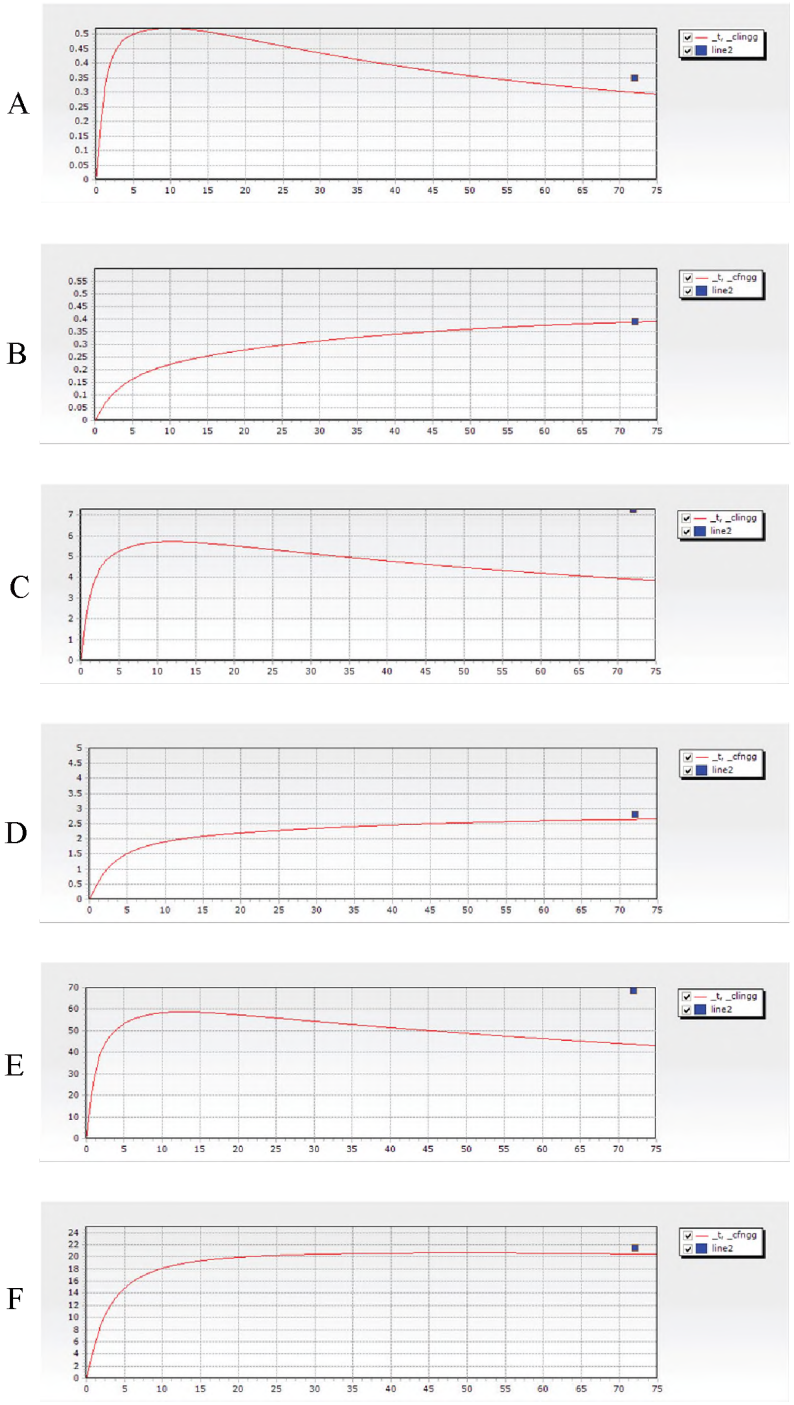
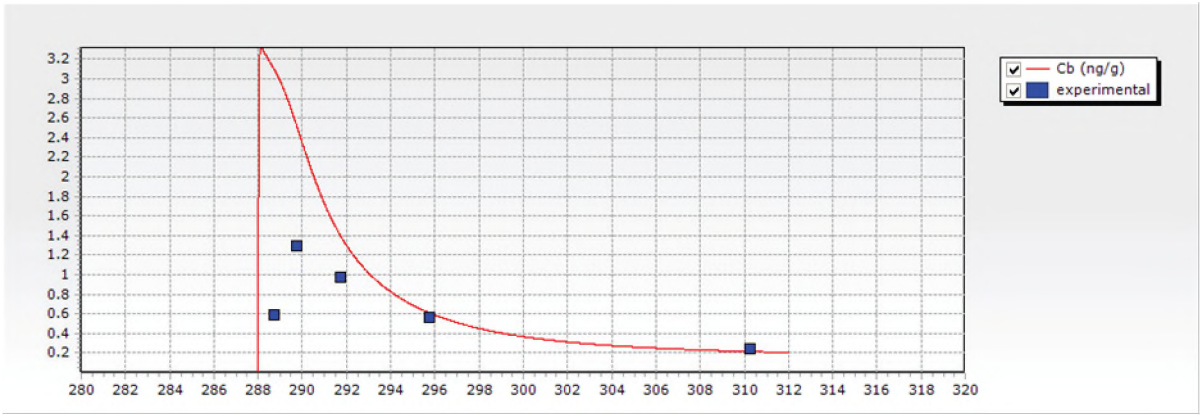
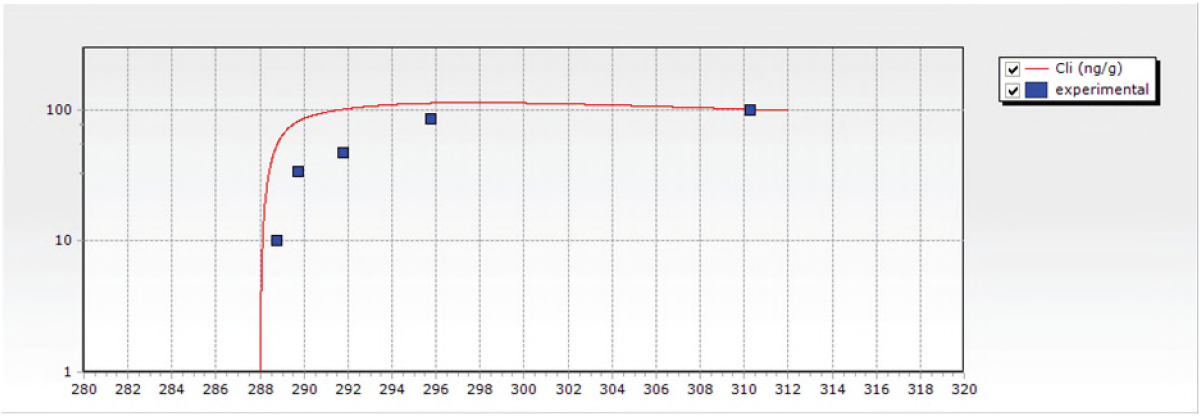


Figure 3-27. PBPK model simulations (solid lines) vs. experimental data (symbols) on the distribution of TCDD after a single acute oral exposure to A−B) 0.1, C−D) 1.0 and E−F) 10 µg of TCDD/kg of body weight in mice.
Liver and adipose concentration for each dose was measured after 72 hours. Y-axis represents the concentration in tissues (ng/g); insets A, C, and E represent liver tissue, whereas B, D, and F correspond to adipose tissue. X-axis represents the time in hours.
Source: experimental data were obtained from Santostefano et al. (1996, 594258).

A



B



C

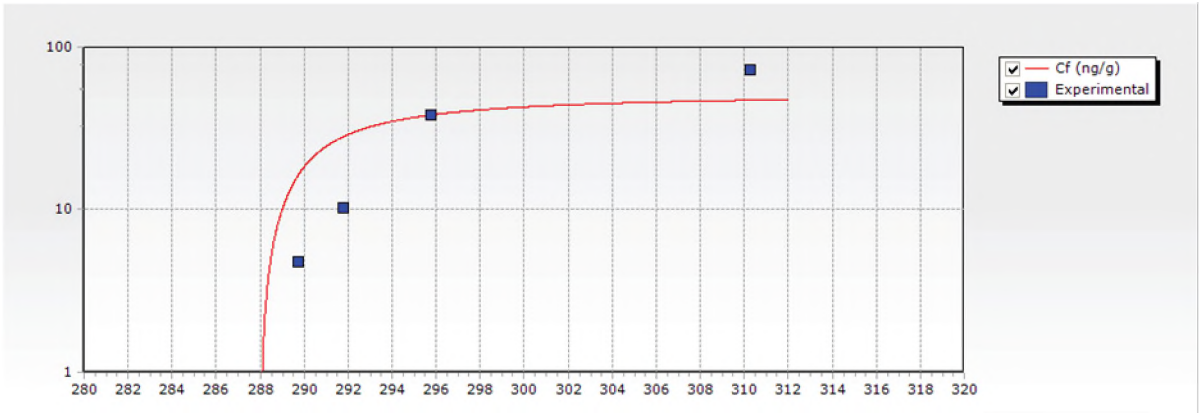


Figure 3-28. PBPK model simulation (solid lines) vs. experimental data (symbols) on the distribution of TCDD after a single dose of 24 µg/kgBW on GD 12 in mice. Concentrations expressed as ng TCDD/g tissue. (A) maternal blood, (B) maternal liver and (C) maternal adipose tissue. Y-axis represents the tissue concentration whereas X-axis represents the time in hours.

.

Source: Experimental data were obtained from (Abbott et al., 1996, 155093).

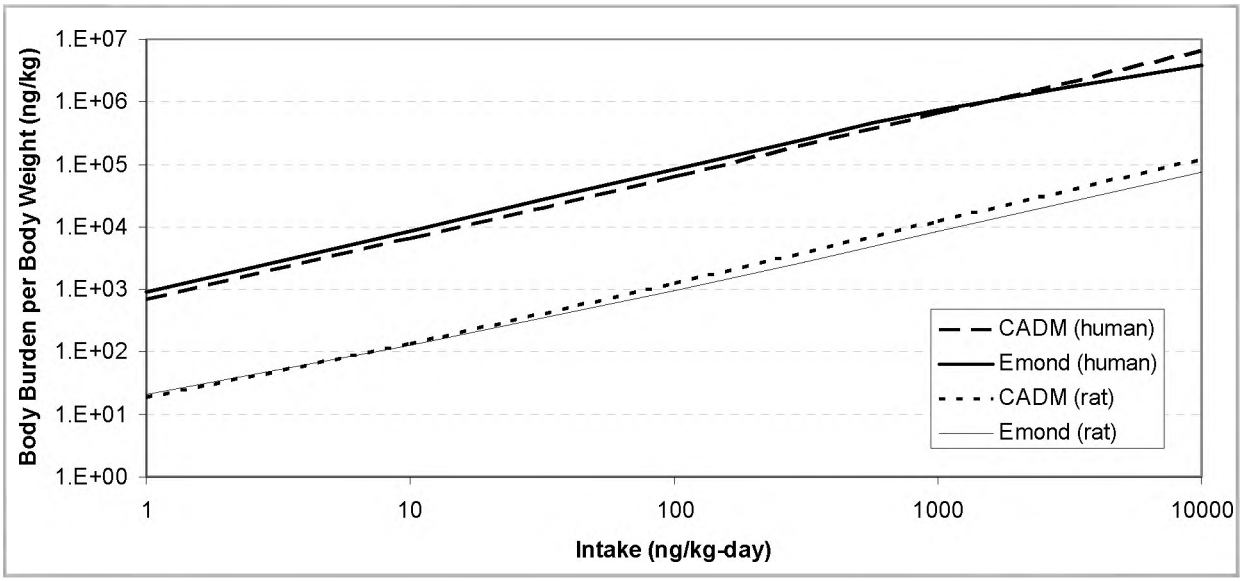


Figure 3-29. Comparison of the near-steady-state body burden simulated with CADM and Emond models for a daily dose ranging from 1 to 10,000 ng/kg-day in rats and humans. The rat model was run for 13 weeks and the human model was run from age 20 to 30. The time-averaged concentration was used for each.

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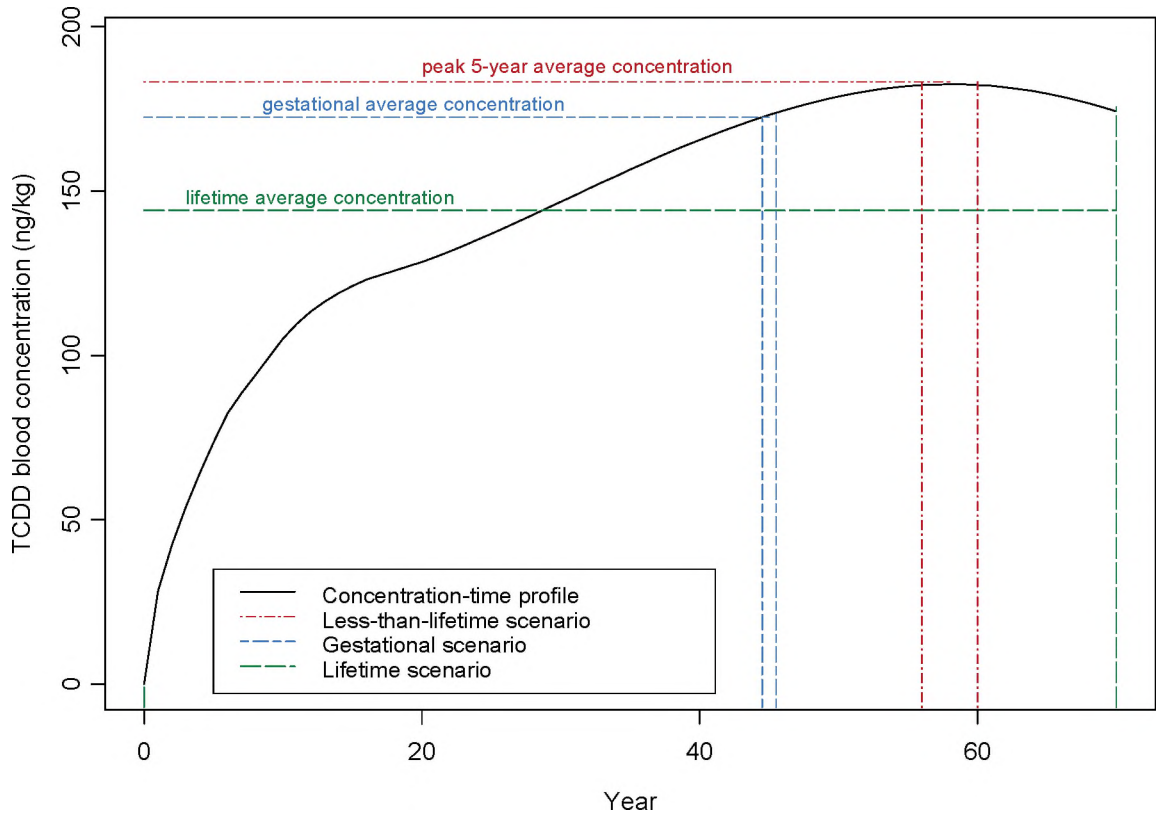


Figure 3-30. TCDD serum concentration-time profile for lifetime, less-than-lifetime and gestational exposure scenarios, with target concentrations shown for each; profiles generated with Emond human PBPK model.

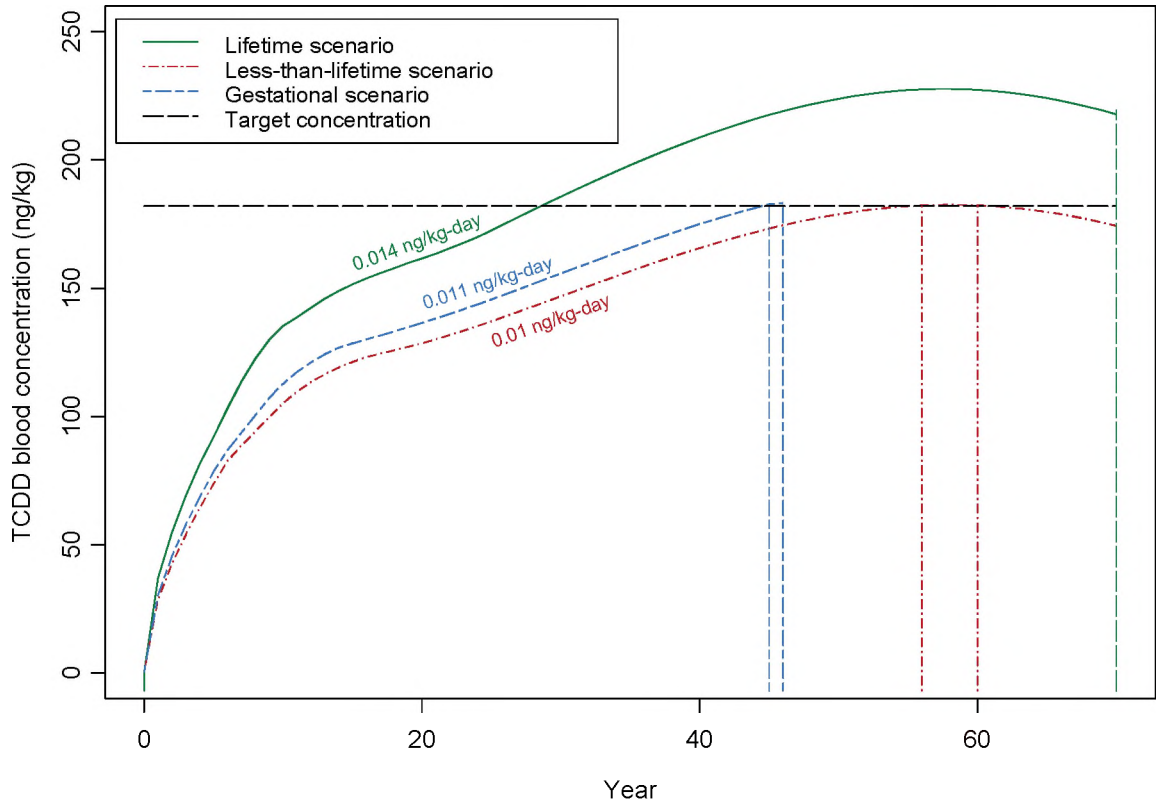


Figure 3-31. TCDD serum concentration-time profile for lifetime, less-than-lifetime and gestational exposure scenarios, showing continuous intake levels to fixed target concentration; profiles generated with Emond human PBPK model.

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4. CHRONIC ORAL REFERENCE DOSE

This section presents U.S. Environmental Protection Agency (EPA)'s response to the National Academy of Sciences (NAS) recommendations that EPA more explicitly discuss the modeling of noncancer endpoints and develop a reference dose (RfD) to address noncancer effects associated with oral 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposures. Section 2 details the selection of the animal studies with the lowest TCDD doses associated with the development of adverse noncancer effects and the selection of relevant epidemiologic studies of adverse noncancer health effects. Section 3 discusses the kinetic modeling and estimation of human equivalent daily oral doses that are used in TCDD RfD development in this section. This section discusses the modeling of noncancer health effects data associated with TCDD exposure and the derivation of an RfD. Specifically, Section 4.1 summarizes the NAS comments on TCDD dose-response modeling and EPA's response, including justification of selected noncancer effects and statistical characterization of modeling results. Section 4.2 presents the TCDD dose-response modeling undertaken for identification of candidate points of departure (PODs) for derivation of an RfD. In Section 4.3, EPA derives an RfD for TCDD. Finally, Section 4.4 describes the qualitative uncertainties in the RfD.

4.1. NAS COMMENTS AND EPA'S RESPONSE ON IDENTIFYING NONCANCER EFFECTS OBSERVED AT LOWEST DOSES

The NAS recommended that EPA identify the noncancer effects associated with low dose TCDD exposures and discuss its strategy for identifying and selecting PODs for noncancer endpoints, including biological significance of the effects.

> With respect to noncancer end points, the committee notes that EPA does not use a rigorous approach for evaluating evidence from studies... (NAS, 2006, 198441p. 47)
>
> The Reassessment should describe clearly the following aspects:
> 1. The effects seen at the lowest body burdens that are the primary focus for any risk assessment—the "critical effects."
> 2. The modeling strategy used for each noncancer effect, paying particular attention to the critical effects, and the selection of a point of comparison based on the biological significance of the effect; if the ED_{01} is retained, then the

> biological significance of the response should be defined and the precision of the estimate given... (NAS, 2006, 198441 p. 187).

In this document, EPA has developed a strategy for identifying the noncancer data sets and PODs that represent the most sensitive and biologically relevant endpoints for derivation of an RfD for TCDD. EPA began this process by using the animal bioassays and human epidemiologic studies that met its study inclusion criteria as sources of these data sets.

For all epidemiologic studies that were identified as suitable for further quantitative dose-response analyses in Section 2.4.3, EPA has chosen to identify PODs (i.e., estimates of a no-observed-adverse-effect level [NOAEL] or lowest-observed-adverse-effect level [LOAEL]; modeling of a benchmark dose lower confidence bound [BMDL] was not possible given the data presented in these studies). Figure 4-1 shows EPA's process to select and identify candidate PODs from these key epidemiologic studies. EPA first evaluated the dose-response information in the study to determine whether it provided an estimate of TCDD dose and an observed noncancer effect that was relevant for RfD derivation. If such data were available, then EPA identified a NOAEL or LOAEL as a candidate POD. For each of these, EPA applied a human kinetic model to estimate the continuous oral daily intake (ng/kg-day) associated with the POD that could be used in the derivation of an RfD (see Section 4.2). If all of this information was available, then the result was included as a candidate POD.

Figure 4-2 summarizes the strategy employed for identifying and selecting candidate PODs from the key animal bioassays identified in Section 2.4.3 for use in noncancer dose-response analysis of TCDD. For each noncancer endpoint, EPA first evaluated the toxicologic relevance of each endpoint, rejecting those judged not to be relevant for RfD derivation. Next, initial PODs (NOAELs, LOAELs, and BMDLs) based on the first-order body burden metric (see Section 3.3.4.2) and expressed as human-equivalent doses (HEDs) were determined for all relevant endpoints (summarized in Table 4-3). Because there were very few NOAELs and BMDL modeling was largely unsuccessful due to data limitations, the next stage of evaluation was carried out using LOAELs only. Within each study, endpoints not observed at the LOAEL (i.e., reported at higher doses) with BMDLs greater than the LOAEL were eliminated from further analysis, as they would not be considered as candidates for the final POD on either a BMDL or NOAEL/LOAEL basis (i.e., the POD would be higher than the PODs of

other relevant endpoints). In addition, all endpoints with HED estimates based on LOAELs ($LOAEL_{HED}$s) beyond a 100-fold range of the lowest identified $LOAEL_{HED}$ were eliminated from further consideration, as they would not be potential POD candidates either (i.e., the POD would be higher than the PODs of other relevant endpoints). For the remaining endpoints, EPA then determined final potential PODs (NOAELs, LOAELs and BMDLs) based on TCDD blood concentrations obtained from the Emond rodent physiologically based pharmacokinetic (PBPK) models. HEDs were then estimated for each of these PODs using the Emond human PBPK model. From these HEDs, a POD_{HED} was selected[19] for each study as the basis for the candidate RfD, to which appropriate uncertainty factors (UFs) were applied following EPA guidelines. The resulting candidate RfDs were then considered in the final selection process for the RfD. Other endpoints occurring at slightly higher doses representing additional effects associated with TCDD exposure (beyond the 100-fold LOAEL range) were evaluated, modeled, and included in the final candidate RfD array[20] to examine endpoints not evaluated by studies with lower PODs. In addition, BMD modeling based on administered dose was performed on all endpoints for comparison purposes. The final array of selected endpoints is shown in Table 4-4 (summary of BMD analysis) and Table 4-5 (candidate RfDs).

The NAS recommended that EPA better justify the selection of response levels for endpoints used to develop risk estimates. The NAS commented on EPA's decision to estimate an ED_{01} (effective dose eliciting a 1% response) for noncancer bioassay/data set combinations as a comparative tool across studies, suggesting that EPA identify and evaluate the levels of change associated with adverse effects to define the benchmark response (BMR) level for continuous noncancer endpoints.

> The committee notes that the choice of the 1% response level as the POD substantially affects … the noncancer analyses…. The committee recommends that the Reassessment use levels of change that represent clinical adverse effects to define the BMR level for noncancer continuous end points as the basis for an appropriate POD in the assessment of noncancer effects (NAS, 2006, 198441, p. 72).

[19]In the standard order of consideration: BMDL, NOAEL, and LOAEL.
[20]However, studies with a lowest dose tested greater than 30 ng/kg-day were not included in the expanded evaluation.

> The committee concludes that EPA did not adequately justify the use of the 1% response level (the ED_{01}) as the POD for analyzing epidemiological or animal bioassay data for … noncancer effects (NAS, 2006, 198441 p. 18).

In the 2003 Reassessment (U.S. EPA, 2003, 537122), EPA was not attempting to derive an RfD when it conducted TCDD dose-response modeling. The 2003 Reassessment developed ED_{01} estimates for noncancer effects in an attempt to compare disparate endpoints on a consistent response scale. Importantly, the 2003 Reassessment defined the ED_{01} as 1% of the maximal response for a given endpoint, not as a 1% change from control. Because RfD derivation is one goal of this document, the noncancer modeling effort undertaken here differs substantially from the modeling in the 2003 Reassessment.

The NAS committee was concerned with the statistical power to determine the shape of the dose-response curve at doses far below observed dose-response information. EPA agrees that the shape of the dose-response curve in the low-dose region cannot be determined confidently when based on higher-dose information. An observed response above background near (or below) the BMR level is needed for discrimination of the shape of the curve and for accurate estimation of an ED_x or BMDL. Although many of the ED_{01}s presented in the 2003 Reassessment were near the lowest dose tested, responses at the lowest doses were often high and much greater than a 1% response (i.e., 1% of the maximum response). The lack of an observed response near the BMR level is often a problem in interpretation of BMD modeling results.

In this document, EPA has used a 10% BMR for dichotomous data for all endpoints; there were no developmental studies that accounted for litter effects, for which a 5% BMR would be used (U.S. EPA, 2000, 052150). For continuous endpoints in this document, EPA has used a BMR of 1 standard deviation from the control mean whenever a specific toxicologically-relevant BMR could not be defined. For the vast majority of continuous endpoints, EPA could not establish unambiguous levels of change representative of adversity, which EPA defines as "a biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge" (U.S. EPA, 2009, 192196). For body and organ weight change, EPA has previously established a BMR of 10% change, which also is used in this document.

The NAS commented on EPA's development of ED_{01} estimates for numerous study/data set combinations in the 2003 Reassessment, suggesting that EPA had not appropriately characterized the statistical confidence around such model predictions in the low-response region of the model.

> It is critical that the model used for determining a POD fits the data well, especially at the lower end of the observed responses. Whenever feasible, mechanistic and statistical information should be used to estimate the shape of the dose-response curve at lower doses. At a minimum, EPA should use rigorous statistical methods to assess model fit and to control and reduce the uncertainty of the POD caused by a poorly fitted model. The overall quality of the study design is also a critical element in deciding which data sets to use for quantitative modeling (NAS, 2006, 198441, p. 18).
>
> EPA should … assess goodness-of-fit of dose-response models for data sets and provide both upper and lower bounds on central estimates for all statistical estimates. When quantitation is not possible, EPA should clearly state it and explain what would be required to achieve quantitation (NAS, 2006, 198441, p. 10).

The NAS also commented that EPA report information describing the adequacy of dose-response model fits, particularly in the low response region. For those cases where biostatistical modeling was not possible, NAS recommended that EPA identify the reasons.

> The Reassessment should also explicitly address the importance of statistical assessment of model fit at the lower end and the difficulties in such assessments, particularly when using summary data from the literature instead of the raw data, although estimates of the impacts of different choices of models would provide valuable information about the role of this uncertainty in driving the risk estimates (NAS, 2006, 198441, p. 73).

To address this concern, in this document EPA has reported the standard suite of goodness-of-fit measures from the benchmark dose modeling software (BMDS 2.1). These include chi-square *p*-values, Akaike's Information Criterion (AIC), scaled residuals at each dose level and plots of the fitted models. In some cases, when restricted parameters hit a bound, EPA used likelihood ratio tests to evaluate whether the improvement in fit afforded by estimating additional parameters could be justified. Goodness-of-fit measures are reported for all key data

sets in Appendix E. (See Section 4.2.4.2 for a more complete description of the benchmark dose modeling criteria for model evaluation.)

4.2. NONCANCER DOSE-RESPONSE ASSESSMENT OF TCDD

This section describes EPA's current effort to conduct an evaluation of TCDD dose-response for the noncancer endpoints from studies that met the study inclusion criteria. Discussions include benchmark dose modeling procedures, kinetic modeling, and POD candidates for derivation of the RfD. Section 4.2.1 discusses the types of endpoints that are considered relevant by EPA's Integrated Risk Information System and lists the study/endpoint combinations that were not considered for the TCDD RfD derivation, with supporting text in Appendix G. Section 4.2.2 describes how EPA has used physiologically-based pharmacokinetic (PBPK) modeling to estimate effective internal exposures as an alternative to using administered doses or body burdens based on first-order kinetics. Section 4.2.3 details the dose-response analysis of the epidemiologic data, with supporting information on kinetic modeling in Appendix D. Section 4.2.4 details the dose-response analysis for the animal bioassay data; Appendix E provides the BMDS input tables (see Section E.1) and output for all modeling, including blood concentrations (see Section E.2) and administered dose (see Section E.3).

4.2.1. Determination of Toxicologically Relevant Endpoints

The NAS committee commented on the low dose model predictions and the need to discuss the biological significance of the noncancer health effects modeled in the 2003 Reassessment. In selecting POD candidates from the animal bioassays for derivation of the candidate RfDs, EPA had to consider the toxicological relevance of the identified endpoint(s) from any given study. Some endpoints/effects may be sensitive, but lack general toxicological significance due to not being clearly adverse (defined in EPA's Integrated Risk Information System glossary as "a biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge" (U.S. EPA, 2009, 192196)), being an adaptive response or not being clearly linked to downstream functional or pathological alterations. For example, CYP induction alone is not considered a significant toxicological effect given that CYPs are induced as part of the hepatic metabolism of xenobiotic agents. Additionally, the role of CYP induction

in hepatotoxicity and carcinogenicity of TCDD is unknown, thus, CYP induction is not considered a relevant POD without obvious pathological significance. Another example is when all oxidative stress markers are significantly affected, but no other indicators of brain pathology are assessed. In this case, it is impracticable to link the markers of oxidative stress to a toxicological outcome in the brain; thus, this endpoint is not considered a relevant POD candidate. It is standard EPA practice for RfD derivation to base a reference value on endpoints that are adverse or are immediate precursors to an adverse effect.

Studies meeting the study selection criteria with endpoints that were not considered for derivation of a candidate RfD (because they were not considered to be toxicologically relevant noncancer effects) are: Kitchin and Woods (1979, 198750), Hassoun et al. (1998, 136626; 2000, 197431; 2002, 543725; 2003, 198726), Burleson et al. (1996, 196998), Kuchiiwa et al. (2002, 198355), Mally and Chipman (2002, 198098), Vanden Heuvel et al. (1994, 197551), Devito et al. (1994, 197278), Lucier et al. (1986, 198398), Sugita-Konishi et al. (2003, 198375), and Sewall et al. (1993, 197889). Appendix G identifies the endpoints from these studies that were not considered to be toxicologically relevant for derivation of an RfD (e.g., cytochrome P450 induction, oxidative stress measures, gap junction disruption, mRNA induction, brain serotonin levels) and provides the rationales for the toxicological relevance decisions on the endpoints. Note that for many of these studies, other endpoints were examined that are toxicologically relevant and were considered in the RfD derivation process.

4.2.2. Use of Toxicokinetic Modeling for TCDD Dose-Response Assessment

Given that TCDD accumulates in fat with continuous exposure and is eliminated slowly from the body, but at very different rates across species, EPA has determined that the standard UF approach or allometric scaling of body weight for interspecies extrapolation is not appropriate. Therefore, EPA has decided to use toxicokinetic modeling to estimate an effective internal dose for equivalence across species. The toxicokinetic models chosen by EPA are the rodent and human PBPK models described by Emond et al. (2004, 197315; 2006, 197316) and modified by EPA for this assessment as described in Section 3.3.4 (hereafter referred to as the "Emond [rodent or human] PBPK model"). Both the rodent and human models have a gestational component, which allow for more relevant exposure comparisons between general adult exposures and the numerous gestational exposure studies. Ideally, a relevant tissue

concentration for each effect would be estimated. However, no models exist for estimation of all relevant tissue concentrations. As virtually all TCDD is found in the adipose fraction of tissues, or bound to specific proteins, a preferred approach to developing a dose metric would be to account for the fat fraction of each tissue and protein binding; however, EPA has decided that the modeling of such estimates is too uncertain and EPA has not found sufficient data to implement this approach. Therefore, EPA has decided to use the concentration of TCDD in blood as a surrogate for tissue concentrations, assuming that tissue concentrations are proportional to blood concentrations. Furthermore, because the RfD is necessarily expressed in terms of average daily exposure, the blood concentrations are expressed as averages over the relevant period of exposure for each endpoint. For the animal bioassay studies, the relevant period of exposure is the duration of dosing, starting at the age of the animals at the beginning of the study. For humans, the relevant period of exposure is generally lifetime, which is defined as 70 years by convention. However, EPA varied the averaging time for the equivalent human blood concentrations to correspond to the test-animal exposure duration in the following manner.

- For correspondence with animal chronic exposures,[21] the human-equivalent TCDD blood concentration is assumed to be the 70-year average.
- For correspondence with animal gestational exposures, the human-equivalent TCDD blood concentration is assumed to be the average over 45 years for a female, beginning at birth, plus 9 months of gestational exposure. The choice of 45 years to beginning of pregnancy is health protective of the population in that the TCDD daily oral intake achieving the target blood concentration is smaller than for shorter averaging times.[22]
- For correspondence with any other animal exposure duration, the human-equivalent TCDD blood concentration is assumed to be the average over the equivalent human exposure duration calculated backward from the peak exposure plateau at or near the end of the 70-year scenario. The average is determined from the terminal end of the human exposure period because the daily oral intake achieving the target blood concentration is smaller than for the same exposure period beginning at birth and is health protective for effects occurring after shorter-term exposure. The determination of equivalent exposure durations across species is problematic and somewhat arbitrary, so EPA uses the average peak blood concentration as the human equivalent for all less-than-chronic animal

[21] Assumed to be ≥75% of nominal lifetime, or about 550 days in rodents.
[22] See Section 3.3.4.2 for a discussion of this issue, including a comparison of the 45-year old pregnancy scenario to one beginning at age 25 in Table 3-15.

exposures (other than gestational).[23] For the first-order kinetics model, the average peak exposure is close to the theoretical steady-state asymptote (see Section 3.3.4.2). However, for the Emond human PBPK model used by EPA in this assessment, the timing of the peak exposure is dose-dependent and tends to decline after 60 years in some cases. Therefore, the 5-year average TCDD blood concentration that includes the peak ("5-year peak") is used as the relevant dose-metric for the PBPK model applications.

4.2.3. Noncancer Dose-Response Assessment of Epidemiological Data

The following four epidemiologic studies describing noncancer endpoints were identified in Section 2.4.3 as studies to be evaluated for development of PODs for derivation of candidate RfDs: Baccarelli et al. (2008, 197059), Mocarelli et al. (2008, 199595), Alaluusua et al. (2004, 197142) and Eskenazi et al. (2002, 197168). Each of these studies described effects observed in the Seveso cohort (see detailed study summaries in Section 2.4.1 and Table 2-5). Each study modeled individual-level human exposure measures and provided information from which EPA could determine an exposure window over which kinetic models could be used to quantify TCDD exposures for dose-response assessment. EPA used kinetic information to estimate group-mean daily TCDD intake rates for the exposure groups presented in these studies (see Appendix D for details). EPA focused on identifying NOAELs and LOAELs for these studies; EPA did not conduct Benchmark Dose modeling because the covariates identified by the study authors could not be incorporated by modeling the grouped response data. EPA's development of PODs for these studies is described in this section and shown in Table 4-1.

4.2.3.1. *Baccarelli et al. (2008, 197059)*

For Baccarelli et al. (2008, 197059), EPA was able to define a LOAEL as the group mean of 39 ppt TCDD in neonatal plasma for thyroid stimulating hormone (TSH) values above 5 μU/mL. (See Section 2.4.1.2.1.5.7 for study details.) Baccarelli et al. (2008, 197059) did not estimate the equivalent oral intake associated with TCDD serum concentrations and gave only neonatal serum TCDD concentrations for the groups above and below the 5 μU/mL standard. The study authors, however, developed a regression model relating the level of TSH in 3-day-old

[23]By comparison to a half-lifetime equivalent (1 year in rodents, 35 years in humans), the ratio of body burden (1st-order kinetic model) to oral intake does not differ significantly from the average-peak scenario; all shorter-term scenarios differ even less (see Section 3.3.4.2). These relationships, with respect to the 5-year peak, hold for the PBPK model results, as well (see Section 3).

neonates to TCDD concentrations in maternal plasma at birth (given as lipid-adjusted serum concentrations, LASC). The authors extrapolated maternal plasma concentrations from previous measurements using a simple first-order pharmacokinetic model. Because there is limited information regarding the relationship between maternal and neonatal serum TCDD levels, EPA determined that there was too much uncertainty in estimating maternal intake from neonatal TCDD serum concentrations, directly. Therefore, EPA determined the maternal intake at the LOAEL from the maternal serum-TCDD/TSH regression model by finding the maternal TCDD LASC at which neonatal TSH exceeded 5 μU/mL. EPA then used the Emond PBPK model under the human gestational scenario (see Section 4.2.2) to estimate the continuous daily oral TCDD intake that would result in a TCDD LASC corresponding to a neonatal TSH of 5 μU/mL at the end of gestation; EPA established the resulting maternal intake (0.024 ng/kg-day) as the LOAEL, shown in Table 4-1 as a candidate POD for derivation of candidate RfDs (PBPK modeling details are shown in Appendix D).

4.2.3.2. *Mocarelli et al. (2008, 199595)*

Mocarelli et al. (2008, 199595) reported decreased sperm concentrations (20%) and decreased motile sperm counts (11%) in men who were 1−9 years old in 1976 at the time of the accident (initial TCDD exposure event) (see Section 2.4.1.2.1.5.8 for study details). Men who were 10−17 years old in 1976 were not adversely affected. Serum (LASC) TCDD levels were measured within one year of the initial exposure. Serum TCDD levels and corresponding responses were reported by quartile, with a reference group of less-exposed individuals assigned a TCDD LASC value of 15 ppt (which was the mean of individuals outside the contaminated area). The lowest exposed group mean was 68 ppt (1st quartile). Because effects were detected only among boys under the age of 10, EPA assumes there is a maximum 10-year critical exposure window for elicitation of these effects. However, for the exposure profile, with a high initial pulse followed by an extended period of elimination with only background exposure, the estimation of an average exposure resulting in the effect is problematic. Therefore, EPA implemented a procedure for the estimation of the continuous daily TCDD intake associated with the LOAEL in the Mocarelli et al. (2008, 199595) study using the following 5-step process:

1. Using the Emond human PBPK model, the initial (peak) blood TCDD concentrations associated with the accident were back-calculated based on the time that had elapsed between the explosion and the serum collection. As serum measurements were taken within 1 year after the event, a lag time of 0.5 years was assumed.
2. The oral exposure associated with the peak blood TCDD concentration (peak exposure) was calculated using the Emond PBPK model.
3. Starting with the peak exposure and accounting for background TCDD intake, the average daily blood TCDD concentration experienced by a representative individual in the susceptible population (boys under 10 years old) was estimated using the Emond PBPK model. Assuming a uniform distribution of subject ages at the time of the event, the average age of the exposed male children would be 5 years. Consequently, a critical exposure window for the cohort was estimated to be, on average, 5 years (i.e., a boy aged 5 years would remain in this exposure window for 5 more years until he was 10 years of age).
4. Using the Emond PBPK model, the average daily TCDD intake rate needed to attain the 5-year average blood TCDD concentration in a boy 10 years old was calculated.
5. The LOAEL POD was calculated as the average of the peak exposure (0.032 ng/kg-day) and the 5-year average exposure (0.0080 ng/kg-day), resulting in LOAEL of 0.020 ng/kg-day, shown in Table 4-1 as a candidate POD for derivation of a candidate RfD. However, neither of the extremes was used because (1) the peak exposure does not account for the continuing internal exposure from TCDD given its slow elimination, and (2) the 5-year average does not reflect the influence of the much higher peak exposure, which may be a significant factor in TCDD toxicity (Kim et al., 2003, 199146).

The PBPK modeling details are shown in Appendix D.

4.2.3.3. *Alaluusua et al. (2004, 197142)*

For Alaluusua et al. (2004, 197142), the approach for estimation of daily oral TCDD intake is virtually identical to the approach used for the Mocarelli et al. (2008, 199595) data. (See Section 2.4.1.2.1.5.5 for study details.) Alaluusua et al. (2004, 197142) reported dental effects in male and female adults who were less than 5 years of age at the time of the initial exposure (1976). For the 75 boys and girls who were less than 5 years old at the time of the accident, 25 (33%) were subsequently diagnosed with some form of dental enamel defect. For the 38 individuals who were older than 5, only 2 (5.3%) suffered dental enamel defects at a later date. A window of susceptibility of approximately 5 years is established. Serum measurements for this cohort were taken within a year of the accident. Serum TCDD levels and corresponding responses were reported by tertile, with a reference group of less-exposed individuals assigned a

TCDD LASC value of 15 ppt (ng/kg); the tertile group means were 130, 383, and 1,830 ppt. The incidence of dental effects for the reference group was 26% (10/39). The incidence of dental effects in the 1st, 2nd and 3rd tertile exposure groups was 10% (1/10), 45% (5/11) and 60% (9/15), respectively. EPA judged that the NOAEL and LOAEL were 130 and 383 ppt TCDD in serum. Following the same procedure used for the Mocarelli et al. (2008, 199595) study (see Section 4.2.3.2), EPA estimated the continuous daily human oral TCDD intake associated with each of the tertiles for both peak and average exposure across the critical exposure window, assuming that the average age of the susceptible cohort at the time of the accident was 2.5 years. Separate estimates for boys and girls were developed based on both the peak intake and average intake across the critical exposure window (PBPK modeling details are shown in Appendix D). The estimated averaged daily oral intakes for the tertiles, averaged for boys and girls, are 0.20, 1.7, and 30 ng/kg-day for the peak exposure and 0.033, 0.15 and 1.5 ng/kg-day for the critical exposure window average. A study NOAEL at the second tertile of 0.12 ng/kg-day was identified as a candidate POD for derivation of a candidate RfD in Table 4-1.

4.2.3.4. *Eskenazi et al. (2002, 197168)*

The approach used to estimate daily TCDD intake in Eskenazi et al. (2002, 197168) combines the approaches EPA used for Baccarelli et al. (2008, 197059), Mocarelli et al. (2008, 199595) and Alaluusua et al. (2004, 197142). Eskenazi et al. (2002, 197168) reported menstrual effects in female adults who were premenarcheal in 1976 at the time of the initial exposure (see Section 2.4.1.2.1.4.1 for study details). In Rigon et al. (2009), the median age at menarche was shown to be 12.4 in Italian females with intergenerational decreases in age at menarche. Thus, EPA established a window of susceptibility of approximately 13 years for this analysis. The average age of the premenarcheal girls at the time of the initial exposure in 1976 was 6.8 years, establishing an average critical-window exposure duration of 6.2 years for this cohort. Serum samples were collected within a year of the accident from this cohort. However, serum TCDD levels and corresponding responses were not reported by percentile and no internal reference group was identified. As for Baccarelli et al. (2008, 197059), Eskenazi et al. (2002, 197168) developed a regression model relating menstrual cycle length to plasma TCDD concentrations (LASC) measured in 1976. The model estimated that menstrual cycle length was increased 0.93 days for each 10-fold increase in TCDD LASC, with a 95% confidence interval of −0.01 to

1.86 days. EPA judged a 1-day increase in menstrual cycle length to be adverse; a normal menstrual cycle length is 28 days. EPA then determined the 1976 TCDD serum level corresponding to a 29-day menstrual cycle length in the exposed cohort from the regression model developed by Eskenazi et al. (2002, 197168). Using this serum level, the peak initial exposure and average exposure over the 6.2 year window were calculated using the Emond human PBPK model, in the same manner as for Mocarelli et al. (2008, 199595) and Alaluusua et al. (2004, 197142). The resulting peak TCDD intake is 3.2 ng/kg-day. The average exposure experienced by this cohort over the critical exposure window is estimated to be 0.12 ng/kg-day. The average of these two estimates is 1.64 ng/kg-day, which is designated as a LOAEL and shown in Table 4-1. Because the LOAEL is almost 2 orders of magnitude higher than the LOAELs for Baccarelli et al. (2008, 197059) and Mocarelli et al. (2008, 199595), it was not considered further as a candidate POD for derivation of the RfD (PBPK modeling details are shown in Appendix D).

4.2.4. Noncancer Dose-Response Assessment of Animal Bioassay Data

EPA followed the strategy illustrated in Figure 4-2 to evaluate the animal bioassay data for TCDD dose-response. For the administered average daily doses (ng/kg-day) in each animal bioassay, EPA identified NOAELs and/or LOAELs based on the original data presented by the study author. Section 2.4.2 identifies these values in the study summaries and in Table 2-7. These became candidate PODs for consideration in the derivation of an RfD for TCDD. The candidate RfD values associated with these candidate PODs are presented in Table 4-5. Additional PODs were identified using BMD modeling. All PODs were converted to HEDs using the Emond PBPK models. The remainder of this Section describes the steps in this process and concludes with the POD candidates from the animal bioassay data that were considered for derivation of the RfD.

4.2.4.1. *Use of Kinetic Modeling for Animal Bioassay Data*

Blood concentrations corresponding to the administered doses in each mouse or rat bioassay qualifying as a final RfD POD candidate were estimated using the appropriate Emond rodent PBPK model. In each case, the simulation was performed using the exposure and observation durations, body weights, and average daily doses from the original studies. For all

multiple exposure protocols, the time-weighted average blood TCDD concentrations over the exposure period were used as the relevant dose metric. For single (gestational and nongestational) exposures, the initial peak blood TCDD concentrations were considered to be the most relevant exposure metric. Gestational exposures were modeled using the species-specific gestational component of the Emond rodent PBPK model. Bioassays employing exposure protocols spanning gestational and postpartum life stages were modeled by sequential application of the gestational and nongestational models.

The Emond PBPK models do not contain a lactation component, so exposure during lactation was not modeled explicitly. Only one bioassay (Shi et al., 2007, 198147) considered as a POD candidate for RfD derivation included exposure during lactation. In Shi et al. (2007, 198147) pregnant animals were exposed weekly to TCDD throughout gestation and lactation. Exposure was continued in the offspring following weaning for 10 months. For assessment of maternal effects, the Emond gestational model was used, terminating at parturition. For assessment of long-term exposure in the offspring, the Emond nongestational model was used, ignoring prior gestational and lactational exposure, with the assumption that the total exposure during these periods was small relative to exposure in the following 10 months. The assumption is conservative in that effects observed in the offspring would be attributed entirely to adult exposure, which is somewhat less than the actual total exposure.

The model code, input files and PBPK modeling results for each bioassay are reported in Appendix C. These predicted TCDD blood concentrations were used for benchmark dose modeling of bioassay response data and determination of NOAELs and LOAELs. BMD modeling was performed, as described in Section 3.5.2.2.1, by substituting the modeled blood concentrations for the administered doses and calculating the corresponding BMDL. For each of these LOAEL, NOAEL, or BMDL blood-concentration equivalents, corresponding HEDs were calculated using the Emond human PBPK model for the appropriate gestational or nongestational scenario as described previously (see Section 4.2.2).

4.2.4.2. *Benchmark Dose Modeling of the Animal Bioassay Data*

Benchmark dose modeling was performed using BMDS 2.1, Build 06/16/09 to estimate BMDs and BMDLs for each study/endpoint combination. The input data tables for these noncancer studies are shown in Appendix E, Section E.1, including both administered doses

(ng/kg-day) and blood concentrations (ng/kg) and either incidence data for the dichotomous endpoints or mean and standard deviations for the continuous endpoints. (See Section 4.2.4.1 and Sections 3.3.4 and 3.3.5 for a description of the development of TCDD blood concentrations using kinetic modeling.)

Evaluation of BMD modeling performance, goodness-of-fit, dose-response data, and resulting BMD and BMDL estimates included statistical criteria as well as expert judgment of their statistical and toxicological properties. For the continuous endpoints, all available models were run separately using both the assumption of constant variance and the assumption of modeled variance. Saturated (0 degrees of freedom) model fits were rejected from consideration. Parameters in models with power or slope parameters were constrained to prevent supralinear fits, which EPA considers not to be biologically plausible and which often have undesirable statistical properties (i.e., the BMDL diverges towards zero). However, if the constrained parameters were estimated at their lower bounds, the unrestricted model was fit to the data, primarily for elucidation of the degree of supralinearity present in the data. Depending on the latter and the magnitude of the BMDL relative to the BMD, unrestricted model fits were occasionally deemed acceptable. Table 4-2 shows each model and any restrictions imposed.

For the quantal/dichotomous endpoints, all primary BMDS dichotomous models were run. The alternative dichotomous models were fit to several data sets, but the results were very sensitive to the assumed independent background response and the fits were not accepted. The confidence level was set to 95% and all initial parameter values were set to their defaults in BMDS. For the continuous endpoints, one standard deviation was chosen as the default for the BMR when a specific toxicologically-relevant BMR could not be defined. For the dichotomous endpoints, a BMR of 10% extra risk was used for all endpoints.[24]

The model output tables in Appendix E show all of the models that were run, both restricted and unrestricted, goodness-of-fit statistics, BMD and BMDL estimates, and whether bounds were hit for constrained parameters. After all models were run, the one giving the best fit was selected using the selection criteria in the current BMDS draft guidance (U.S. EPA, 2000, 052150) where possible. Acceptable model fits were those with chi-square goodness-of-fit *p*-values greater than 0.1. For continuous endpoints, a preference was held for models with an asymptote term (plateau for high-dose response) because continuous measures do not continue to

[24]There were no developmental studies that accounted for litter effects, for which a 5% BMR would be used.

rise (or fall) with dose forever; this phenomenon is particularly evident for TCDD. Unbounded models, such as the power model, must account for the plateauing effect entirely in the shape parameter, generally resulting in an abnormally supralinear fit. Also, for the continuous endpoints, the p-value for the homogenous variance test (Test 2) was used to determine whether constant variance ($p > 0.1$) or modeled (nonconstant) variance ($p < 0.1$) should be used. As BMDS offers only one variance model, model fits for nonconstant variance models were not necessarily rejected if the variance model did not fit well (Test 3 p-value < 0.05). Within the group of models with acceptable fits, the selected model was generally the one with the lowest BMDL, unless the AIC was much higher (ca. +2) than another model. However, particularly for continuous models, the fit of the model to the control mean and standard deviation and in the lower response range was assessed. Models with higher BMDLs or AICs but much better fit to the lower response data were often chosen over the nominally best-fitting model.

For many data sets, no models satisfied the acceptance criteria and no clear BMD/BMDL selection could be made. In theses cases, model fits were examined on an individual basis to determine the reasons for the poor fits. On occasion, high doses were dropped and the models were refit. Also, if a poor fit to the control mean was evident, the model was refit to the data after fixing the control mean by specifying the relevant parameter in BMDS. However, these techniques rarely resulted in better fits. If the fit was still not acceptable, the NOAEL/LOAEL approach was applied to the study/data set combination. Most of the problems with BMD modeling were a consequence of lack of response data near the BMR; many of the TCDD data sets failed to show a response near the BMR, whether it was a 10% dichotomous relative change or a continuous 1 standard deviation change. Responses at the lowest doses were generally much higher than the BMR, resulting in a lack of anchoring at the critical response levels of interest causing numerical problems in the estimation of BMDLs.

4.2.4.3. *POD Candidates from Animal Bioassays Based on HED and BMD Modeling Results*

Table 4-3 summarizes the PODs that EPA estimated for each key animal study included for TCDD noncancer dose-response modeling. After estimating the blood TCDD concentration associated with a particular toxicity measure (NOAEL, LOAEL, or BMDL) obtained from a rodent bioassay, EPA estimated a corresponding HED using the Emond human PBPK model (described in Section 3). Table 4-3 summarizes the NOAEL, LOAEL, or BMDL (ng/kg) based

on the administered animal doses for each key bioassay/data set combination. Table 4-3 also summarizes the continuous daily HED corresponding to these administered doses as 1st order body burdens and as blood concentrations. The doses in Table 4-3 are defined as follows, all in units of ng/kg-day:

- Administered Dose NOAEL: Average daily dose defining the NOAEL for the test species in the animal bioassay
- Administered Dose LOAEL: Average daily dose defining the LOAEL for the test species in the animal bioassay
- Administered Dose BMDL: BMDL for the test species based on modeling of the administered doses from the animal bioassay
- First-Order Body Burden HED NOAEL: Average daily dose defining the NOAEL for humans derived from the animal bioassay using the first-order kinetics body-burden model
- First-Order Body Burden HED LOAEL: Average daily dose defining the LOAEL for humans derived from the animal bioassay using the first-order kinetics body-burden model
- First-Order Body Burden HED BMDL: Human-equivalent BMDL from BMD modeling of the animal bioassay data using first-order body burdens
- Blood Concentration HED NOAEL: Average daily dose defining the NOAEL for humans derived from the animal bioassay using the Emond human PBPK model
- Blood Concentration HED LOAEL: Average daily dose defining the LOAEL for humans derived from the animal bioassay using the Emond human PBPK model
- Blood Concentration HED BMDL: Human-equivalent BMDL from BMD modeling of the animal bioassay data using the Emond human PBPK model

An evaluation of key BMD analyses is presented in Table 4-4. Tables showing the best model fit for each study/endpoint combination and the associated BMD/BMDL are shown in Appendix E. As described above in Section 4.3.4.2, the BMD modeling was largely unsuccessful, primarily because of a lack of response data near the BMR, poor modeled representation of control values, or nonmonotonic responses yielding poor fits. The comments column in Table 4-4 lists reasons for poor results.

4.3. RfD DERIVATION

Table 4-5 lists all the studies and endpoints considered for derivation of the RfD. These studies were chosen from the entire list of candidate study/data set combinations (see Section 2.4.3) based on the toxicologic relevance of the endpoints and covering a range of the most conservative RfD candidates that includes three of the four human studies.[25] Figure 4-3 (exposure-response array) shows all of the endpoints listed in Table 4-5 graphically in terms of PODs in human-equivalent intake units (ng/kg-day). The human study endpoints are shown at the far left of the figure and the rodent endpoints are arranged by category to the right. (Note the two studies in guinea pigs were estimated using first-order body burden kinetics which are not directly comparable to the PODs based on the mouse, rat and human studies that were generated from the Emond PBPK model. There are no published models for TCDD disposition in guinea pigs and EPA did not develop one for this assessment.) Figure 4-4 demonstrates the same endpoints, arrayed by RfD value, showing the POD, applicable UFs and candidate RfD.

Table 4-5 illustrates the study, species, strain and sex, study protocol, and toxicologic endpoints observed at the lowest TCDD doses. The table also identifies the human-equivalent BMDLs (when applicable), NOAELs and LOAELs, as well as the composite UF that applies to the specific endpoint, and finally, the corresponding candidate RfD.[26] The NOAELS, LOAELs, and BMDLs are presented as HEDs, based on the assumption that blood concentration is the toxicokinetically-equivalent TCDD dose metric across species and serves as a surrogate for tissue concentration.[27] For rats and mice, these estimates relied on the two Emond PBPK models—one for the relevant rodent species and one for the human—as described previously (see Section 3.3.4.3). The two guinea pig studies that are included in Table 4-5 are given in HED units based on the first-order body burden model described in Section 3.3.4.2; there is currently no TCDD PBPK model for the guinea pig. The values listed for guinea pigs are not directly comparable to those for rats and mice but are probably biased low, as first-order body burden HED estimates for rats and mice are generally 2- to 5-fold lower than the corresponding PBPK model estimates. The LOAELs for the human studies also rely on the Emond PBPK model, as described in Sections 4.2.2 and 4.2.3.

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[25]The RfD derived from the study of Eskenazi et al. (2002, 197168) was outside the RfD range presented in Table 4-5.

[26]Extra significant digits are retained for comparison prior to rounding to one significant digit for the final RfD.

[27]The procedures for estimating HEDs based on TCDD blood concentration are described in the preceding section.

As is evident from the Table 4-5, very few NOAELs and even fewer BMDLs have been established for low-dose TCDD studies. BMD modeling was unsuccessful for all of the endpoints without a NOAEL, primarily because of the lack of dose-response data near the BMR (see discussion in Section 4.2). Therefore, the RfD assessment rests largely on evaluation of LOAELs to determine the POD.

The rows in Table 4-5 are arranged in order of increasing candidate RfD magnitude. Endpoints projected to occur at higher exposure levels are still considered for qualitative support of the effects shown in Table 4-5.

4.3.1. Toxicological Endpoints

As can be seen in Table 4-5, a wide array of toxicological endpoints has been observed following TCDD exposure, ranging from subtle developmental effects to overt chronic liver toxicity. Developmental effects in rodents include dental defects, delayed puberty in males, and several neurobehavioral effects. Reproductive effects reported in rodents include altered hormone levels in females and decreased sperm production in males. Immunotoxicity endpoints such as decreased response to SRBC challenge in mice and decreased delayed-type hypersensitivity response in guinea pigs are also observed. Longer durations of TCDD exposure in rodents elicit results such as organ and body weight changes, renal toxicity, and liver and lung lesions. Adverse effects in human studies are also observed, which include male reproductive effects, increased TSH in neonates, and dental defects in children. Analogous results have been observed in animal bioassays for each of these human endpoints.

All but two of the study/endpoint combinations from animal bioassays listed in Table 4-5 are on TCDD-induced toxicity observed in mice and rats; the other two study/endpoint combinations are effects in guinea pigs. Although the effects of TCDD have been investigated in several other species (i.e., hamsters, monkeys, and mink), those studies were not included for final POD consideration because the effect levels were greater than those in Table 4-5, or because the effects could not be attributed solely to TCDD exposure (i.e., confounding by dioxin-like compounds [DLCs]).

Three human studies were also included for final POD consideration in the derivation of an RfD and are presented in Table 4-5 as candidate RfDs. All three human study/endpoint combinations are from studies on the Seveso cohort. The developmental effects observed in

these studies were associated with TCDD exposures either in utero or in early childhood between 1 and 10 years of age. Baccarelli et al. (2008, 197059) reported increased levels of TSH in newborns exposed to TCDD in utero, indicating a possible dysregulation of thyroid hormone metabolism. Mocarelli et al. (2008, 199595) reported decreased sperm concentrations and decreased motile sperm counts in men who were 1–9 years old in 1976 at the time of the Seveso accident (initial TCDD exposure event). Alaluusua et al. (2004, 197142) reported dental effects in adults who were less than 9.5 years of age at the time of the initial exposure (1976).

4.3.2. Exposure Protocols of Candidate PODs

The studies in Table 4-5 represent a wide variety of exposure protocols, involving different methods of administration and exposure patterns across virtually all exposure durations and life stages. Both dietary and gavage administration have been used in rodent studies, with gavage being the predominant method. Gavage dosing protocols vary quite widely and include single gestational exposures, multiple daily exposures (for up to 2 weeks, intermittent schedules that include 5 days/week, once weekly, or once every 2 weeks), and loading/maintenance dose protocols, in which a relatively high dose is initially administered followed by lower weekly doses. The intermittent dosing schedules require dose-averaging over time periods as long as 2 weeks, which introduces uncertainty in the effective exposures. In other words, the high unit dose may be more of a factor in eliciting the effect than the average TCDD tissue levels over time. Although the loading/maintenance dose protocols are designed to maintain a constant internal exposure, these protocols are somewhat inconsistent with the constant daily TCDD dietary exposures associated with human ingestion patterns.

The epidemiologic studies conducted in the Seveso cohort represent exposures over different life stages including gestation, childhood, and young adulthood. The Seveso exposure profile is essentially a high initial pulse TCDD exposure followed by a 20–30 year period of elimination. Effects are realized, or measured, 10–20 years following the initial exposure; the critical exposure window for susceptibility varies with effect and is often unknown. Therefore, the effective exposure profiles for the Seveso cohort studies vary considerably. For the Mocarelli et al. (2008, 199595) and Alaluusua et al. (2004, 197142) studies where early childhood exposures proximate to the initial event are associated with the outcomes, there is some uncertainty as to the magnitude of the effective doses. Although the effects are associated

with TCDD exposure in the first 10 years of life, it is not clear to what extent the initial peak exposure is primarily responsible for the effects. It is also not clear if averaging exposure over the critical window is appropriate given the large difference between initial TCDD body burden and body burden at the end of the critical exposure window. The LOAELs for both Mocarelli et al. (2008, 199595) and Alaluusua et al. (2004, 197142) are calculated as the average of the peak exposure and average exposure across the critical exposure window (see Section 4.2 for details).

For the gestational exposure study (Baccarelli et al., 2008, 197059), the critical exposure window is strictly defined and relatively short (9 months) and occurs long after the initial exposure (15−20 years). In addition, the maternal serum TCDD concentrations were measured 10−15 years after the initial exposure and are proximate to the actual pregnancies; consequently, there is less uncertainty in the kinetic extrapolation between time of measurement and time of birth (i.e., the critical exposure window). The narrow critical exposure window at a much later time than the initial exposure (where the TCDD elimination curve is flattening) is assumed to lead to a relatively steady-state exposure over the critical time period with much less uncertainty in the magnitude of the effective dose. With the exception of Eskenazi et al. (2002, 197168) (see Section 4.2), the effective doses for other effects reported for the Seveso cohort (see Section 2.4.1.1.1.4) have not been quantified and are not represented in Table 4-5 because no critical exposure windows can be identified or individual exposure estimates were not reported.

4.3.3. Uncertainty Factors (UFs)

The UF column in Table 4-5 shows the composite (total) UF that would be applied to the POD for each endpoint. For the animal bioassays, a UF of 3 for the toxicodynamic component of the interspecies extrapolation factor (UF_A) was applied to all PODs. For both animal and human studies, when a NOAEL was used as the POD, a factor of 10 was applied for human interindividual variability (UF_H). For all of the animal bioassay endpoints lacking a NOAEL, a UF of 10 for the LOAEL-to-NOAEL UF (UF_L) was included. For the human LOAELs, a UF_L of 3 was applied because sensitive populations were identified. A subchronic-to-chronic UF (UF_S) of 1 and a database factor (UF_D) of 1 are applied to all endpoints. A rationale for each UF is provided for the derivation of the RfD below.

4.3.4. Choice of Human Studies for RfD Derivation

For selection of the POD, the human studies are given the highest consideration, as quality human data are always preferred by the EPA to animal data of comparable quality. The human studies included in Table 4-5 (Alaluusua et al., 2004, 197142; Baccarelli et al., 2008, 197059; Mocarelli et al., 2008, 199595) each evaluate a segment of the Seveso civilian population (i.e., not an occupational cohort) exposed directly to TCDD released from an industrial accident. (The identification of PODs from these studies is detailed in Sections 4.3.4.1, 4.3.4.2, and 4.3.4.3.) Thus, exposures were primarily to TCDD, the chemical of concern, with apparently minimal DLC exposures beyond those associated with background intake,[28] making these studies highly appropriate for use in RfD derivation for TCDD. In addition, health effects associated with TCDD exposures were observed in humans, the species of concern whose health protection is represented by the RfD, eliminating the uncertainty associated with interspecies extrapolation. The cohort members who were evaluated included infants (exposed in utero) and adults who were exposed when they were less than 10 years of age. These studies considered together associate TCDD exposures with health effects in potentially vulnerable population subgroups. Their inclusion among the RfDs derived also may characterize noncancer health effects associated with TCDD exposures in potentially vulnerable populations, thus accounting for some part of the intraspecies uncertainty in the RfD. Finally, the two virtually identical RfDs from different endpoints in different studies provide an additional level of confidence in the use of these data for derivation the RfD for TCDD.

Although the human data are preferred, Table 4-5 presents a number of animal studies with RfDs that are lower than the human RfDs. To a large extent, this is expected because a 10-fold interspecies uncertainty factor is generally used to extrapolate from test-animal species to humans, intended to provide a conservative estimate of an RfD that would be derived directly from human data. Two of the rat bioassays among this group of studies—Bell et al. (2007, 197041; RfD = 1.4E−9 mg/kg day based on delay in the onset of puberty) and NTP (2006, 197605; RfD = 4.6E−10 mg/kg day based on liver and lung lesions)—are of particular note. Both studies were recently conducted. Both were very well designed and conducted, using 30 or

[28]As an example, note the lack of statistically significant effects reported by Baccarelli et al. (2008, 197059; Figure 2 C and D) in regression models based on either maternal plasma levels of noncoplaner PCBs or total TEQ on neonatal TSH levels.

more animals per dose group (see Table 4-6 for a discussion of these studies' strengths and weaknesses); both also are consistent with and, in part, have helped to define the current state of practice in the field. Bell et al. (2007, 197041) evaluated several reproductive and developmental endpoints, initiating TCDD exposures well before mating and continuing through gestation. NTP (2006, 197605) is the most comprehensive evaluation of TCDD chronic toxicity in rodents to date, evaluating dozens of endpoints at several time points in all major tissues. Thus, proximity of the RfDs derived from these two high quality, recent studies provide additional support for the use of the human data for RfD derivation.

There are several animal bioassay candidate RfDs at the lower end of the RfD range in Table 4-5 that are more than 10-fold below the human-based RfDs. Two of these studies report effects that are analogous to the endpoints reported in the three human studies and support the RfDs based on human data. Specifically, decreased sperm production in Latchoumydandane and Mathur (2002, 197498) is consistent with the decreased sperm counts and other sperm effects in Baccarelli et al. (2008, 197059), and missing molars in Keller et al. (2007, 198526; 2008, 198531; 2008, 198033) are similar to the dental defects seen in Alaluusua et al. (2004, 197142). Thus, because these endpoints have been associated with TCDD exposures in humans, these animal studies would not be selected for RfD derivation in preference to human data showing the same effects.

Another characteristic of the remaining studies in the lower end of the candidate RfD distribution is that they are dominated by mouse studies (comprising 6 of the 8 lowest rodent-based RfDs). EPA considers the candidate RfD estimates based on mouse data to be much more uncertain than either the rat or human candidate RfD estimates. The EPA considers the Emond mouse PBPK model to be the most uncertain of toxicokinetic models used to estimate the PODs because of the lack of key mouse-specific data, particularly for the gestational component (see Section 3.3.4.3.2.5). The $LOAEL_{HED}$s identified in mouse bioassays are low primarily because of the large toxicokinetic interspecies extrapolation factors used for mice, for which there is more potential for error. The ratio of administered dose to HED (D_a:HED) ranges from 65 to 1,227 depending on the duration of exposure. The D_a:HED for mice is, on average, about four times larger than that used for rats. In addition, each one of the mouse studies has other qualitative limitations and uncertainties (discussed above and in Table 4-6) that make them less desirable candidates as the basis for the RfD than the human studies.

4.3.4.1. *Identification of POD from Baccarelli et al. (2008, 197059)*

Baccarelli et al. (2008, 197059) reported increased levels of TSH in newborns exposed to TCDD in utero, indicating a possible dysregulation of thyroid hormone metabolism. The study authors related TCDD concentrations in neonatal blood to TSH levels, reporting group mean TCDD concentrations associated with TSH levels above or below 5 μ-Units TSH per mL of serum (5 μU/mL).

The World Health Organization (WHO, 1994) established the 5 μU/mL standard as an indicator of potential iodine deficiency and potential thyroid problems in neonates. Increased TSH levels are indicative of decreased thyroid hormone (T4 and/or T3) levels. The 5 μU/mL "cutoff" for TSH measurements in neonates was recommended by WHO (1994) for use in population surveillance programs as an indicator of iodine deficiency disease (IDD). In explaining this recommendation, WHO (1994) stated that:

> "While further study of iodine replete populations is needed, a cutoff of 5μU/ml whole blood… may be appropriate for epidemiological studies of IDD [iodine deficiency disease.] Populations with a substantial number of newborns with TSH levels above the cutoff could indicate a significant IDD problem."

For TCDD, the toxicological concern is not likely to be iodine uptake inhibition, but rather increased metabolism and clearance of T4, as evidenced in a number of animal studies (e.g., Seo et al., 1995, 197869). Clinically, a TSH level of >4 μU/mL in a pregnant woman is followed up by an assessment of free T4, and treatment with L-thyroxine is prescribed if T4 levels are low (Glinoer and Delange, 2000). This is to ensure a sufficient supply of T4 for the fetus, which relies on maternal T4 exclusively during the 1st half of pregnancy (Chan et al., 2005; (Calvo et al., 2002, 051690; Morreale et al., 2000, 019231).

Adequate levels of thyroid hormone also are essential in the newborn and young infant as this is a period of active brain development (Glinoer and Delange, 2000; Zoeller and Rovet, 2004). Smaller reserves, higher demand, and shorter half-life of thyroid hormones in newborns and young infants also could make this population more susceptible to the impact of insufficient levels of T4 (Savin et al., 2003(Greer et al., 2002, 051202; Van Den et al., 1999, 016478).

Thyroid hormone disruption during pregnancy and in the neonatal period can lead to neurological deficiencies. However, the exact relationship between TSH increases and adverse

neurodevelopmental outcome is not well defined. A TSH level above 20 μU/L in a newborn infant is cause for immediate intervention to prevent mental retardation, often caused by a malformed or ectopic thyroid gland in the newborn (Glinoer and Delange, 2000; Rovet, 2002; WHO, 2007). Recent epidemiological data indicate concern for even lower level thyroid hormone perturbations during pregnancy. For example, Haddow et al. (1999, 002176) reported that women with subclinical hypothyroidism, with a mean TSH of 13.2 μU/L had children with IQ deficits of up to 4 IQ points on the Wechsler IQ scale. Neonatal TSH within the first 72 hours of birth (as was evaluated by Baccarelli et al., 2008, 197059)is a sensitive indicator of both neonatal and maternal thyroid status (DeLange et al., 1983). Animal models have recently indicated that very modest perturbations in thyroid status for even a relatively short period of time can lead to altered brain development (e.g., Auso et al., 2004; Lavado-Autric et al., 2003; Sharlin et al., 2008, 2010; Royland et al., 2008).

Baccarelli et al. (2008, 197059) discount iodine status in the population as a confounder, as exposed and referent populations all lived in a relatively small geographical area. It is unlikely that there was iodine deficiency in one population and not in the other population based on iodine levels in the soil.

Baccarelli et al. (2008, 197059) also showed, in graphical form, how the TSH distribution in each of three categorical exposure groups (reference, zone A, and zone B—representing increasing TCDD exposure) shifted to higher TSH values with increasing exposure. The individuals comprising the above 5 μU/mL group were from all three categorical exposure groups, not just from the highest exposure group. Therefore, EPA was able to designate a LOAEL independently of the nominal categorical exposure groups; the LOAEL is designated as the group mean of 39 ppt TCDD in neonatal plasma as a LOAEL for TSH values above 5 μU/mL. Using the Emond human PBPK model, the daily oral intake at the LOAEL is estimated to be 0.024 ng/kg-day (see Section 4.2.3.1). A NOAEL is not defined because it is not clear what maternal intake should be assigned to the group below 5 μU/mL.

4.3.4.2. *Identification of POD from Mocarelli et al. (2008, 199595)*

Mocarelli et al. (2008, 199595) reported decreased sperm concentrations (20%) and decreased motile sperm counts (11%) in men who were 1−9 years old in 1976 at the time of the Seveso accident (initial TCDD exposure event). The sperm concentrations and motile sperm

counts in men who were 10−17 years old in 1976 were not affected. Serum (LASC) TCDD levels were measured within one year of the initial exposure. Serum TCDD levels and corresponding responses were reported by quartile, with a reference group of less-exposed individuals assigned a TCDD LASC value of 15 ppt (which was the mean of the TCDD LASC reported in individuals outside the contaminated area). The lowest exposed group mean was 68 ppt (1st-quartile). Mean sperm concentrations and motile sperm counts were reduced about 20% from the reference group. Further decrease in these values in the groups exposed to more than 68 ppt was slight and reached a maximum of about 33%.

Although a decrease in sperm concentration of 20% likely would not have clinical significance for an individual EPA's concern with the reported decreases in sperm concentration and total number of motile sperm (relative to the comparison group) is that such decreases associated with TCDD exposures could lead to shifts in the distributions of these measures in the general population. Such shifts could result in decreased fertility in men at the low end of these population distributions. While there is no clear cut-off indicating male fertility problems for either of these measured effects. A sperm concentration of 20 million/ml is typically used as a cut-off by clinicians to indicate follow-up for potential reproductive impact in affected individuals. Low sperm counts are typically accompanied by poor sperm quality (morphology and motility). For fertile men, between 50% and 60% of sperm are motile (Swan et al., 2003; Slama et al., 2002; Wijchman et al., 2001). Any impacts on these reported levels could become functionally significant.

For the 22−31 year-old men exposed to TCDD as a consequence of the Seveso accident, the mean total sperm concentration was reported by Mocarelli et al. (2008, 199595) to be 53.6 million/ml, with a value of 21.8 million/ml at one standard deviation below the mean. In the comparison group that consisted of men not exposed to TCDD by the Seveso explosion and of the same age as the exposed men, the mean total sperm concentration was 72.5 million/ml (31.7 million/ml at one standard deviation below the mean). In the group exposed due to the Seveso accident, individuals one standard deviation below the mean are just above the cut-off used by clinicians, indicating a that a number of individuals in the exposed group likely had sperm concentrations less than 20 million/ml; EPA could not obtain the individual data to determine the exact number of men in this category. EPA judged that the impact on sperm

concentration and quality reported by Mocarelli et al. (2008, 199595) is biologically significant given the potential for functional impairment.

EPA has designated the lowest exposure group (68 ppt) as a LOAEL, which translates to a continuous daily oral intake of 0.020 ng/kg-day (see Section 4.2.3.2). The reference group is not designated as a NOAEL because there is no clear zero-exposure measurement for any of these endpoints, particularly considering the contribution of background exposure to DLCs, which further complicates the interpretation of the reference group response as a true "control" response (see discussion in Section 4.4). However, males less than 10 years old can be designated as a sensitive population by comparison to older males who were not affected.

4.3.4.3. *Identification of POD from Alaluusua et al. (2004, 197142)*

Alaluusua et al. (2004, 197142) reported dental effects in male and female adults who were less than 9.5 years of age, but not older, at the time of the initial exposure (1976) in Seveso. EPA used the same approach to estimate daily TCDD intake as was used for the Mocarelli et al. (2008, 199595) data; a window of susceptibility of about 5 years was established. Serum measurements for this cohort were taken within a year of the accident. Serum TCDD levels and corresponding responses were reported by tertile, with a reference group of less-exposed individuals assigned a TCDD LASC value of 15 ppt (ng/kg); the tertile group means were 130, 383, and 1,830 ppt. Both a NOAEL and LOAEL can be defined for this study. The NOAEL is 0.12 ng/kg-day, corresponding to the TCDD LASC of 130 ppt at the first tertile. The LOAEL is 0.93 ng/kg-day at the second tertile. The children in this cohort less than 5 years old can be designated as a sensitive population by comparison to older individuals who were not affected relative to the reference group.

4.3.5. Derivation of the RfD

The two human studies, Baccarelli et al. (2008, 197059) and Mocarelli et al. (2008, 199595), have similar LOAELs of 0.024 and 0.020 ng/kg-day, respectively. Together, these two studies constitute the best foundation for establishing a POD for the RfD, and are designated as coprincipal studies. Therefore, increased TSH in neonates in Baccarelli et al. (2008, 197059) and male reproductive effects (decreased sperm count and motility) in Mocarelli et al. (2008, 199595) are designated as cocritical effects. Although the exposure estimate used in

determination of the LOAEL for Mocarelli et al. (2008, 199595) is more uncertain than the Baccarelli et al. (2008, 197059) exposure estimate, the slightly lower LOAEL of 0.020 ng/kg-day from Mocarelli et al. is designated as the POD. A composite UF of 30 is applied to account for lack of a NOAEL ($UF_L = 10$) and human interindividual variability ($UF_H = 3$); the resulting RfD in standard units is 7×10^{-10} mg/kg-day. Table 4-7 presents the details of the RfD derivation.

4.4. UNCERTAINTY IN THE RfD

Exposure assessment is a key limitation of the epidemiologic studies (of the Seveso cohort) used to derive the RfD. The Seveso cohort exposure profile consists of an initial high dose followed by a drop in body burden to background levels over a period of about 20 years, at which time the effects were observed. This exposure scenario is a mismatch with the constant daily intake scenario addressed by the RfD methodology. The determination of an effective average daily dose from the Seveso exposure scenario requires an understanding of the critical time-window of susceptibility and the influence of the peak exposure on the occurrence of the observed effects, particularly when the peak exposure is high relative to the average exposure over the critical exposure window. For one of the principal studies (Mocaelli et al., 2008, 199595), a maximum susceptibility exposure window can be identified based on the age of the population at risk. However, the influence of the peak exposure on the effects observed 20 years later is unknown and the biological significance of averaging the exposure over several years, with internal exposure measures spanning a 4.5-fold range, is unknown. EPA, in this assessment, has averaged intermittent exposures for rodent bioassays over weekly dosing intervals, but the peak and average body burdens varied by less than 50%. EPA has not developed guidance for larger-interval averaging. Furthermore, because there is an assumption of a threshold level of exposure below which the effects are not expected to occur, averaging over large intervals could include below-threshold exposures. The process used by EPA to estimate the LOAEL exposure for the Mocarelli study is a compromise between the extremes; as such, there is some uncertainty in the estimate, perhaps in the range of 3- to 10-fold in either direction. This uncertainty also holds for the LOAEL determined for the dental effects reported in Alaluusua et al. (2004, 197142) and the increased menstrual cycle length reported in Eskenazi et al. (2002, 197168 see Section 4.2.3.4); in both of those studies, the uncertainty is greater, as

the difference between peak and average internal exposures is an order of magnitude or more. The LOAEL for increased TSH in neonates (Baccarelli et al., 2008, 197059), however, is less uncertain because the critical exposure window is much narrower (9 months) and the developmental exposures occurred 10 to 15 years after the initial exposure, when internal TCDD concentrations for the pregnant women likely were leveling off; that is, exposure over the critical window was more constant and estimation of the relevant exposures was less uncertain. However, there is some uncertainty in the magnitude of the exposures because they were estimated from measurements in sera taken several years prior to pregnancy.

Another source of uncertainty using human epidemiologic data is the lack of completely unexposed populations. The available TCDD epidemiologic data were obtained by comparing populations that experienced elevated TCDD exposures to populations that experienced lower exposures, rather than to a population with no TCDD exposure. An additional complicating factor is coexposure to DLCs, which can behave in the same way as TCDD. Although the accidental exposure to the Seveso women's cohort was primarily to TCDD, background exposure was largely to DLCs.[29] Eskenazi et al. (2004, 197160) reported that TCDD comprised only 20% of the total toxicity equivalence (TEQ) in the serum of the reference group that was not exposed as a result of the factory explosion, which implies that the effective background TEQ exposure was approximately 5-fold higher.

The higher background exposure could be significant at the lower TCDD exposure levels, with the effect diminishing as TCDD exposure increased. For dose-response modeling, the effect of a higher background dose (i.e., total TEQ), if included, would be to shift the response curve to the right (responses associated with higher exposures) but, primarily, would reduce the spread of the exposures, which would tend to alter the shape of the dose response towards sublinear. Both the right shift and the more sublinear shape would result in higher ED_x estimates, such as BMDs and BMDLs, from fitting dose-response models. However, for determination of a LOAEL, which is the case for all the human studies in Table 4-5, the impact may be minimal, as the LOAEL depends only on establishing that an effect of sufficient

[29]Moccarelli (2001, 197002) reported the release from the Seveso plant to contain a mixture of TCDD, ethylene glycol and sodium hydroxide. As these chemicals are not thought to persist in the environment or in the body, coexposure to these additional contaminants along with TCDD would not have a significant impact on longer-term TCDD dose-response. For acute exposure, male reproductive or thyroid hormone effects are not evident for ethylene glycol (U.S. EPA, 2009, 192196). It is unlikely that sodium hydroxide, being primarily a caustic agent, would cause these effects.

magnitude was observed at some TCDD exposure level. In this case, the effect of the increased effective background exposure would be to inflate the "control" (zero-TEQ) response, providing the threshold for the response had been exceeded. The potential impact of an inflated control response would be to mask a significant effect of the added TCDD exposure, when the latter effect is determined by comparison to the reference group response. To compensate for this, EPA has been somewhat conservative in interpreting the magnitude of responses defining LOAELs for the Seveso cohort studies. The actual magnitude of the impact of the DLC background exposure is impossible to assess without knowing the true (TEQ-free) background response.

A primary strength of the TCDD database is that analogous effects have been observed in animal bioassays for most of the human endpoints, increasing the overall confidence in the relevance to humans of the effects reported in rodents and the association of TCDD exposure with the effects reported in humans. Table 4-5 shows that low dose TCDD exposures are associated with a wide array of toxicological endpoints in rodents including developmental effects, reproductive effects, immunotoxicity and chronic toxicity. Effects reported in human studies are similar, including male reproductive effects, increased TSH in neonates and dental defects in children; other human health effects such as female reproductive effects and chloracne have been observed at higher exposures (see Section 2.4.1). Other effects reported in rodent studies such as liver toxicity and overt immunological endpoints have not been reported in human studies. However, with respect to immunological effects, Baccarelli et al. (2002, 197062; 2004, 197045) evaluated immunoglobin and complement levels in the sera of TCDD-exposed individuals from the Seveso cohort and found slightly reduced immunoglobulin in the highest exposure groups but no effect on other immunoglobulins or on C3 or C4 complement levels. The latter finding indicates that at least one immunological measure in humans is not a sensitive endpoint, as it is for mice, with large reductions in serum complement at low exposure levels (White et al., 1986, 197531).

Although there is a substantial amount of qualitative concordance of effects between rodents and humans, quantitative concordance is not evident in Table 4-5. The differential sensitivity of mice and humans for the serum complement endpoint is one example. Other examples of differential sensitivity are developmental dental effects and thyroid hormonal dysregulation. Developmental dental defects are relatively sensitive effects in rodents, appearing

at exposure levels in mice (Keller et al., 2007, 198526; Keller et al., 2008, 198531; Keller et al., 2008, 198033) more than an order of magnitude lower than effect levels in humans (Alaluusua et al., 2004, 197142). In contrast, thyroid hormone effects are seen in rats (Crofton et al., 2005, 197381) at 30-fold higher exposures than for humans (Baccarelli et al., 2008, 197059). Male reproductive effects (sperm production) occur in rats (Latchoumycandane and Mathur, 2002, 197498) and humans (Mocaelli et al., 2008, 199595) at about the same dose. To what extent these differential sensitivities depend on specifics of the comparison, such as species (mouse vs. rat), life-stage (e.g., fetal vs. adult), endpoint measure (e.g., thyroxine [T4] vs. TSH) or magnitude of the lowest dose tested, cannot be determined, so strong conclusions about quantitative concordance cannot be made.

A number of qualitative strengths and limitations/uncertainties are associated with the top animal bioassays listed in Table 4-5, as articulated in Table 4-6. Considering the issue of lowest tested dose, the general lack of NOAELs and acceptable BMDLs is a primary weakness of the rodent bioassay database. None of the 6 most sensitive rodent studies in Table 4-5, spanning a 30-fold range of LOAELs, had defined NOAELs or BMDLs. NOAELs or BMDLs were established for only 4 of the next 10 rodent studies. In addition, many of these LOAELs are characterized by relatively high responses with respect to the control population, so it is not certain that a 10-fold lower dose (based on the application of UF_L of 10) would be approximately equivalent to a NOAEL. A major reason for the failure of BMD modeling was that the responses were not "anchored" at the low end (i.e., first response levels were far from the BMR [see Table 4-4]). Another major problem with the animal bioassay data was nonmonotone and flat response profiles. The small dose-group sizes and large dose intervals probably contributed to many of these response characteristics that prevented successful BMD modeling. Larger study sizes with narrower dose intervals at lower doses are still needed to clarify rodent response to TCDD.

Lower TCDD doses have been tested in rodents but almost entirely for investigation of specialized biochemical endpoints[30] that EPA does not consider to be adverse health effects (see Appendix G). There is, however, a fundamental limit to the lowest dose of TCDD that can be tested meaningfully, as TCDD is present in feed stock and accumulates in unexposed animals prior to the start of any study. This issue is illustrated by the presence of TCDD in tissues of

[30]Enzyme induction, oxidative stress indicators, mRNA levels, etc.

unexposed control animals, often at significant levels relative to the lowest tested dose in low dose studies (Bell et al., 2007, 197041; Ohsako et al., 2001, 198497) (Vanden Heuvel et al., 1994, 594318, see Text Box 4-1). Some DLCs also have been measured in animal feeds and are anticipated to accumulate in unexposed test animals further complicating the interpretation of low dose studies.

Text Box 4-1. Background levels of TCDD in Control Group Animals

TCDD tissue levels in control animals are rarely reported either explicitly or implicitly. Vanden Heuvel et al. (1994, 197551), however, reported TCDD concentrations in livers of control animals (10-week-old female Sprague-Dawley rats) of 0.43 ppt (ng/kg) compared to 0.49 ppt in the livers of animals given a single oral TCDD dose of 0.1 ng/kg. Assuming proportionality of liver concentration to total body burden, the body burden of untreated animals was 87.8% of that of treated animals. The equivalent administered dose for untreated animals (d_0) can be calculated as equal to $0.878 \times (0.1 + d_0)$, assuming proportionality of body burden to administered dose and that all animals started with the same TCDD body burdens. The calculation yields a value of 0.72 ng/kg for d_0, which represents the accumulated TCDD from all sources in these animals prior to being put on and during test. This value would raise the nominal 0.1 ng/kg TCDD dose 8-fold to 0.82 ng/kg. The next higher dose of 1 ng/kg would be nearly doubled to 1.72 ng/kg. The impact on higher doses would be negligible, because the ratio of treatment dose to apparent background exposure levels increases with higher treatment levels. Bell et al. (2007, 197041) reported slightly higher levels (0.66 ppt) in the livers of slightly older untreated pregnant female Sprague-Dawley rats (mated at 16−18 weeks of age and tested 17 days later).

Ohsako et al. (2001, 198497) reported TCDD concentrations in the fat of offspring of untreated pregnant Holtzman rats that were 46% of the TCDD fat concentrations in animals exposed in utero to 12.5 ng/kg (single exposure on GD 15). This level of TCDD would imply a very large background exposure, but quantitation based on simple kinetic assumptions probably would not reflect the more complicated indirect exposure scenario

Bell et al. (2007, 197041) also reported concentrations of 0.1 and 0.6 ppt TCDD measured in two samples of feed stock. Assuming that the average of 0.35 ppt is representative of the entire supply of feed stock and a food consumption factor of 10% of body weight per day, the average daily oral exposure from feed to these animals would be 0.035 ng/kg. Discrimination of outcomes from longer-term repeated exposures might be problematic at exposure levels around 0.1 ng/kg-day. Background exposure was not much of an issue for Bell et al. (2007, 197041), as the lowest TCDD exposure level was 2.4 ng/kg-day (28-day dietary exposure).

NTP (2006, 543749) reported TCDD concentrations in the liver and fat of untreated female S-D rats after 2 years on test that were 1% and 2.5% of the levels in the liver and fat of the low-dose TCDD treatment group (2.14 ng/kg-day; (NTP, 2006, 197605)), respectively. Assuming proportionality of fat concentration and oral intake, control animal exposure would have been approximately 0.05 ng/kg-day, similar to the estimate from Bell et al. (2007, 197041). As for the latter study, background intake for the NTP (2006, 197605) study animals would not have a large effect on the dose-response assessment given the lowest exposure level of 2.14 ng/kg-day.

In all of these studies, except the 28-day exposure in Bell et al. (2007, 197041), control animals were gavaged with corn oil vehicle. TCDD concentrations in corn oil were not reported in any of the studies.

Table 4-1. POD candidates for epidemiologic studies of TCDD

Study	POD (ng/kg-day)	Critical effects
Alaluusua et al. (2004, 197142)	1.2E−01[a] (NOAEL)	Dental effects in adults exposed to TCDD in childhood
Baccarelli et al. (2008, 197059)	2.4E−02[b] (LOAEL)	Elevated TSH in neonates
Eskenazi et al. (2002, 197168)	1.64E+00[c] (LOAEL	Increased length of menstrual cycle in women exposed to TCDD in childhood
Mocarelli et al. (2008, 199595)	2.0E−02[d] (LOAEL)	Decreased sperm count and motility in men exposed to TCDD in childhood

[a]Mean of peak exposure (0.15 ng/kg-day) and average exposure over 10-year critical window (0.0093 ng/kg-day).
[b]Maternal exposure corresponding to neonatal TSH concentration exceeding 5 µU/mL.
[c]Mean of peak exposure (3.2 ng/kg-day) and average exposure over 10-year critical window (0.12 ng/kg-day).
[d]Mean of peak exposure (0.035 ng/kg-day) and average exposure over 10-year critical window (0.0078 ng/kg-day).

Table 4-2. Models run for each study/endpoint combination in the animal bioassay benchmark dose modeling

Model	Restrictions imposed
Continuous models	
Exponential M2-M5, not grouped	Adverse direction specified according to the response data; power ≥1
Hill	Adverse direction is automatic; $n > 1$
Linear	Adverse direction is automatic; degree of polynomial = 1
Polynomial	Adverse direction is automatic; degree of polynomial unrestricted; restrict the sign of the power to nonnegative or nonpositive, depending on the direction of the responses
Power	Adverse direction is automatic; power ≥1
Dichotomous models	
Gamma	Power ≥1
Logistic	None
Log-Logistic	Slope ≥1
Log-Probit	None
Multistage	Beta ≥0, 2nd degree polynomial
Probit	None
Weibull	Power ≥1

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Table 4-3. Summary of key animal study PODs (ng/kg-day) based on three different dose metrics: administered dose, first-order body burden HED, and blood concentration

Study	Endpoint	Administered dose[a]			1st-order body burden HED[b]			Blood concentration HED[c]		
		NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]
Amin et al. (2000, 197169)	Saccharin preference ratio, female	–	2.50E+01	5.10E+01	–	2.49E−02	5.08E−02	–	1.71E−01	3.20E−01
Bell et al. (2007, 197041)	Balano-preputial separation in male pups	–	2.40E+00	2.87E+00	–	1.26E−02	1.50E−02	–	8.83E−02	4.33E−02
Cantoni et al. (1981, 197092)	Urinary coproporhyrins	–	1.43E+00	1.25E−01	–	1.24E−02	1.09E−03	–	6.51E−02	1.60E−03
Chu et al. (2001, 521829)	Tissue weight changes	2.50E+02	1.00E+03	–	7.55E−01	3.02E+00	–	–	–	–
Chu et al., 2007	Liver lesions	2.50E+00	2.50E+01	–	7.55E−03	7.55E−02	–	3.56E−02	5.76E−01	–
Crofton et al. (2005, 197381)	Serum T4	3.00E+01	1.00E+02	3.01E+01	1.92E−02	6.40E−02	1.92E−02	1.72E−01	7.61E−01	1.40E−01
Croutch et al. (2005, 197382)	Decreased body weight	5.43E+01	2.17E+02	–	2.22E−01	8.89E−01	–	–	–	–
DeCaprio et al. (1986, 197403)	Decreased body weight	6.10E−01	4.90E+00	–	4.11E−03	3.30E−02	–	–	–	–
Fattore et al. (2000, 197446)	Decreased hepatic retinol	–	2.00E+01	–	–	1.23E−01	–	–	8.01E−01	–
Fox et al. (1993, 197344)	Increased liver weight	5.70E−01	3.27E+02	–	1.42E−03	8.12E−01	–	–	–	–
Franc et al. (2001, 197353)	Organ weight changes	1.00E+01	3.00E+01	1.59E+00	6.62E−02	1.99E−01	1.05E−02	4.60E−01	1.45E+00	3.37E−02
Franczak et al. (2006, 197354)	Abnormal estrous cycle	–	7.14E+00	–	–	5.95E−02	–	–	3.25E−01	–
Hojo et al. (2002, 198785)	DRL response per min	–	2.00E+01	2.70E−01	–	5.26E−03	7.11E−05	–	5.50E−02	7.37E−05
Hutt et al. (2008, 198268)	Embyrotoxicity	–	7.14E+00	–	–	4.67E-02	–	–	2.57E-01	–

Table 4-3. Summary of key animal study PODs (ng/kg-day) based on three different dose metrics: administered dose, 1st-order body burden HED and blood concentration HED (continued)

Study	Endpoint	Administered dose[a]			1st-order body burden HED[b]			Blood concentration HED[c]		
		NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]
Ikeda et al. (2005, 197834)	Sex ratio	–	1.65E+01	–	–	1.05E−01	–	–	2.75E+00	–
Ishihara et al. (2007, 197677)	Sex ratio	1.00E−01	1.00E+02	–	3.18E−04	3.18E−01	–	–	–	–
Kattainen et al. (2001, 198952)	3rd molar length	–	3.00E+01	2.14E+00	–	7.89E−03	5.64E−04	–	8.99E−02	1.71E−03
Keller et al. (2007, 198526; 2008, 198531; 2008, 198033)	Missing mandibular molars	–	1.00E+01	1.88E+01	–	2.58E−03	4.85E−03	–	9.81E−03	1.70E−02
Kociba et al. (1976, 198594)	Liver and hematologic effects and body weight changes	7.14E+00	7.14E+01	–	4.53E−02	4.53E−01	–	2.68E−01	3.10E+00	–
Kociba et al. (1978, 001818)	Liver and lung lesions, increased urinary porphyrins	1.00E+00	1.00E+01	7.30E−01	1.07E−02	1.07E−01	7.84E−03	6.46E−02	6.46E−01	2.00E−02
Latchoumycandane and Mathur (2002, 197498)	Sperm production	–	1.00E+00	1.56E−02	–	3.87E−03	6.03E−05	–	1.67E−02	3.83E−05
Li et al. (1997, 199060)	Increased serum FSH	3.00E+00	1.00E+01	3.60E+03	7.89E−04	2.63E−03	9.47E−01	2.97E−03	1.72E−02	2.38E+01
Li et al. (2006, 199059)	Hormone levels (serum estradiol)	–	2.00E+00	1.08E+02	–	9.85E−04	5.33E−02	–	1.57E−03	3.46E−01
Markowski et al. (2001, 197442)	FR2 revolutions	–	2.00E+01	7.34E+00	–	6.25E−03	2.29E−03	–	5.14E−02	1.18E−02
Maronpot et al. (1993, 198386)	Increased relative liver weight	1.07E+01	3.50E+01	–	8.97E−02	2.93E−01	–	–	–	–

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Table 4-3. Summary of key animal study PODs (ng/kg-day) based on three different dose metrics: administered dose, 1st-order body burden HED and blood concentration HED (continued)

Study	Endpoint	Administered dose[a]			1st-order body burden HED[b]			Blood concentration HED[c]		
		NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]
Miettinen et al. (2006, 198266)	Cariogenic lesions in pups	–	3.00E+01	1.05E+01	–	7.89E−03	2.77E−03	–	8.93E−02	9.32E−03
Murray et al. (1979, 197983)	Fertility index in f2 generation	1.00E+00	1.00E+01	1.63E+00	9.43E−03	9.43E−02	1.54E−02	2.96E−02	3.88E−01	4.05E−02
NTP (1982, 200870)	Liver lesions	–	1.39E+00	4.68E+00	–	6.47E−03	2.18E−02	–	2.21E−02	5.20E−02
NTP (2006, 197605)	Liver and lung lesions	–	2.14E+00	5.04E−01	–	2.34E−02	5.50E−03	–	1.39E−01	7.38E−03
Nohara et al. (2000, 200027)	Decreased spleen cellularity	8.00E+02	–	–	2.10E−01	–	–	5.34E+00	–	–
Ohsako et al. (2001, 198497)	Anogenital distance in pups	1.25E+01	5.00E+01	9.75E+00	3.29E−03	1.32E−02	2.57E−03	2.75E−02	1.78E−01	1.84E−02
Seo et al. (1995, 197869)	Decreased thymus weight	2.50E+01	1.00E+02	–	2.49E−02	9.96E−02	–	1.67E−01	9.15E−01	–
Sewall et al. (1995, 198145)	Serum T4	1.07E+01	3.50E+01	5.16E+00	8.97E−02	2.93E−01	4.33E−02	5.15E−01	1.76E+00	1.84E−01
Shi et al. (2007, 198147)	Serum estradiol in female pups	1.43E−01	7.14E−01	2.24E−01	1.23E−03	6.13E−03	1.92E−03	4.71E−03	2.75E−02	4.95E−03
Simanainen et al. (2002, 201369)	Decreased serum T4	1.00E+02	3.00E+02	–	2.63E−02	7.89E−02	–	–	–	–
Simanainen et al. (2003, 198582)	Decreased thymus weight and change in EROD activity	1.00E+02	3.00E+02	–	2.63E−02	7.89E−02	–	–	–	–
Simanainen et al. (2004, 198948)	Decreased daily sperm production	1.00E+02	3.00E+02	–	2.63E−02	7.89E−02	–	–	–	–
Smialowicz et al. (2004, 198948)	Decreased antibody response to SRBCs	3.00E+02	1.00E+03	–	7.73E−02	2.58E−01	–	–	–	–
Smialowicz et al. (2008, 198341)	PFC per 10^6 cells	–	1.07E+00	4.09E−01	–	5.00E−03	1.91E−03	–	6.38E−03	2.00E−03

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Table 4-3. Summary of key animal study PODs (ng/kg-day) based on three different dose metrics: administered dose, 1st-order body burden HED and blood concentration HED (continued)

Study	Endpoint	Administered dose[a]			1st-order body burden HED[b]			Blood concentration HED[c]		
		NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]
Toth et al. (1979, 197109)	Skin lesions	–	1.00E+00	2.15E+02	–	3.70E−03	7.94E−01	–	1.00E−02	2.18E−01
VanBirgelen et al. (1995, 198052)	Decreased liver retinyl palmitate	–	1.40E+01	9.89E+02	–	8.63E−02	6.09E+00	–	5.25E−01	5.00E+00
Vos et al. (1973, 198367)	Decreased delayed-type hypersensitivity response to tuberculin	1.14E+00	5.71E+00	–	6.43E−03	3.22E−02	–	–	–	–
White et al. (1986, 197531)	Decreased serum complement	–	1.00E+01	3.59E+01	–	2.23E−02	7.98E−02	–	2.83E−02	4.65E−02
Yang et al. (2000, 198590)	Increased endometrial implant survival	1.79E+01	–	–	6.74E−01	–	–	–	–	–

[a]Average administered daily dose over the experimental exposure period.
[b]HED based on 1st-order body burden model described in Section 3.2.4.4.
[c]HED based on Emond rodent and human PBPK models described in Section 3.3.6.
[d]BMR = 0.1 for quantal endpoints and 1 standard deviation control mean for continuous endpoints, except for body and organ weights, where BMR = 10% relative deviation from control mean.

– = value not established or not modeled.

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg)[a]

Study	NOAEL/ LOAEL	Endpoint	Control response	First response[b]	Max response[c]	Model fit detail	BMD/ BMDL	Comments
Amin et al. (2000, 197169) (rat)	– 3.38E+00	Saccharin consumed, female, (0.25%) (n = 10)	–	22% ↓ (0.3 SD)	66% ↓	Continuous linear, nonconstant variance (p = 0.55)	9.15E+00 6.09E+00	BMDL > LOAEL; restricted power model, constrained parameter hit lower bound
						Continuous power, nonconstant variance, unrestricted (p = NA)	8.37E+00 3.42E+00	Saturated model; supralinear fit (power = 0.74)
		Saccharin consumed, female (0.50%) (n = 10)	–	49% ↓ (0.7 SD)	80% ↓	Continuous linear, nonconstant variance (p = 0.06)	1.02E+01 6.57E+00	Restricted power model, constrained parameter hit lower bound
						Continuous power, nonconstant variance, unrestricted (p = NA)	6.57E+00 1.15E+00	Saturated model; supralinear fit (power = 0.40)
		Saccharin preference ratio, female (0.25%) (n = 10)	–	29% ↓ (1.8 SD)	33% ↓	Continuous linear, nonconstant variance (p = 0.002)	1.16E+01 5.57E+00	BMDL > LOAEL; no response near BMR; near maximal response at LOAEL
		Saccharin preference ratio, female (0.50%) (n = 10)	–	39% ↓ (1.1 SD)	54% ↓	Continuous linear, constant variance (p = 0.14)	8.14E+00 5.11E+00	BMDL > LOAEL; near maximal response at LOAEL; restricted power model, constrained parameter hit lower bound
						Continuous power, constant variance, unrestricted (p = NA)	2.60E+00 1.06E−14	Saturated model; supralinear fit (power = 0.28)

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg[a]) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Bell et al. (2007, 197041) (rat)	– 2.20E+00	Balano-preputial separation in male pups (n = 30 [dams])	1/30	5/30	15/30	Dichotomous log-logistic, restricted (p = 0.78)	2.25E+00 1.39E+00	Adequate fit; constrained parameter bound hit; not litter based; selected
						Dichotomous log-logistic, unrestricted (p = 0.50)	2.00E+00 2.80E−01	Supralinear fit (slope = 0.93); selected
Cantoni et al. (1981, 197092) (rat)	– 1.85E+00	Urinary uroporhyrins (n = 4)	–	2.4-fold ↑ (5.7 SD)	87-fold ↑	Continuous exponential (M2), nonconstant variance (p = 0.0003)	3.76E+00 2.76E+00	No response near BMR; poor fits for all nonconstant variance models; constant variance poor representation of control SD; BMDL > LOAEL
		Urinary coproporhyrins (n = 4)	–	2.4-fold ↑ (3.1 SD)	4.0-fold ↑	Continuous exponential (M4), nonconstant variance (p = 0.49)	5.34E−01 1.80E−01	No response near BMR
						Continuous power, nonconstant variance, unrestricted (p = 0.61)	2.77E−02 2.03E−05	Supralinear fit (n = 0.30); poor model choice for plateau effect
Crofton et al. (2005, 197381) (rat)	3.46E+00 9.26E+00	Serum T4, (n = 4−14)	–	29% ↓ (1.9 SD)	51% ↓	Continuous exponential (M4), constant variance (p = 0.94)	5.19E+00 3.03E+00	No response near BMR

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg[a]) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Franc et al. (2001, 197353) (rat)	6.58E+00 1.45E+01	S-D Rats, Relative Liver Weight	–	8.1% ↑ (0.58 SD)	55% ↑	Continuous power, constant variance (p = 0.84)	9.47E+00 4.59E+00	Acceptable fit
		L-E Rats, Relative Liver Weight	–	6.3% ↑ (0.63 SD)	22% ↑	Continuous Hill, nonconstant variance, restricted (p = 0.83)	7.72E+00 1.22E+00	Constrained parameter hit lower bound; otherwise acceptable fit; selected
						Continuous Hill, nonconstant variance, unrestricted (p = N/A)	7.22E+00 1.15E+00	Supralinear fit (power = 0.55)
		S-D Rats, Relative Thymus Weight	–	9.0% ↓ (0.11 SD)	77% ↓	Continuous exponential (M4), nonconstant variance (p = 0.72)	1.88E+00 9.22E−01	Poor fit for responses in controls and lowest exposure group
						Continuous polynomial, nonconstant variance (p = 0.40)	4.78E+00 3.89E+00	Acceptable fit
		L-E Rats, Relative Thymus Weight	–	7.7% ↓ (0.15 SD)	66% ↓	Continuous exponential (M4), constant variance (p = 0.23)	2.08E+00 5.93E−01	Poor fit for responses in controls and lowest exposure group; dose-response relationship not significant
		H/W Rats, Relative Thymus Weight	–	3.7% ↓ (0.10 SD)	51% ↓	Continuous exponential (M2), constant variance (p = 0.70)	5.09E+00 3.13E+00	

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg[a]) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Hojo et al. (2002, 198785) (rat)	– 1.62E+00	DRL reinforce per min (n = 12)	–	55% ↑ (1.0 SD)	80% ↑	Continuous exponential (M4), constant variance (p = 0.054)	1.32E+00 2.37E−03	Poor fit; near maximal response at lowest dose, BMD/BMDL ratio »100
		DRL response per min (n = 12)	–	105% ↓ (2.4 SD)	105% ↓	Continuous exponential (M4), constant variance (p = 0.48)	3.81E−01 1.55E−02	No response data near BMR; maximal response at lowest dose, BMD/BMDL ratio »20
Kattainen et al. (2001, 198952) (rat)	– 2.23E+00	3rd molar length in pups (n = 4−8)	–	15% ↓ (4.2 SD)	27% ↓	Continuous Hill, nonconstant variance, restricted (p = 0.02)	3.13E−01 1.68E−01	No response data near BMR; Constrained parameter lower bound hit
						Continuous Hill, nonconstant variance, unrestricted (p < 0.001)	1.21E−02 –	BMDL could not be calculated
		3rd molar eruption in pups (n = 4−8)	1/16	3/17	13/19	Dichotomous log-logistic, restricted (p = 0.98)	2.40E+00 1.33E+00	Constrained parameter lower bound hit
						Dichotomous log-logistic, unrestricted (p = 0.95)	1.93E+00 1.84E−01	Supralinear fit (slope = 0.91)
Keller et al. (2007, 198526; 2008, 198531; 2008, 198033) (mouse)	– 5.37E−01	Missing molars (n = 23−36)	0/29	2/23	30/30	Dichotomous 1° multistage (p = 0.26)	1.09E+00 7.62E−01	Poor fit at first response level; not most sensitive endpoint; other endpoints not amenable to BMD modeling

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg[a]) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Kociba et al. (1978, 001818) (rat)	1.55E+00 7.15E+00	Uroporphyrin per creatinine, females (n = 5)	–	15% ↑ (0.48 SD)	89% ↑	Continuous linear, constant variance (p = 0.79)	1.31E+01 9.29E+00	BMDL > LOAEL; otherwise adequate fit
		Urinary coproporphyrins, females (n = 5)	–	67% ↑ (5.1 SD)	78% ↑	Continuous exponential (M4), nonconstant variance (p = 0.01)	1.57E+00 7.18E−01	Poor fit; no response near BMR
		Liver lesions (n = 50)						No data presented
		Lung lesions (n = 50)						No data presented
Latchoumy-candane and Mathur (2002, 197498) (rat)	– 7.85E−01	Daily sperm production (n = 6)	–	29% ↓ (1.0 SD)	41% ↓	Continuous Hill, constant variance, restricted (p = 0.96)	1.17E−01 1.32E−02	Near maximal response at LOAEL; constrained parameter bound hit; standard deviations given in paper interpreted as standard errors
						Continuous Hill, constant variance, unrestricted (p = N/A)	9.96E−02 1.23E−09	Slightly supralinear fit (n = 0.92)
Li et al. (1997, 199060) (rat)	2.66E−01 7.99E−01	FSH in female rats (n = 10)	–	3.6-fold ↑ (2.0 SD)	19-fold ↑	Continuous power, nonconstant variance, restricted (p < 0.01)	2.00E+02 1.36E+02	Power hit lower bound
						Continuous power, nonconstant variance, unrestricted (p = 0.003)	1.96E−01 2.48E−02	supralinear fit (power = 0.31)

Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg[a]) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Li et al. (2006, 199059) (mouse)	– 1.59E−01	Serum estradiol (n = 10)	–	2.0-fold ↑ (0.8 SD)	2.4-fold ↑	Continuous linear, constant variance (p = 0.16)	1.61E+01 5.38E+00	BMDL > LOAEL; high control CV (1.25); near maximal response at low dose; nonmonotonic response; other model fits are step-function-like
		Serum progesterone (n = 10)	–	33% ↓ (2.0 SD)	61% ↓	Continuous Hill, nonconstant variance (p = 0.39)	9.46E−04 8.01E−11	No response data near BMR; large CVs (>1) for treatment groups; poor fit for variance model; Hill coefficient at lower bound (step-function)
Markowski et al. (2001, 197442) (rat)	– 1.56E+00	FR5 run opportunities (n = 4−7)	–	10% ↓ (0.21 SD)	51% ↓	Continuous Hill, constant variance (p = 0.94)	1.72E+00 9.08E−01	Constrained parameter upper bound hit
						Continuous power, constant variance, unrestricted (p = 0.13)	2.67E+00 1.03E−14	Saturated model; supralinear fit (power = 0.39); BMD/BMDL ratio »100
		FR2 revolutions (n = 4−7)	–	9% ↓ (0.15 SD)	43% ↓	Continuous Hill, constant variance (p = 0.65)	1.84E+00 5.99E−01	Constrained parameter bound hit (upper bound)
						Continuous power, constant variance, unrestricted (p = 0.16)	5.74E+00 1.03E−14	Supralinear fit (power = 0.32)
		FR10 run opportunities (n = 4−7)	–	15% ↓ (0.24 SD)	57% ↓	Continuous exponential (M2) , constant variance (p = 0.30)	8.57E+00 2.89E+00	BMDL > LOAEL

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg[a]) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Miettinen et al. (2006, 198266) (rat)	– 2.22E+00	Cariogenic lesions in pups (n = 4–8)	25/42	23/29	29/32	Dichotomous log-logistic, restricted (p = 0.60)	1.43E+00 5.17E−01	Constrained parameter lower bound hit; near maximal response at LOAEL; high control response
						Dichotomous log-logistic, unrestricted (p = 0.73)	4.94E−02 –	Supralinear fit (slope = 0.47); BMDL could not be calculated
Murray et al. (1979, 197983) (rat)	1.12E+00 5.88E+00	Fertility in f2 gen. (no litters) (n = 20)	4/32	0/20	9/20	Dichotomous multistage (p = 0.08)	2.73E+00 1.37E+00	Poor fit; nonmonotonic response; no response data near BMR
NTP (1982, 200870) (mouse)	– 7.67E−01	Toxic hepatitis; males (n = 50)	1/73	5/49	44/50	Dichotomous multistage (p = 0.04)	2.78E+00 1.34E+00	No acceptable model fits; lowest BMDL shown
NTP (2006, 197605) (rat)	– 2.56E+00	Hepatocyte hypertrophy (n = 53–54)	0/53	19/54	52/53	Dichotomous multistage (p = 0.02)	9.27E−01 7.91E−01	Poor fits for all models
		Alveolar metaplasia (n = 52–54)	2/53	19/54	46/52	Dichotomous log-logistic (p = 0.72)	6.50E−01 3.75E−01	No response near BMR
		Oval cell hyperplasia (n = 53–54)	0/53	4/54	53/53	Dichotomous probit (p = 0.23)	5.67E+00 4.79E+00	Relatively poor fit for control and low dose groups; negative response intercept (same for logistic); BMDL > LOAEL
						Dichotomous Weibull (p = 0.08)	5.72E+00 4.09E+00	Marginal fit; BMDL > LOAEL

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg[a]) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
NTP (2006, 197605) (rat) (continued)	– 2.56E+00 (continued)	Gingival hyperplasia (n = 53–54)	1/53	7/54	16/53	Dichotomous log-logistic, restricted (p = 0.06)	5.85E+00 3.73E+00	Poor fit; constrained parameter bound hit; BMDL > LOAEL
						Dichotomous log-logistic, unrestricted (p = 0.66)	7.05E−01 1.26E−05	Supralinear fit (slope = 0.37)
		Eosinophilic focus, multiple (n = 53–54)	3/53	8/54	42/53	Dichotomous probit (p = 0.46)	5.58E+00 4.86E+00	Relatively poor fit to control response; BMDL > LOAEL
		Liver fatty change, diffuse (n = 53–54)	0/53	2/54	48/53	Dichotomous Weibull (p = 0.72)	3.92E+00 2.86E+00	BMDL > LOAEL; otherwise adequate fit
		Liver necrosis (n = 53–54)	1/53	4/54	17/53	Dichotomous log-probit, unrestricted (p = 0.80)	7.50E+00 3.50E+00	Adequate fit; slightly supralinear; BMDL > LOAEL
		Liver pigmentation (n = 53–54)	4/53	9/54	53/53	Dichotomous log-probit (p = 0.96)	2.46E+00 1.89E+00	Adequate fit
		Toxic hepatopathy (n = 53–54)	0/53	2/54	53/53	Dichotomous multistage (p = 0.69)	3.98E+00 3.06E+00	BMDL > LOAEL; otherwise adequate fit
Ohsako et al. (2001, 198497) (rat)	1.04E+00 3.47E+00	Ano-genital distance in male pups (n = 5)	–	12% ↓ (1.0 SD)	17% ↓	Continuous Hill, constant variance, restricted (p = 0.15)	2.88E+00 8.03E−01	Constrained parameter lower bound hit; near maximal response at LOAEL
						Continuous Hill, constant variance, unrestricted (p = 0.056)	3.49E+00 3.05E−01	Supralinear fit (n = 0.59)

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg[a]) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Sewall et al. (1995, 198145) (rat)	7.11E+00 1.66E+01	Serum T4 (n = 9)	–	9.1% ↓ (0.6 SD)	40% ↓	Continuous Hill, constant variance, restricted (p = 0.90)	1.03E+01 3.60E+00	Constrained parameter hit lower bound; otherwise acceptable fit; selected
						Continuous Hill, constant variance, unrestricted (p = 0.86)	9.71E+00 1.97E+00	Supralinear fit (power = 0.57)
Shi et al. (2007, 198147) (rat)	3.42E−01 1.07E+00	Serum estradiol in female pups (n = 10)	–	38% ↓ (0.4 SD)	62% ↓	Continuous exponential (M4), nonconstant variance (p = 0.69)	8.07E−01 3.54E−01	Adequate fit; selected
Smialowicz et al. (2008, 198341) (mouse)	– 4.38E−01	PFC per spleen (n = 15)	–	24% ↓ (0.5 SD)	89% ↓	Continuous power, unrestricted, nonconstant variance (p = 0.27)	1.19E+01 3.76E+00	BMDL > LOAEL; fit at control and low dose inconsistent with data; constrained parameters in other models hit lower bounds
		PFC per 10^6 cells (n = 8−15)	–	24% ↓ (0.5 SD)	9.3-fold ↓	Continuous power unrestricted, constant variance (p = 0.48)	1.90E+00 2.16E−01	Constant variance test failed; observed control variance underestimated by 35%; poor fits for all nonconstant variance models
Toth et al. (1979, 197109) (mouse)	– 5.73E−01	Skin lesions (n = 38−44)	0/38	5/44	25/43	Dichotomous log-logistic, restricted (p = 0.08)	6.41E+00 4.02E+00	Constrained parameter lower bound hit
						Dichotomous log-logistic, unrestricted (p = 0.74)	5.97E−01 6.77E−02	Supralinear fit (slope = 0.48)

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg[a]) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Toth et al. (1979, 197109) (mouse) (continued)	– 5.73E−01 (cont.)	Dermal amyloidosis (n = 38−44)	0/38	5/44	17/43	Dichotomous log-logistic, restricted (p = 0.05)	1.50E+01 8.75E+00	Poor fit; constrained parameter lower bound hit; BMDL > LOAEL
						Dichotomous log-logistic, unrestricted (p = 0.90)	4.84E−01 5.31E−03	Supralinear fit (slope = 0.33)
Van Birgelen et al. (1995, 198052) (rat)	– 7.20E+00	Hepatitic retinol (n = 8)	–	44% ↓ (0.74 SD)	96% ↓	Continuous exponential (M4), nonconstant variance (p < 0.01)	2.49E+01 3.36E+00	Poor fit
						Continuous power, nonconstant variance, unrestricted (p = 0.01)	3.80E−01 1.39E−02	Poor fit; supralinear fit (power = 0.14)
		Hepatitic retinyl palmitate (n = 8)	–	80% ↓ (1.4 SD)	99% ↓	Continuous exponential (M4), nonconstant variance (p < 0.01)	1.42E+02 3.65E+01	Poor fit; no response near BMR
						Continuous power, nonconstant variance, unrestricted (p = 0.24)	5.26E−02 5.89E−05	Supralinear fit (power = 0.06)

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg[a]) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
White et al. (1986, 197531) (mouse)	– 1.09E+00	Total hemolytic complement activity (CH50) (n = 8)	–	41% ↓ (2.6 SD)	81% ↓	Continuous Hill, nonconstant variance, restricted (p = 0.002)	8.63E+00 1.50E+00	Poor fit; no response near BMR; constrained parameter bound hit; BMDL > LOAEL
						Continuous Hill, nonconstant variance, unrestricted (p = 0.07)	1.48E−01 4.35E−03	Supralinear fit (n = 0.25)

[a]Animal whole blood concentrations were used to determine the HEDs in Table 4-5.
[b]Magnitude of response at first dose where response differs from control value (in the adverse direction); continuous response magnitudes given as relative to control plus change relative to control standard deviation; quantal response given as number affected/total number.
[c]Magnitude of response maximally differing from control value (in the adverse direction).

S-D = Sprague-Dawley.
SD = standard deviation.

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Table 4-5. Candidate points of departure for the TCDD RfD using blood-concentration-based human equivalent doses

Study	Species, strain (sex, if not both)	Protocol	Endpoint	$NOAEL_{HED}$ (N) or $BMDL_{HED}$ (B) (ng/kg-day)	$LOAEL_{HED}$ (ng/kg-day)	UF[a]	RfD (mg/kg-day)
Li et al. (2006, 199059)	Mouse, NIH (F)	Gavage GD 1−3; n = 10	Hormone levels in pregnant dams (decreased progesterone, increased estradiol)	–	1.6E−03	300	5.2E−12
Smialowicz et al. (2008, 198341)	Mouse, B6C3F1 (F)	90-day gavage; n = 8−15	Decreased SRBC response	–	6.4E−03	300	2.1E−11
Keller et al. (2007, 198526; 2008, 198531; 2008, 198033)[b]	Mouse, CBA/J and C3H/HeJ	Gavage GD 13; n = 23−36 (pups)	Missing molars, mandibular shape changes in pups	–	9.8E−03	300	3.3E−11
Toth et al. (1979, 197109)	Mouse, Swiss/H/Riop (M)	1-year gavage; n = 38−44	Dermal amyloidosis, skin lesions	–	1.0E−02	300	3.3E−11
Latchoumycandane and Mathur (2002, 197498)	Rat, Wistar (M)	45-day oral pipetting; n = 6	Decreased sperm production	–	1.7E−02	300	5.6E−11
NTP (1982, 200870)	Mouse, B6C3F1 (M)	2-year gavage; n = 50	Liver lesions	–	2.2E−02	300	7.4E−11
White et al. (1986, 197531)	Mouse, B6C3F1 (F)	14-day gavage; n = 6−8	Decreased serum complement	–	2.8E−02	300	9.4E−11
Li et al. (1997, 199060)	Rat, S-D (F, 22 day-old)	Single gavage; n = 10	Increased serum FSH	3.0E−03 (N)	1.7E−02	30[c]	9.9E−11
DeCaprio et al. (1986, 197403)	Guinea pig, Hartley	90-day dietary; n = 10	Decreased body weight, organ weight changes (liver, kidney, thymus, brain)	4.1E−03[d] (N)	3.3E−02[d]	30[c]	1.4E−10
Shi et al. (2007, 198147)	Rat, S-D (F)	11-month gavage; n = 10	Decreased serum estradiol	4.7E−03 (N) 5.0E−03 (B)	2.8E−02	30[c]	1.6E−10
Markowski et al. (2001, 197442)	Rat, Holtzman	Gavage GD 18; n = 4−7	Neurobehavioral effects in pups (running, lever press, wheel spinning)	–	5.1E−02	300	1.7E−10

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Table 4-5. Candidate points of departure for the TCDD RfD using blood-concentration-based human equivalent doses (continued)

Study	Species, strain (sex, if not both)	Protocol	Endpoint	$NOAEL_{HED}$ (N) or $BMDL_{HED}$ (B) (ng/kg-day)	$LOAEL_{HED}$ (ng/kg-day)	UF[a]	RfD (mg/kg-day)
Hojo et al. (2002, 198785)	Rat, S-D	Gavage GD 8; n = 12	Food-reinforced operant behavior in pups	–	5.5E−02	300	1.8E−10
Vos et al. (1973, 198367)	Guinea pig, Hartley (F)	8-week gavage; n = 10	Decreased delayed-type hypersensitivity response to tuberculin	6.4E−03[d] (N)	3.2E−02[d]	30[c]	2.1E−10
Cantoni et al. (1981, 197092)	Rat, CD-COBS (F)	45-week gavage; n = 4	Increased urinary porhyrins	–	6.5E−02	300	2.2E−10
Miettinen et al. (2006, 198266)	Rat, Line C	Gavage GD 15; n = 3−10	Cariogenic lesions in pups	–	8.9E−02	300	3.0E−10
Kattainen et al. (2001, 198952)	Rat, Line C	Gavage GD 15; n = 4−8	Inhibited molar development in pups	–	9.0E−02	300	3.0E−10
NTP (2006, 197605)	Rat, S-D (F)	2-year gavage; n = 53	Liver and lung lesions	–	1.4E−01	300	4.6E−10
Amin et al. (2000, 197169)	Rat, S-D	Gavage GD 10−16; n = 10	Reduced saccharin consumption and preference	–	1.7E−01	300	5.7E−10
Mocarelli et al. (2008, 199595)	**Human (M)**	**Childhood exposure; n = 157**	**Decreased sperm concentration and sperm motility, as adults**	–	**2.0E−02[e]**	**30[f]**	**6.7E−10**
Baccarelli et al. (2008, 197059)	**Human infants**	**Gestational exposure; n = 51**	**Increased TSH in newborn infants**	–	**2.4E−02[g]**	**30[f]**	**8.2E−10**
Hutt et al. (2008, 198268)	Rat, S-D (F)	13-week dietary; n = 3	Embryotoxicity	–	2.6E+00	300	8.6E−10
Ohsako et al. (2001, 198497)	Rat, Holtzman	Gavage GD 15; n = 5	Decreased ano-genital distance in male pups	2.8E−02 (N)	1.8E−01	30[c]	9.2E−10
Murray et al. (1979, 197983)	Rat, S-D	3-generation dietary	Reduced fertility and neonatal survival (f 0 and f 1)	3.0E−02 (N)	3.9E−01	30[c]	9.9E−10

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Table 4-5. Candidate points of departure for the TCDD RfD using blood-concentration-based human equivalent doses (continued)

Study	Species, strain (sex, if not both)	Protocol	Endpoint	$NOAEL_{HED}$ (N) or $BMDL_{HED}$ (B) (ng/kg-day)	$LOAEL_{HED}$ (ng/kg-day)	UF[a]	RfD (mg/kg-day)
Franc et al. (2001, 197353)	Rat, Long-Evans (F)	22-week gavage; $n = 8$	Increased Relative Liver Weight; decreased relative thymus weight	4.6E−01 (N) 3.4E−02 (B)	1.45E+00	30[c]	1.1E−09
Chu et al., 2007	Rat, S-D (F)	28-day gavage, $n = 5$	Liver lesions	3.6E−02 (N)	5.8E−01	30[c]	1.2E−09
Bell et al. (2007, 197041)	Rat, CRL:WI (Han) (M)	17-week dietary; $n = 30$	Delay in onset of puberty	4.3E−02 (B)	8.8E−02	30[c]	1.4E−09
Van Birgelen et al. (1995, 198052)	Rat, S-D (F)	13-week dietary; $n = 8$	Decreased liver retinyl palmitate	–	5.3E−01	300	1.8E−09
Kociba et al. (1978, 001818)	Rat, S-D (F)	2-year dietary; $n = 50$	Liver and lung lesions, increased urinary porhyrins	6.5E−02 (N)	6.5E−01	30[c]	2.2E−09
Fattore et al., (2000, 197446)	Rat, S-D	13-week dietary; $n = 6$	Decreased hepatic retinol	–	8.0E−01	300	2.7E−09
Seo et al. (1995, 197869)	Rat, S-D	Gavage GD 10−16; $n = 10$	Decreased serum T4 and thymus weight	1.7E−01 (N)	9.1E−01	30[c]	5.6E−09
Crofton et al. (2005, 197381)	Rat, Long-Evans (F)	4-day gavage; $n = 4$−14	Decreased serum T4	1.7E−01 (N)	7.6E−01	30[c]	5.7E−09
Sewall et al. (1995, 198145)	Rat, S-D (F)	30-week gavage; $n = 9$	Decreased serum T4	5.2E−01 (N) 1.8E−01 (B)	1.8E+00	30[c]	6.1E−09
Alaluusua et al. (2004, 197142)	Human	Childhood exposure; $n = 48$	Dental defects	1.2E−01[h] (N)	9.3E−01[i]	3[j]	3.9E−08

[a]Except where indicated, $UF_A = 3$ (for dynamics), $UF_H = 10$, $UF_L = 10$.
[b]Results from 3 separate studies with identical designs combined.
[c]$UF_L = 1$ (NOAEL or BMDL).
[d]HED determined from 1st-order body burden model; no PBPK model available for guinea pigs.
[e]Mean of peak exposure (0.0319 ng/kg-day) and average exposure over 10-year critical window (0.00802 ng/kg-day).
[f]$UF_H = 3$, $UF_L = 10$.
[g]Maternal exposure corresponding to neonatal TSH concentration exceeding 5 µU/mL.

Table 4-5. Candidate points of departure for the TCDD RfD using blood-concentration-based human equivalent doses (continued)

[h]Mean of peak exposure (0.200 ng/kg-day) and average exposure over 10-year critical window (0.0335 ng/kg-day).
[i]Mean of peak exposure (1.71 ng/kg-day) and average exposure over 10-year critical window (0.153 ng/kg-day).
[j]$UF_H = 3$.

S-D = Sprague-Dawley.

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Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate points-of-departure for the TCDD RfD

Study	Strengths	Limitations	Remarks
Bell et al. (2007, 197041)	• Large sample size of both rat dams and offspring/dose employed • Several developmental effects tested	• Batch-to-batch variation of up to 30% in TCDD concentration in the diet • Longer-term dosing of dams does not accurately define gestational period when fetus is especially sensitive to TCDD-induced toxicity	Study is a significant addition to a substantial database on the developmental toxicity of TCDD in laboratory animals
Cantoni et al. (1981, 197092)	• Experiments were designed to test qualitative and quantitative composition and the course of urinary excretion in TCDD-induced porphyria	• Small sample size of rats/dose employed (n = 4) • Concurrent histological changes with tissue porphyrin levels were not examined • TCDD used for dosing was of unknown purity	Early study on porphyrogenic effects of TCDD
DeCaprio et al. (1986, 197403)	• Subchronic oral dosing duration up to 90 days. • Male and female guinea pigs tested	• Relatively small sample size of guinea pigs/dose employed (n = 10) • No histopathological analyses performed • TCDD used for dosing was of unknown purity	Limited subchronic study; PBPK model not available for estimation of HED
Franc et al. (2001, 197353)	• Three different rat strains with varying sensitivities to TCDD were utilized (Sprague-Dawley, Long Evans, Han/Wistar) • Longer-term oral dosing up to 22 weeks	• Relatively small sample size of rats/dose employed (n = 8) • Only female rats were tested • Concurrent liver histopathological changes with liver weight changes were not examined • Gavage exposure was only biweekly	Limited subchronic study
Hojo et al. (2002, 198785)	• Low TCDD dose levels used allowed for subtle behavioral deficits to be identified in rat offspring • Preliminary training sessions in operant chamber apparatuses were extensive • Neurobehavioral effects are exposure-related and cannot be attributed to presence of learning or discrimination deficits	• Relatively small sample size of rat dams/dose employed (n = 12) • Small sample size of rat offspring/dose evaluated (n = 5–6) • Neurobehavioral effects induced by TCDD at earlier or later gestational dosing dates are unknown because of single gavage administration on GD 8 • Although BMD analysis was conducted, the model parameters were not constrained according to EPA guidance, so the results cannot be used	One of a few neurobehavioral toxicity studies; somewhat limited study size

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Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate points-of-departure for the TCDD RfD (continued)

Study	Strengths	Limitations	Remarks
Keller et al. (2007, 198526; 2008, 198531; 2008, 198033)	• Six different inbred mouse strains were utilized • Large sample size of mouse offspring/dose/strain evaluated • Low TCDD dose levels used compared to typical mouse studies allowed for identification of subtle sensitivity differences in presence of absence of third molars, variant molar morphology, and mandible structure in offspring	• Unknown sample size of mouse dams/dose/strain employed • All inbred strains possessed sensitive *b* allele at the *Ahr* locus (i.e., a potentially resistant subpopulation was not evaluated for comparison purposes) • Morphological dental and mandibular changes induced by TCDD at earlier or later gestational dosing dates are unknown because of single gavage administration on GD 13 • Difficulties breeding A/J mice led to abandonment of that strain in the analysis (Keller et al., 2008a, b)	Endpoint similar to effects observed at higher exposure levels in humans; HED highly uncertain using mouse PBPK model
Latchoumy-candane and Mathur (2002, 197498)	• Compared to epididymal sperm counts, the testicular spermatid head count provides better quantitation of acute changes in sperm production and can indicate pathology	• Small sample size of rats/dose employed (n = 6) • Oral pipette administration of TCDD may be a less efficient dosing method than gavage	Endpoint has human relevance, similar to critical effects in principal human study for RfD
Li et al. (2006, 199059)	• Female reproductive effects (i.e., early embryo loss and changes in serum progesterone and estradiol) were tested at multiple exposure times—early gestation, preimplantation, and peri- to postimplantation	• Small sample size of dams/dose (n = 10) • Large dose-spacing interval (25-fold at lowest 2 doses)	Endpoint has human relevance but HED highly uncertain using mouse PBPK model
Markowski et al. (2001, 197442)	• Low TCDD dose levels used allowed for subtle behavioral deficits to be identified in rat offspring • Several training sessions on wheel apparatuses were extensive • Neurobehavioral effects are exposure-related and cannot be attributed to motor or sensory deficits	• Unknown sample size of rat dams/dose employed. • Small sample size of rat offspring/dose evaluated (n = 4−7) • TCDD used for dosing was of unknown purity and origin • Only 2 treatment levels • Neurobehavioral effects induced by TCDD at earlier or later gestational dosing dates are unknown because of single gavage administration on GD 18	One of a few neurobehavioral toxicity studies; somewhat limited study size

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Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate points-of-departure for the TCDD RfD (continued)

Study	Strengths	Limitations	Remarks
NTP (1982, 200870)	• Large sample size of mice and rats/dose employed • Comprehensive 2-year bioassay that assessed body weights, clinical signs, and pathological changes in multiple tissues and organs	• Elevated background levels of hepatocellular tumors in untreated male mice • Gavage exposure was only 2 days/week • Only 2 treatment levels	Comprehensive chronic toxicity evaluations of TCDD in rodents; HED highly uncertain using mouse PBPK model
NTP (2006, 197605)	• Chronic exposure duration with several interim sacrifices • Large number of dose groups with close spacing • Large number of animals per dose group • Comprehensive suite of endpoints evaluated • Comprehensive biochemical, clinical and histopathological tests and measures • Detailed reporting of results, with individual animal data presented as well as group summaries	• Single species, strain and sex • Lowest dose tested too high for establishing NOAEL	Study is the most comprehensive chronic TCDD toxicity evaluation in rats to date
Shi et al. (2007, 198147)	• Study design evaluated TCDD effects on aging female reproductive system (i.e., exposure began in utero and spanned across reproductive lifespan) • Several female reproductive endpoints were evaluated, including cyclicity, endocrinology, serum hormone levels, and follicular reserves	• Relatively small sample size of rats/dose employed (n = 10)	Endpoint similar to effects observed at higher exposure levels in humans
Smialowicz et al. (2008, 198341)	• Sheep red blood cell (SRBC) plaque forming cell assay is highly sensitive and reproducible across laboratories when examining TCDD	• Small sample size of animals/dose (n = 8) • Only female mice were tested • Thymus and spleen weights were only other immune response-related endpoints tested	Limited immunotoxicity study
Toth et al. (1979, 197109)	• Large sample size of mice/dose employed • Chronic exposure duration	• Reporting of findings is terse and lacks sufficient detail (e.g., materials and methods, thorough description of pathological findings, etc.) • Limited number of endpoints examined • Only male mice were tested	Limited chronic study; HED highly uncertain using mouse PBPK model

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Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate points-of-departure for the TCDD RfD (continued)

Study	Strengths	Limitations	Remarks
Vos et al. (1973, 198367)	• Three different animal species tested (guinea pigs, mice, and rats) • Effects of TCDD tested on both cell-mediated and humoral immunity	• Small sample size of animals/dose employed in each experiment (n = 5−10) • Only female guinea pigs and rats were tested, and only male mice were tested • Only one experimental assay was utilized to assess cell-mediated and humoral immunity in each animal species; humoral immunity was only investigated in guinea pigs • TCDD used for dosing was of unknown purity	Endpoints relevant to humans but study size limited; PBPK model not available for estimation of HED
White et al. (1986, 197531)	• Total hemolytic complement (CH50) is representative functional assay of the complete complement sequence	• Small sample size of rats/dose employed (n = 6−8) • Individual complement factors may be significantly depleted without affecting CH50 activity (only C3 is measured) • TCDD used for dosing was of unknown purity	Endpoint similar to effects observed at higher exposure levels in humans; HED highly uncertain using mouse PBPK model

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Table 4-7. Basis and derivation of the TCDD reference dose

Principal study detail		
Study	**POD (ng/kg-day)**	**Critical effects**
Mocarelli et al. (2008, 199595)	0.020 (LOAEL)	Decreased sperm count (20%) and motility (11%) in men exposed to TCDD during childhood
Baccarelli et al. (2008, 197059)	0.024 (LOAEL)	Elevated TSH (> 5 μU/mL) in neonates

RfD derivation	
POD	0.020 ng/kg-day (2.0E−8 mg/kg-day)
UF	30 (UF_L = 10, UF_H = 3)
RfD	7×10^{-10} (7E-10) mg/kg-day (2.0E−8 ÷ 30)

Uncertainty factors		
LOAEL-to-NOAEL (UF_L)	10	No NOAEL established; cannot quantify lower exposure group in Baccarelli et al. (2008, 197059); magnitude of effects at LOAEL sufficient to require a 10-fold factor.
Human interindividual variability (UF_H)	3	A factor of 3 ($10^{0.5}$) is used because the effects were elicited in sensitive populations. A further reduction to 1 was not made because the sample sizes were relatively small, which, combined with uncertainty in exposure estimation, may not fully capture the range of interindividual variability.
Interspecies extrapolation (UF_A)	1	Human study.
Subchronic-to-chronic (UF_S)	1	Chronic effect levels are not well defined for humans; however, animal bioassays indicate that developmental effects are the most sensitive, occurring at doses lower than other effects noted in chronic studies. Considering that exposure in the principal studies encompasses the critical window of susceptibility associated with development, an UF to account for exposure duration is not warranted.
Database sufficiency (UF_D)	1	The database for TCDD contains an extensive range of human and animal studies that examine a comprehensive set of endpoints. There is no evidence to suggest that additional data would result in a lower reference dose.

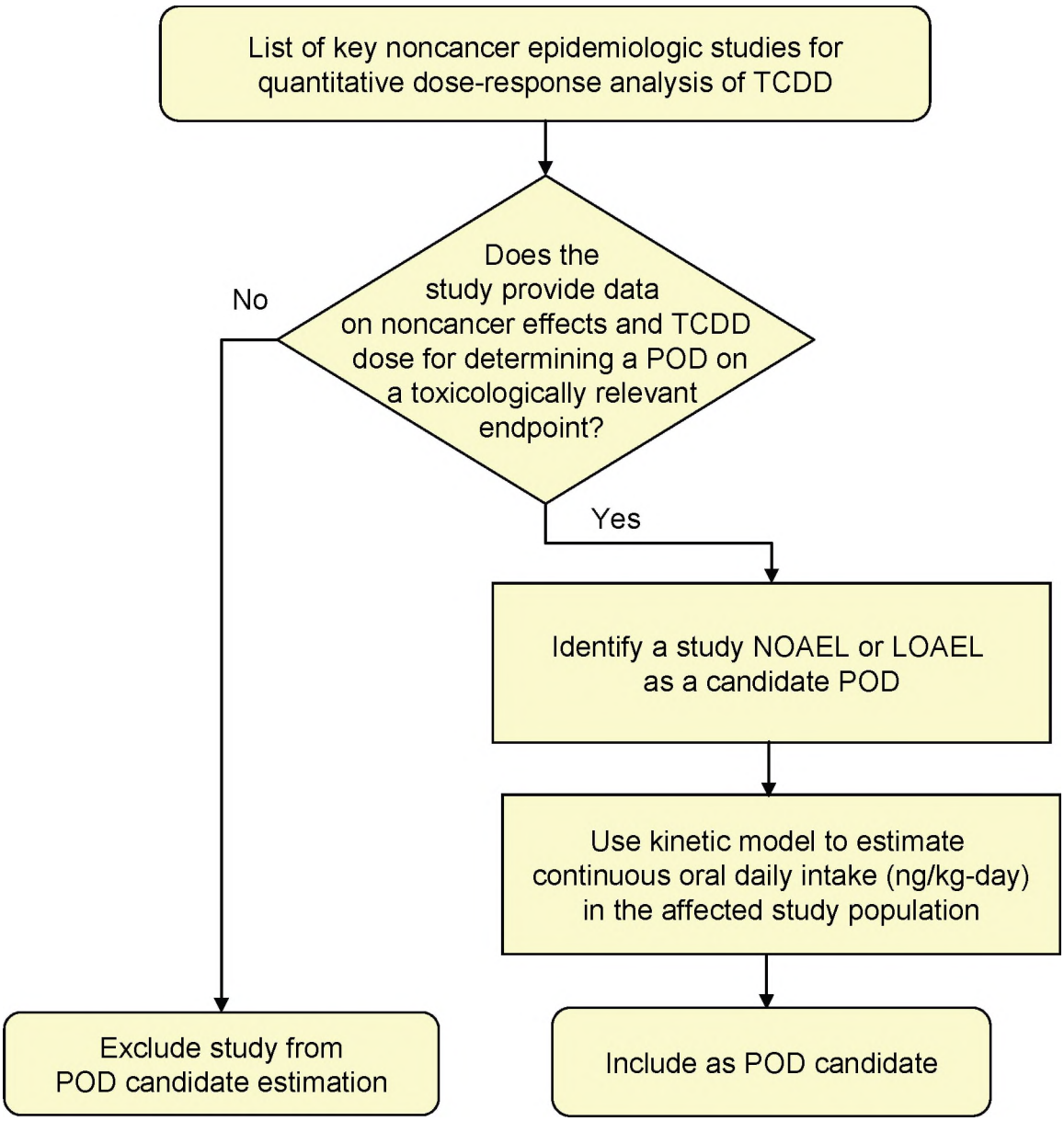


Figure 4-1. EPA's process to select and identify candidate PODs from key epidemiologic studies for use in the noncancer risk assessment of TCDD. For each noncancer study that qualified for TCDD dose-response assessment using the study inclusion criteria, EPA first evaluated the dose-response information developed by the study authors for whether the study provided noncancer effects and TCDD dose data for a toxicologically relevant endpoint. If such data were available, then EPA identified a NOAEL or LOAEL as a candidate POD. Then, EPA used a human kinetic model to estimate the continuous oral daily intake (ng/kg-day) for the candidate POD that could be used in the derivation of an RfD based on the study data. If all of this information was available, then the result was included as a candidate POD.

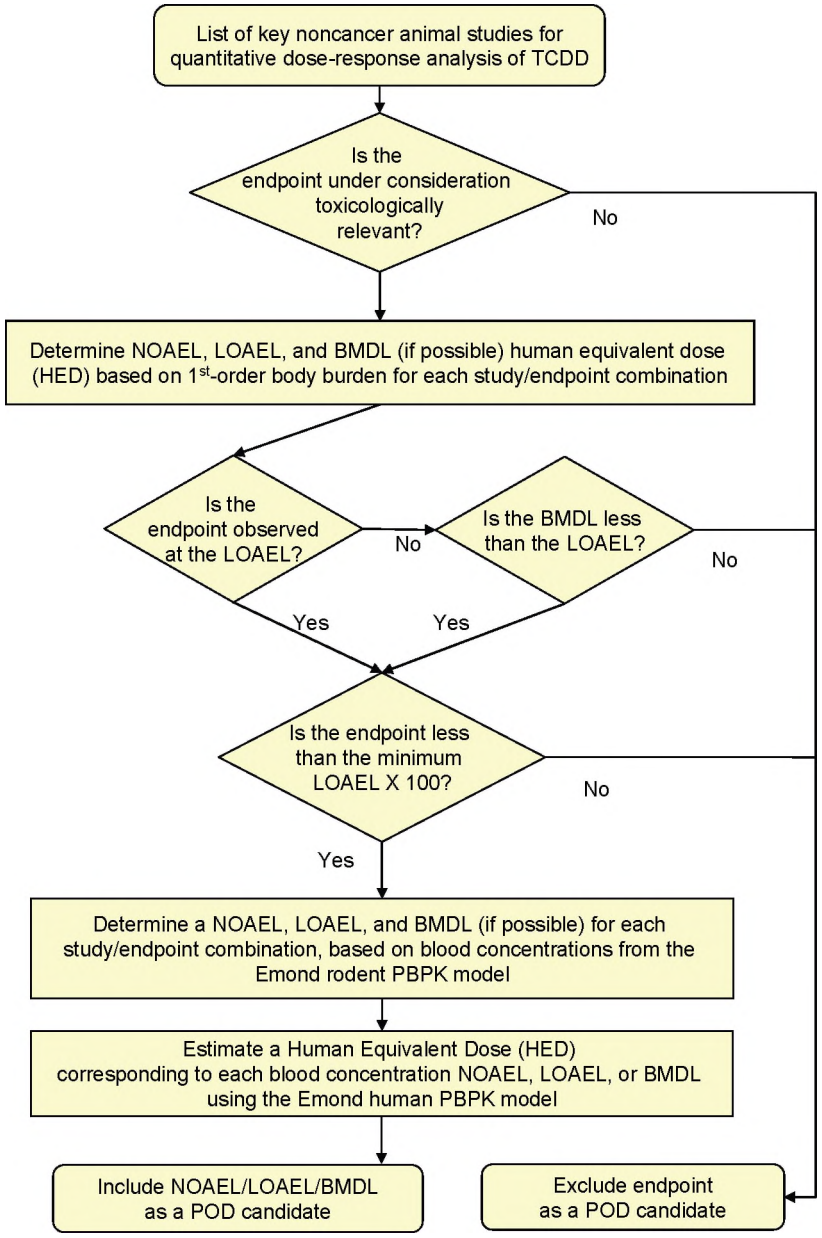


Figure 4-2. EPA's process to select and identify candidate PODs from key animal bioassays for use in noncancer dose-response analysis of TCDD. For each noncancer endpoint reported in the studies that qualified for TCDD dose-response assessment using the study inclusion criteria, EPA evaluated the endpoint and eliminated it if it was not toxicologically relevant for RfD deriation. Then, relevant endpoints not observed at the LOAEL (i.e., reported at higher doses) with BMDLs greater than the LOAEL were eliminated from further analysis. Endpoints with LOAELS greater than the minimum LOAEL times 100 also were eliminated from further analysis. Using kinetic modeling, EPA developed human equivalent doses for each remaining NOAEL/LOAEL/BMDL associated with selected endpoints and included these as candidate PODs.

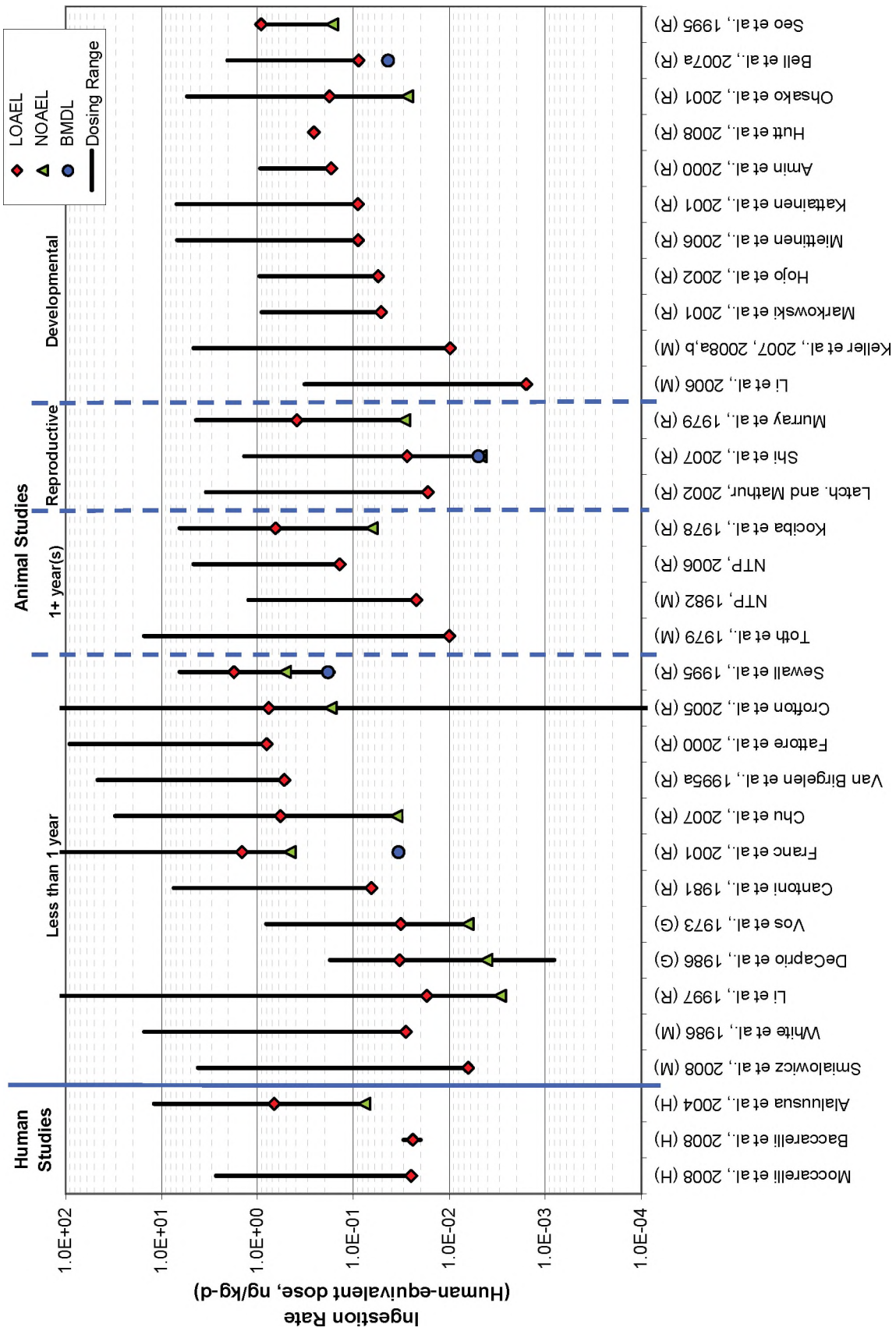


Figure 4-3. Exposure-response array for ingestion exposures to TCDD.

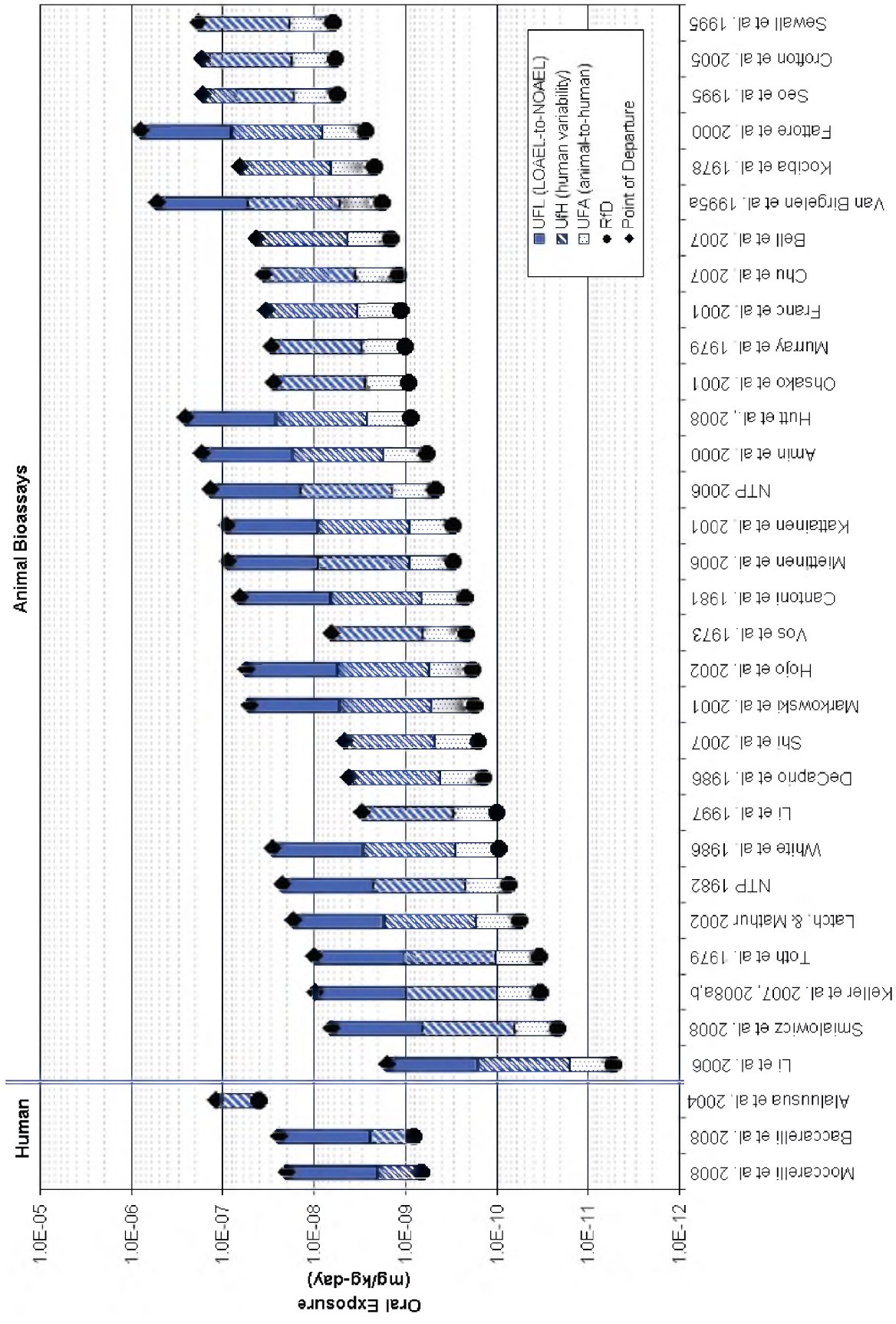


Figure 4-4. Candidate RfD array.

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5. CANCER ASSESSMENT

5.1. QUALITATIVE WEIGHT-OF-EVIDENCE CARCINOGEN CLASSIFICATION FOR 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)

5.1.1. Summary of National Academy of Sciences (NAS) Comments on the Qualitative Weight-of-Evidence Carcinogen Classification for 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD)

In its charge, the National Academy of Sciences (NAS) was requested to comment specifically on U.S. Environmental Protection Agency (EPA)'s conclusion that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is best characterized as "carcinogenic to humans." While indicating that distinction between the categories of "carcinogenic to humans" and "likely to be carcinogenic to humans" is "…based more on semantics than on science…" (NAS, 2006, 198441, p. 141) and recommending that EPA "…spend its energies and resources on more carefully delineating the assumptions used in quantitative risk estimates for TCDD…" (NAS, 2006, 198441, p. 141) rather than on the qualitative cancer descriptor for TCDD, the NAS provided the following comments:

> …the classification of dioxin as "carcinogenic to humans" versus "likely to be carcinogenic to humans" depends greatly on the definition and interpretation of the specific criteria used for classification, with the explicit recognition that the true weight of evidence lies on a continuum with no bright line that easily distinguishes between these two categories. The committee agreed that, although the weight of epidemiological evidence that dioxin is a human carcinogen is not strong, the human data available from occupational cohorts are consistent with a modest positive association between relatively high body burdens of dioxin and increased mortality from all cancers. Positive animal studies and mechanistic data provide additional support for classification of dioxin as a human carcinogen. However, the committee was split on whether the weight of evidence met all the necessary criteria described in the cancer guidelines for classification of dioxin as "carcinogenic to humans." EPA should summarize its rationale for concluding that dioxin satisfies the criteria set out in the most recent cancer guidelines for designation as either "carcinogenic to humans" or "likely to be carcinogenic to humans (NAS, 2006, 198441, p. 140).
>
> If EPA continues to designate dioxin as "carcinogenic to humans," it should explain whether this conclusion reflects a finding that there is a strong association between dioxin exposure and human cancer or between dioxin exposure and a key precursor event of dioxin's mode of action (presumably AhR binding). If EPA's finding reflects the latter association, EPA should explain why that end point

(e.g., AhR binding) represents a "key precursor event (NAS, 2006, 198441, p. 141).

5.1.2. EPA's Response to the NAS Comments on the Qualitative Weight-of-Evidence Carcinogen Classification for TCDD

A cancer descriptor is used to express the conclusion of the weight of evidence regarding the carcinogenic hazard potential of a compound. EPA agrees with the NAS committee that cancer descriptors represent points along a continuum of evidence. Relatedly, EPA acknowledges that there are gradations and borderline situations that cannot be communicated through a descriptor and are best clarified by a full weight of evidence narrative.

The 2003 Reassessment contains a detailed discussion of TCDD carcinogenicity in both humans (Part II, Chapter 7a; 8) and animals (Part II, Chapter 6; 8) as well as an overall summary of TCDD carcinogenicity (Part III, Chapter 2.2.1). Since the release of the 2003 Reassessment, the database pertaining to TCDD carcinogenicity has been strengthened and expanded by numerous publications (U.S. EPA, 2008, 519261), including a new chronic bioassay in female rats (NTP, 2006, 543749) and several new follow-up epidemiological investigations (see Section 2.4.1 and references therein). Many of these studies have been published subsequent to the NAS review. These new data are summarized and evaluated in Section 2.4 of this document.

As noted by the NAS, the 2003 Reassessment was released prior to EPA's publication of the U.S. EPA *Guidelines for Carcinogen Risk Assessment* ("2005 Cancer Guidelines"; U.S. EPA, 2005, 086237). Using EPA's guidance at the time of its release (U.S. EPA, 1996, 198087), the 2003 Reassessment determined that the available evidence was sufficient to classify TCDD as a "human carcinogen." The 1996 guidance suggested "human carcinogen" to be an appropriate descriptor of carcinogenic potential when there is an absence of conclusive epidemiologic evidence to clearly establish a cause-and-effect relationship between human exposure and cancer, but there are compelling carcinogenicity data in animals and mechanistic information in animals and humans demonstrating similar modes of carcinogenic action.

The 2005 Cancer Guidelines (U.S. EPA, 2005, 086237) are intended to promote greater use of the increasing scientific understanding of the mechanisms that underlie the carcinogenic process. The 2005 Cancer Guidelines expand upon earlier guidance applied in the 2003 Reassessment and encourage the use of chemical- and site-specific data versus default options, the consideration of mode of action information and understanding of biological changes, fuller

characterization of carcinogenic potential, and consideration of differences in susceptibility. The 2005 Cancer Guidelines also emphasize the importance of weighing all of the available evidence in reaching conclusions about the human carcinogenic potential of an agent. As noted above, additional information on TCDD carcinogenicity has been published since the release of the 2003 Reassessment. This information has expanded the TCDD database and provided additional support for conclusions made in the 2003 Reassessment regarding the carcinogenic potential of TCDD.

Under the 2005 Cancer Guidelines (U.S. EPA, 2005, 086237), TCDD is characterized as *carcinogenic to humans*, based on the available data as of 2009. The 2005 Cancer Guidelines indicate that this descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer or when all of the following conditions are met (a) there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action, but not enough for a causal association, and (b) there is extensive evidence of carcinogenicity in animals, and (c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and (d) there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information.

As noted above, the NAS commented that EPA should "...explain whether this conclusion reflects a finding that there is a strong association between dioxin exposure and human cancer or between dioxin exposure and a key precursor event of dioxin's mode of action (presumably AhR binding)" (NAS, 2006, 198441). When evaluating the carcinogenic potential of a compound, EPA employs a weight of evidence approach in which all available information is evaluated and considered in reaching a conclusion. The following sections provide a summary of EPA's weight of evidence evaluation for TCDD.

5.1.2.1. *Summary Evaluation of Epidemiologic Evidence of TCDD and Cancer*

The available occupational epidemiologic studies provide convincing evidence of an association between TCDD exposure and all cancer mortality. Among the strongest of these are the studies of over 5,000 U.S. chemical manufacturing workers (the National Institute for Occupational Safety and Health [NIOSH] cohort) (Aylward et al., 1997, 594365; Cheng et al.,

2006, 523122; Collins et al., 2009, 197627; Fingerhut et al., 1991, 197301; Steenland et al., 1999, 197437; Steenland et al., 2001, 198589); a study of nearly 2,500 German workers involved in the production of phenoxy herbicides and chlorophenols (the Hamburg cohort) (Becher et al., 1996, 197121; Becher et al., 1998, 197173; Flesch-Janys et al., 1995, 197261; Flesch-Janys et al., 1998, 197339; Manz et al., 1991, 199061; Nagel et al., 1994, 594369); a study of more than 2,000 Dutch workers in two plants involved in the synthesis and formulation of phenoxy herbicides and chlorophenols (the Dutch cohort) (Bueno et al., 1993, 196993; Hooiveld et al., 1998, 197829); a smaller study of roughly 250 workers involved in a chemical accident cleanup (the BASF cohort) ed in a chemical accident cleanup (the BASF cohort) (Ott and Zober, 1996, 198101; Thiess et al., 1982, 064999; Zober et al., 1990, 197604); and an international study of more than 18,000 workers exposed to phenoxy herbicides and chlorophenols (Kogevinas et al., 1997, 198598; Saracci et al., 1991, 199190) including newer studies of smaller subsets of these workers (McBride, 2009, 198490; McBride et al., 2009, 197296; t' Mannetje et al., 2005, 197593). The findings from these studies have been thoroughly described either in the 2003 Reassessment or in Section 2.4.1 of this document.

As noted in Section 2.4, there are considerable challenges inherent in addressing potential sources of confounding from smoking and co-exposure to other carcinogens, (which could produce inflated or spurious associations), the healthy worker effect, (which could result in attenuated effects through comparison with a referent background with an inappropriately high background risk), and quantifying exposure to the populations included in many of these retrospective studies. The more recent studies of these cohorts have made significant advances in reducing the potential for bias from the healthy worker effect through use of internal cohort analyses and/or controlling for potential confounders through statistical adjustment, restriction, and use of internal comparisons. Although some exposure assessment uncertainties remain, some of these studies have also collected individual-level TCDD exposure estimates that allow quantification of effective dose necessary for dose-response modeling. Overall, the occupational data provide consistent support for an association between exposure to TCDD and increased cancer mortality.

Additional epidemiologic evidence supporting an association between TCDD exposure and cancer comes from studies investigating the morbidity and mortality of residents exposed to TCDD following an accidental release from a chemical plant near Seveso, Italy (the Seveso

cohort) (Bertazzi et al., 1989, 197013; Bertazzi et al., 1993, 192445; Bertazzi et al., 1997, 197097; Bertazzi et al., 2001, 197005; Consonni et al., 2008, 524825; Pesatori et al., 1998, 523076; Pesatori et al., 2003, 197001; Warner et al., 2002, 197489). Pesatori et al. (2003, 197001) and Consonni et al. (2008, 524825) were not available at the time the 2003 Reassessment was released. Among individuals with relatively high exposure at Seveso (Zones A and B combined), all-cancer mortality in the 20-year post-accident period and all-cancer incidence in the 15-year post-accident period failed to exhibit significant departures from the expected 197001). However, an increased risk of all-cancer mortality was noted among men 15–20 years after first exposure; not only is the association similar in magnitude to other studies (relative risk [RR] = 1.3; 95% confidence interval [CI] = 1.0–1.7) but also emphasizes the importance of consideration of latency (Bertazzi et al., 2001, 197005). Furthermore, associations between TCDD and some specific cancer sites were detected in this cohort, including increased incidence (based on 15 years of follow-up) and mortality (based on 20 years follow-up) from lymphatic and hematopoietic neoplasms in both males and females from Zones A and B (Consonni et al., 2008, 524825). This excess was primarily due to non-Hodgkin's lymphoma. Additionally, there was an increase in lung and rectal cancer mortality in men (Bertazzi et al., 2001, 197005) and limited evidence of increased liver cancer incidence in women based on the 15-year follow-up study (Bertazzi et al., 1993, 192445). In a separate analysis of 981 women in Zone A, breast cancer incidence (n = 15) was associated (a 2-fold increase for a 10-fold increase in serum TCDD) with TCDD measurements first collected in 1976 and 1977 (Warner et al., 2002, 197489). The authors also reported a 2–3-fold increase in all cancer incidence (n = 21) for the two upper quartiles of TCDD exposure.

Overall, the newer studies of the Seveso cohort have reported significant increases in cancer incidence and elevations in cancer mortality that were not evident in earlier studies of this cohort. While these studies demonstrate an association between TCDD exposure and different types of cancer, one of the main limitations is the small number of cancer cases to assess site-specific associations with TCDD exposure. Ongoing studies in that cohort should help further elucidate potential risk for specific cancer types (and other endpoints) associated with TCDD exposures among this population.

5.1.2.1.1. *Evidence for causality.*

The evidence for causality for cancer from the human studies is briefly summarized in the paragraphs that follow and is based on recommendations from the 2005 Cancer Guidelines. It should be noted that there are methodological limitations of the epidemiologic studies that may temper some of the conclusions regarding causality. These limitations include limited statistical power, exposure assessment uncertainty, and lack of control of confounders (e.g., dioxin-like compounds and smoking) in some studies. There also is additional uncertainty in the evidence for causality due to the lack of organ specificity in TCDD associated cancers, as the most consistent results occurred for all-cancer mortality; however, this would be consistent with a hypothesized carcinogenic mode of action of TCDD as a promoter. Despite these uncertainties, many of the more recent studies have greatly improved exposure assessments compared to earlier studies of the same cohorts and have addressed the potential for confounding and other types of biases.

Temporality—exposure must precede the effect for causal inference. Given the long induction period for many types of cancers, exposure should precede the effect with a sufficient latency (i.e., typically 15−20 years for environmental carcinogens). In all the occupational studies reviewed (with the exception of (McBride, 2009, 198490)), TCDD exposure has preceded the effect with sufficient latency to be considered causally associated. In the studies of the Seveso cohort, the follow-up exposure period has now reached 20 years, a latency sufficient to address some carcinogenic endpoints. Since most of the studies are based on occupational exposures or accidental releases into the environment, temporality is more readily established due to the obvious determination of the specific exposure windows prior to disease onset.

Strength of Association—refers to the magnitude of measures of association such as the ratio of incidence or mortality (e.g., standardized mortality ratio [SMRs], standardized incidence ratios, RRs, or odds ratios) in addition to statistical significance considerations. Effect estimates that are large in magnitude are less likely to be due to chance, bias, or confounding. Reports of modest risk, however, do not preclude a causal association and may reflect an agent of lower potency, lower levels of exposure or attenuation due to nondifferential exposure misclassification. The four occupational cohorts with the highest exposures (NIOSH, Hamburg, Dutch, and BASF) consistently showed statistically significant, although moderate, elevations in cancer mortality. When the data were combined, the SMR for all four subcohorts was 1.4

[95% CI = 1.2−1.6] (IARC, 1997, 537123). Based on findings from the International Agency for Research on Cancer (IARC) Working Group, increases in all cancer (combined) mortality of the magnitude reported for TCDD have rarely been found in occupational cohort studies (IARC, 1997, 537123). Although these estimates are higher than the all-cancer mortality results among Seveso men (RR = 1.1; 95% CI = 1.0−1.3), they are comparable to the risk estimated in this population (RR = 1.3; 95% CI = 1.0-1.7) 15−20 years after first exposure.[31] These consistent results comparable in magnitude from the occupational cohorts and Seveso population are not likely due to chance.

The occupational cohort studies also show an increased risk for lung cancer in the previously mentioned four subcohorts. The relative risk for lung cancer in the combined highly exposed subcohorts was estimated to be 1.4 (95% CI = 1.1−1.7) (IARC, 1997). This is consistent with the lung cancer mortality findings for the highest exposed group of men in Seveso (RR = 1.3; 95% CI = 1.0−1.7). Additionally, there was an increase in rectal cancer mortality in the Seveso cohort (RR = 2.4; 95% CI = 1.2−4.6) (Bertazzi et al., 2001, 197005) with a corresponding increase in incidence. Consistent relative risks of more than two were also detected for rectal cancer in the Hamburg and New Zealand cohorts, but increased risks were not found in the other cohorts. Although there was limited evidence of increased incidence or morality from hepatobiliary cancers across the cohorts, liver cancer incidence was elevated in the 15-year post accident period among women in the Seveso cohort (RR = 2.4; 95% CI = 1.1−5.1, (Warner et al., 2002, 197489)). An association in this population was also detected for between breast cancer incidence (RR = 2.1; 95% CI = 1.0–4.6) and serum TCDD levels (per a 10-fold increase in serum TCDD). Although findings were based on small numbers, three- and four-fold increased risks of soft tissue sarcoma were detected among the NIOSH (Collins et al., 2009, 197627) and New Zealand cohorts (McBride, 2009, 198490). No other cases of this very rare cancer were detected in the exposed populations from the other cohorts.

[31] In addition to consideration of statistical significance to address the possibility of random variability (i.e., chance), many other factors are important to consider when assessing causality using a weight of evidence determination. As noted in the EPA's Cancer Guidelines, a number of factors besides statistical significance are relevant for assessing evidence of adverse health effects based on human data. These include strength of association, temporality, biological gradient (i.e., dose-response concordance), biological plausibility, etc.). In analyzing the body of information in the literature, the consistency of the magnitude of reported risk estimates (across different studies) is considered when addressing causality; rather than relying solely on statistical significance.

Elevated risk of lymphohemopoietic cancer mortality was noted among the Seveso cohort (RR = 1.7; 95% CI = 1.2, 2.5) (Consonni et al., 2008, 524825). Increased SMRs for lymphohemopoietic cancer comparable in magnitude (range: 1.6–2.2) were also detected among the Hamburg and New Zealand occupational cohorts, but limited evidence (range: 1.0 to 1.2) of increased mortality was found in the BASF, NIOSH and Ranch Hands employees (Akhtar et al., 2004, 197141; Ott and Zober, 1996, 198101; Steenland et al., 1999, 197437). Most of the lymphohemopoietic cancer mortality risk was reportedly due to non-Hodgkin's lymphoma in most of the cohorts. Relative risks for non-Hodgkin's lymphoma among TCDD exposed populations from the NIOSH, Hamburg, New Zealand, Dutch, and Seveso cohorts ranged from 1.2 to 3.8. Although statistical power was limited in most of these studies, relative risks exceeded 3.0 for non-Hodgkin's lymphoma in three of these cohorts (Consonni et al., 2008, 524825; Flesch-Janys et al., 1998, 197339; Hooiveld et al., 1998, 197829).

Consistency—the observation of the same site-specific effect across several independent study populations strengthens an inference of causality. Despite differences across occupational cohorts, most studies have consistently reported increases in all-cancer mortality with TCDD exposure. Several of these studies have also reported increases in lung cancer related to TCDD exposure. As noted above, there is also suggestive evidence of an increased risk in all-cancer and lung cancer mortality among the Seveso cohort consistent in magnitude to the occupational cohorts. Elevated risk of lymphohemopoietic cancer mortality consistent in magnitude (range: 1.6–2.2) was also detected among the Seveso, Hamburg and New Zealand cohorts. An increased risk for non-Hodgkin's lymphoma was found in two of the occupational cohorts as well as in the Seveso cohort, although the relative risks largely did not achieve statistical significance. Among those studies detecting an association, consistent two-fold relative risks were found for rectal cancer (Bertazzi et al., 2001, 197005; Flesch-Janys et al., 1998, 197339; McBride, 2009, 198490) and relative risks in excess of three were detected for soft tissue sarcoma (Collins et al., 2009, 197627; McBride, 2009, 198490).

Biological Gradient—refers to the presence of a dose-response and/or duration-response between a health outcome and exposure of interest. Several of the occupational cohort studies (Flesch-Janys et al., 1998, 197339; Manz et al., 1991, 199061; Michalek and Pavuk, 2008, 199573; Ott and Zober, 1996, 198101; Steenland et al., 1999, 197437) found evidence of a dose-response relationship for all cancers and various TCDD exposure measures. The SMR

analyses based on internal comparisons within the occupational cohorts show a biological gradient by comparing highly TCDD exposed workers to low or unexposed workers. A biological gradient was also demonstrated in the Seveso cohort by comparing highly exposed individuals (Zones A and B) to individuals in lower exposure zones (Zones C and R). Warner et al. (2002, 197489) also reported evidence of a dose-response trend for breast cancer and increasing TCDD exposures.

Biological Plausibility—refers to the observed effect having some biological link to the exposure. Most evidence suggests that toxic effects of TCDD are mediated by interaction with the aryl hydrocarbon receptor (AhR). AhR is a highly conserved protein among mammals, including humans (Fujii-Kuriyama et al., 1995, 543727; Harper et al., 2002, 198124; Nebert et al., 1991, 543728). Several hypothesized modes of action have been presented for TCDD-induced tumors in rodents, all involving AhR activation. The available evidence does not preclude the relevance of these hypothesized modes of action to humans.

Specificity—as originally intended, refers to increased inference of causation if a single site effect, as opposed to multiple effects, is observed and associated with exposure. Based on current biological understanding, this is now considered one of the weaker guidelines for causality. As stated in the 2005 Cancer Guidelines, given the current understanding that many agents cause cancer at multiple sites, and cancers have multiple causes, the absence of specificity does not detract from evidence for a causal effect. Given that the most consistent findings associating TCDD and cancer are for all-cause cancer mortality, epidemiological evidence suggests that TCDD lacks specificity for particular tumor sites. A key event in TCDD's mode of action is binding to and activating AhR; however, downstream events leading to tumor formation are uncertain and may likely be tissue specific. Given that the AhR is highly conserved among species and is expressed in various human tissues, the lack of tumor site specificity does not preclude a determination of causality.

In summary, EPA finds the available epidemiological information provides strong evidence of an association between TCDD exposure and human cancer that cannot be reasonably attributed to chance or confounding and other types of bias, and with a demonstration of temporality, strength of association, consistency, biological plausibility, and a biological gradient. Additional evidence from animal studies and from mechanistic studies (described below) provides additional support for the classification of TCDD as carcinogenic to humans.

5.1.2.2. *Summary of Evidence for TCDD Carcinogenicity in Experimental Animals*

An extensive database on the carcinogenicity of TCDD in experimental animals is described in detail in Part II, Chapter 6 of the 2003 Reassessment. There is substantial evidence that TCDD is carcinogenic in experimental animals based on long-term bioassays conducted in both sexes of rats and mice (Kociba et al., 1978, 001818; NTP, 1982, 594255; NTP, 2006, 543749) and in male hamsters (Rao et al., 1988, 199032). Additionally, National Toxicology Program (NTP, 2006, 543749) has completed a new chronic bioassay in female Sprague Dawley rats. These studies are summarized in Section 2.4.2 of this document. All studies have produced positive results, with TCDD increasing the incidence of tumors at sites distant from the site of treatment and at doses well below the maximum tolerated dose. In both sexes of rodents, when administered by different routes and at low doses, TCDD caused tumors at multiple sites; tumors were observed in liver, lung, lymphatic system, soft tissue, nasal turbinates, hard palate, thyroid, adrenal, pancreas, and tongue. The most consistent and best characterized carcinogenic responses to TCDD are in the rodent liver, lung, and thyroid (discussed below in Section 5.1.2.3).

5.1.2.3. *TCDD Mode of Action*

The 2005 Cancer Guidelines defines the term "mode of action" as "a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation." A "key event" is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element. Mode of action is contrasted with "mechanism of action," which implies a more detailed understanding and description of events, often at the molecular level. In the case of TCDD, the terms 'mechanism of action' and 'mode of action' are often used interchangeably in the scientific literature in reference to TCDD's interaction with the AhR. A thorough discussion of TCDD's interaction with the AhR can be found in the 2003 Reassessment (Part II, Chapter 2; Part III, Chapter 3), and is summarized below (see Section 5.1.2.3.1).

Most evidence suggests that the majority of toxic effects of TCDD are mediated by interaction with the AhR. EPA considers interaction with the AhR to be a necessary, but not sufficient, event in TCDD carcinogenesis. The sequence of key events following binding of

TCDD to the AhR and that ultimately leads to the development of cancer is unknown. Therefore, in the strictest sense, TCDD's interaction with the AhR does not constitute a "mode of action" as defined by the 2005 Cancer Guidelines because information about the progression of necessary events is lacking. However, AhR binding and activation by TCDD is considered to be a key event in TCDD carcinogenesis.

5.1.2.3.1. *The aryl hydrocarbon receptor (AhR).*

While substantial evidence suggests that most toxic effects of TCDD are mediated by interaction with the AhR, less is known about the complex responses that result in tumor formation. Nonetheless, a picture is emerging wherein TCDD is considered a "receptor-mediated carcinogen" in laboratory animals (see Figure 5-1), acting in a manner similar to peroxisome proliferators, phorbol esters, or estrogen (Woods et al., 2007, 543735).

TCDD activates the AhR, a member of the basic helix-loop-helix, Per-Arnt-Sim (bHLH-PAS) family of transcription factors. AhR is present in most cell types and in the inactivated state is cytosolic and exists in a complex with chaperone proteins, such as heat shock protein 90 (Hsp90). Binding of TCDD to AhR leads to nuclear translocation and heterodimerization with its partner protein Arnt, another bHLH-PAS family member. The AhR:Arnt heterodimer binds to specific cognate DNA sequence elements known as dioxin/xenobiotic response elements (DRE/XRE) present in the regulatory region of specific genes. Binding of the AhR:Arnt heterodimer to these elements, and subsequent recruitment of tissue specific transcriptional coactivator complexes, leads to increased transcription of specific genes, known as "target genes." There is a battery of genes affected in this manner and targets include certain xenobiotic-metabolizing enzymes, such as cytochrome P450 (CYP)1A1, CYP1A2, CYP2B1, and UDP-glucuronosyltransferase (UGT)1A6 (reviewed in Schwartz and Appel, 2005, 543737). In addition, genes affected by the TCDD/AhR-complex code for both inhibitory and stimulatory growth factors; their gene products affect cellular growth, differentiation and homeostasis and have been shown to contribute to carcinogenicity as well as other forms of toxicity (reviewed in Popp et al., 2006, 197074).

Detailed molecular biology research has been performed to identify the extent of the genes regulated by AhR (Woods et al., 2007, 543735); however a complex and still ill-defined profile remains. The basic physiology of AhR signaling is still poorly understood, despite being

highly conserved among vertebrate species (reviewed in Hahn, 2002, 099302). In fact, it is now known that the AhR recognizes a large number of chemical structures, including nonaromatic and nonhalogenated compounds (Denison and Nagy, 2003, 197226), which supports the biological role of the AhR as a receptor that helps regulate the expression of genes necessary for biotransformation of environmental chemicals (i.e., CYP1A1). However, the endogenous physiological role of AhR is complicated, as evidenced by the numerous studies examining AhR null (ArH -/-) mice, which demonstrate alterations in the liver, immue system, ovary, heart and other organs (reviewed in Hahn, 2009, 477460). The endogenous function of AhR remains unknown.

Given that the AhR is expressed in most tissues (Dolwick et al., 1993, 543762) with tissue-specificity in terms of level of expression and the profile of target genes, there is substantial complexity and difficulty associating TCDD-mediated transcription of specific target genes and tissue-specific toxic responses, including cancer. It is important to note that the extent of the response of individual TCDD target genes does not correlate with site-specific tumorigenicity. For example, while TCDD is ineffective as a tumor promoter in ovariectomized rats and does not stimulate liver cell proliferation in these animals, it is still capable of inducing CYP1A2 in roughly the same magnitude as in the intact female rats (Lucier, 1991, 198691). Similarly, CYP1A1 induction by TCDD is very similar in male and female rats even though males are almost completely resistant to TCDD carcinogenicity (Wyde et al., 2002, 197009).

Some of AhR's effects on gene expression may be the result of interaction with other transcription factors (such as the retinoblastoma protein(Ge and Elferink, 1998, 197702), NF-κB (Tian et al., 1999, 198378) or with the tyrosine kinase c-Src (Blankenship and Matsumura, 1997, 543751) rather than via direct interaction with DNA. By far the most extensive studies involving cross-talk between AhR and another transcription factor are those involving the estrogen receptor alpha (ERα). The anti-estrogenic properties of TCDD have been well-documented, beginning with the observations that TCDD repressed estradiol function in rat uterus and liver. The AhR-ERα cross-talk can be manifested at several levels including direct protein interaction, association of the receptors with the other's response element and altered metabolism of estradiol by AhR ligand (Takemoto et al., 2004, 543753). The interactions between AhR/Arnt- and estrogen receptor-dependent signaling pathways, which mediate anti-estrogenic effects of dioxins and dioxin-like polychlorinated biphenyls (PCBs; Bock, 1994, 543755), is probably

causal for the well-documented gender-specificity of the carcinogenic effects of these agents (e.g., hepatocarcinogenicity of TCCD in female as opposed to male rats) (Lucier, 1991, 198691). In addition, cross-talk between AhR/Arnt and other nuclear receptors, their coactivators, and corepressors, has been described. In fact, cross-talk has been reported for AhR and numerous signaling pathways involved in a broad range of physiological processes. The molecular mechanisms by which the AhR interferes with these signaling networks are multifaceted and occur at multiple levels of regulation (many beyond transcriptional control) (Haarmann-Stemmann et al., 2009, 197874). It remains unknown how any of these molecular pathways involving AhR signaling are linked to TCDD-mediated carcinogenesis.

Pertinent to human risk assessment, there are wide inter- and intraspecies differences in the toxicological responses to TCDD (Ema et al., 1994, 197313; Poland and Glover, 1990, 543759; Poland et al., 1994, 198439) some of which can be explained by polymorphisms in AhR. For instance, there is a 10-fold difference in susceptibility to TCDD-induced toxicity between the TCDD-sensitive C57BL/6 and the TCDD-resistant DBA/2 strains of mice (Poland and Glover, 1980, 543761) that can be explained by polymorphic variations in the ligand-binding domain and in the C-terminal region of the AhR molecule of each strain (Dolwick et al., 1993, 543762). Depending on the system examined, the estimated affinity of binding of TCDD (and related compounds) to the human AhR is about 10-fold lower than that observed to the AhR from "responsive" rodent species and is comparable to that observed to the AhR from "nonresponsive" mouse strains (Ramadoss and Perdew, 2004, 198824). This reduced affinity is due, in part, to a single amino acid substitution within the ligand binding domain of the human and "nonresponsive" mouse AhRs (Ramadoss and Perdew, 2004, 198824). Although the affinity of binding of TCDD and related compounds to the human AhR is reduced compared with rodent AhRs, the qualitative and quantitative rank-order potency of these chemicals is similar. The considerable tissue and species variability in response to TCDD cannot be ascribed solely to polymorphisms of the AhR gene (Geyer et al., 1997, 543768; Pohjanvirta and Tuomisto, 1994, 543767), further complicating this key event in TCDD-mediated carcinogenesis.

5.1.2.3.1.1. ***Other AhR considerations.***

In addition to the potent agonist TCDD, there are many other exogenous ligands for the AhR, including certain polycyclic aromatic hydrocarbons, polychlorinated dibenzofurans, and

PCBs (Bock, 1994, 543755). Several natural and endogenous compounds are also regulators of AhR (Chiaro et al., 2008, 543771). The classes of endogenous compounds that have been shown to induce CYP1 and/or activate AhR include: (a) tryptophan metabolites, other indole-containing molecules, and phenylethylamines (Gielen and Nebert, 1971, 543775); (b) tetrapyrroles such as bilirubin and biliverdin; (c) sterols such as 7-ketocholesterol and the horse steroid equilenin; (d) fatty acid metabolites, including at least six different prostaglandins (Seidel et al., 2001, 543776) and lipoxin A4; and (e) the ubiquitous second messenger cAMP (reviewed in McMillan and Bradfield (2007, 543777) and Barouki et al. (2007, 543778)). Several of these endogenous and exogeous compounds, including bilirubin, biliverdin, and β-naphthoflavone, that also bind to the AhR are not carcinogenic in rodent models, therefore, some other key precursor event(s) need to be identified. Further, the existence of multiple ligands with varying affinity and responses suggests that "selective receptor modulators" (or SRMs) of the AhR exist. SRMs are ligands for a receptor that, upon binding, elicit a conformational change in the receptor that results in differential recruitment of coregulatory molecules to the target gene promoter region, thereby imparting a different biological activity relative to the prototypical ligand. This phenomenon has been most studied for nuclear receptors such as the ERα with the classic example being tamoxifen, which has estrogen-like activity in the uterus but anti-estrogen-like effects in the breast. Thus, the relative abilities of compounds to stimulate gene expression or other effects vary in promoter- and cell type-specific manners. It is now apparent that SRMs exist for the AhR as well (SAhRMs, Fretland et al., 2004, 197357). For example, 6-methyl-1,3,8-trichlorodibenzofuran (6-MCDF), a SAhRM whose structure is similar to that of TCDD, can induce CYP1A1 gene expression in liver but does not lead to the toxic responses associated with TCDD (Fritz et al., 2009, 594372). The existence of SAhRMs further complicates the role of TCDD binding to AhR as a key event in TCDD-mediated carcinogenicity, and suggests that additional information is necessary to elucidate the carcinogenic mode of action of TCDD.

TCDD may have dose-dependent modes of action. It has been demonstrated that AhR-deficient (AhR-/-) mice show no signs of toxicity at doses of TCDD approximating the lethal dose eliciting 50% response (LD_{50}) dose (200 μg/kg) in AhR +/+ mice (Fernandez-Salguero et al., 1996, 197650). However, a single high exposure of 2,000 μg/kg to AhR-deficient mice produced several minor lesions including scattered necrosis and vasculitis in

the liver and lungs. These data suggest that a pathway leading to toxicity exists, albeit at very high doses, that is independent of the AhR. However, these data also indicate that, at least in mice, the major in vivo effects of TCDD are mediated through the AhR. The finding of carcinogenicity in hamsters (Rao et al., 1988, 199032) is of special interest since hamsters have been found to be relatively resistant to the lethal effects of TCDD (Henck et al., 1981, 543779; Olson et al., 1980, 197976). To date, there have been no chronic bioassay studies of TCDD carcinogenicity in AhR-deficient transgenic animals.

There are additional insights into the complexity of TCDD's mechanism of action involving AhR. Some biochemical responses to TCDD treatment in isolated cells have been reported in cells lacking Arnt, in cells expressing a mutated Arnt protein and in cells with highly reduced levels of AhR (Kolluri et al., 1999, 548721; Puga et al., 1992, 543784), implying either a non nuclear role of the AhR in mediating these events or an AhR-independent process.

Additionally, recent studies have linked AhR activation in the absence of exogenous ligand to a multitude of biological effects, ranging from control of mammary tumorigenesis to regulation of autoimmunity (Hahn et al., 2009, 548725). Finally, constitutively activated AhR in rodents has been shown to induce stomach tumors (Andersson et al., 2002, 197101). This indicates that AhR activation alone (i.e., in the absence of ligand) is sufficient to induce tumors.

5.1.2.3.2. *TCDD as a tumor promoter.*

The role of TCDD as a tumor promoter is discussed in the 2003 Reassessment (Part II, Chapter 6). The following is a brief summary of the information regarding TCDD as a tumor promoter.

Numerous studies have examined the tumor promoting potential of TCDD. Using the traditional two-stage initiation-promotion study design in the liver, studies have demonstrated that TCDD is a dose- and duration-dependent liver tumor promoter (Dragan and Schrenk, 2000, 197243; Maronpot et al., 1993, 198386; Pitot et al., 1980, 197885; Teeguarden et al., 1999, 198274; Walker et al., 2000, 198733)(Walker et al., 1998). TCDD has also tested positive for tumor promoting ability in the two-stage models of mouse skin tumorigenesis (Dragan and Schrenk, 2000, 197243; IARC, 1997, 537123), and in the lung (Anderson et al., 1991, 201761; Beebe et al., 1995, 548754). Overall, the data demonstrate that TCDD is a tumor promoter and potentially harbors only weak initiating activity.

TCDD is typically designated as a nongenotoxic and nonmutagenic carcinogen because it does not damage DNA directly through the formation of DNA adducts, is negative in most short-term assays for genotoxicity, and is a potent tumor promoter and a weak initiator or noninitiator in multistage models for chemical carcinogenesis (Clark et al., 1991, 594378; Flodstrom and Ahlborg, 1991, 548728; Graham et al., 1988, 594375; Lucier, 1991, 198691; Pitot et al., 1980, 197885; Poland et al., 1982, 199756). However, mechanisms have been proposed that support the possibility that TCDD might be indirectly genotoxic, either through the induction of oxidative stress or by altering the DNA-damaging potential of exogenous and endogenous compounds, such as estrogens. In addition, there have been numerous reports demonstrating TCDD-induced modifications of growth factor signaling pathways and cytokines in experimental animals and cell culture systems. Some of the altered signaling pathways include those for epidermal growth factor, transforming growth factor alpha, glucocorticoids, estrogen, tumor necrosis factor-alpha, interleukin 1-beta, plasminogen inactivating factor-2, and gastrin. Many of these pathways are involved in cell homeostasis, proliferation, and differentiation and provide plausible mechanisms responsible for the carcinogenic actions of TCDD. Unfortunately, information on the etiology of the different tumor types is lacking to equivocally link tumor promotion or indirect genotoxic action of TCDD to a specific mechanism or mode of TCDD carcinogenesis.

5.1.2.3.3. *Hypothesized modes of action of TCDD in rodents.*

TCDD has been shown to consistently induce multiple tumors in both sexes in several rodent species. These tumors are observed in various tissues, including (but not limited to): liver, lung, thyroid, lymphatic system, soft tissue, nasal turbinates, hard palate, adrenal, pancreas, and tongue. While the mode of action of TCDD in producing cancer has not been elucidated for any tumor type, the best characterized carcinogenic actions of TCDD are in rodent liver, lung, and thyroid. The hypothesized mode of action for each of these three tumor types is briefly discussed below and is described in Figure 5-2. The hypothesized sequence of events following TCDD interaction with the AhR is markedly different for each of these three tumor types. No detailed hypothesized mode of action information exists for any of the other reported tumor types. Further, no single definitive mode of action of TCDD-mediated carcinogenicity has been identified.

5.1.2.3.3.1. *Liver tumors.*

The mode of action of TCDD in producing liver cancer in rodents has not been elucidated. One hypothesized mode of carcinogenic action of TCDD in the liver is mediated through hepatotoxicity. Generically speaking, TCDD activation of the AhR leads to a variety of changes in gene expression, which then lead to hepatotoxicity, followed by compensatory regenerative cellular proliferation and subsequent tumor development (see Figure 5-2). The details of the mechanism of TCDD-induced hepatotoxicity have not been fully determined but both CYP induction and oxidative stress have been postulated to be involved (Maronpot et al., 1993, 198386; Viluksela et al., 2000, 198968). The enhanced cell proliferation arising from either altered gene expression or hepatotoxicity, or both, may lead to the promotion of hepatocellular tumors (Whysner and Williams, 1996, 197556). The sensitivity of female rat liver to TCDD, which apparently does not extend to the mouse, depends on ovarian hormones (Lucier, 1991, 198691; Wyde et al., 2001, 198575). This sensitivity has been ascribed to induction of estradiol metabolizing enzymes (Graham et al., 1988, 594375) and is hypothesized to lead either to generation of reactive metabolites of endogenous estrogen or to active oxygen species of estrogens. Oxidative DNA damage has been implicated in liver tumor promotion (Umemura et al., 1999, 198001).

A dose-response relationship exists for TCDD-mediated hepatotoxicity, and this parallels the dose-response relationship for tumor formation (or formation of foci of cellular alteration as a surrogate of tumor formation). However, the dose-response relationship for other TCCD-induced responses such as enhanced gene expression is different from the dose-response for tumor formation in terms of both efficacy and potency (see Popp et al. (2006, 197074) for review). It is important to note that differences in potency between events (i.e., gene expression versus cell proliferation) does not necessary imply alternative mechanisms of action.

5.1.2.3.3.2. *Lung tumors.*

The mode of action of TCDD in producing lung cancer in rodents (predominantly keratinizing squamous cell carcinoma, (Larsen, 2006, 548744)) has not been elucidated. One hypothesized mechanism of the carcinogenic action of TCDD in the lung involves disruption of retinoid homeostasis in the liver (see Figure 5-2). Retinoic acids and their corresponding nuclear receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs), work together

to regulate cell growth, differentiation, and apoptosis. It is hypothesized that TCDD, through activation of the AhR, can affect parts of the complex retinoid system and/or other signaling systems regulated by, and/or cross-talking with, the retinoid system (reviewed in (Nilsson and Håkansson, 2002, 548746)). These effects are then hypothesized to lead to lung tumor development; however, the mechanisms underlying this hypothesis are not well-defined. Pulmonary squamous proliferative lesions have been reported following oral exposure to TCDD in rats (Tritscher et al., 2000, 197265). In general, squamous metaplasia with some inflammation is associated with significant forms of injury via inhalation of toxic compounds but is also seen with vitamin A deficiency (Tritscher et al., 2000, 197265) and gives some credence to this hypothesis.

Another hypothesized mechanism for the carcinogenic action of TCDD in the lung is through induction of metabolic enzymes. Through activation of AhR and subsequent induction of metabolizing enzymes (such as CYP1A1), TCDD may enhance bioactivation of other carcinogens in lung (Tritscher et al., 2000, 197265). There have been few studies to support this hypothesis; however, in a long-term continuous-application study of carcinogenesis using airborne particulate extract (APE), squamous cell carcinoma occurred in 8 of 17 AhR+/+ mice (47%) while no tumors were found in AhR-/- mice (Matsumoto et al., 2007, 548748). In addition CYP1A1 was induced in AhR+/+ mice but not in AhR-/- mice in this study. These results suggest that AhR plays a significant role in APE-induced carcinogenesis in AhR+/+ mice and CYP1A1 activation of carcinogenic polycyclic aromatic hydrocarbons (the primary carcinogenic component of APE) is also of importance.

5.1.2.3.3.3. ***Thyroid tumors.***

The mode of action of TCDD in producing thyroid cancer in rodents has not been elucidated. It is hypothesized that TCDD increases the incidence of thyroid tumors through an extrathyroidal mechanism (see Figure 5-2). The prevailing hypothesis for the induction of thyroid tumors by TCDD involves the disruption of thyroid hormone homeostasis via induction of Phase II enzymes UGTs in the liver (reviewed in Brouwer et al., 1998, 201801) by an AhR-dependent transcriptional mechanism (Bock et al., 1998, 548752; Nebert et al., 1990, 548756). This induction of hepatic UGT results in increased conjugation and elimination of thyroxine (T4), leading to reduced serum T4 concentrations. T4 synthesis is controlled by the

thyroid stimulating hormone (TSH) which is under negative and positive regulation from the hypothalamus, pituitary, and thyroid via thyrotrophin-releasing hormone, TSH, T4, and triiodothyronine. Consequently, the reduced serum T4 concentrations lead to a decrease in the negative feedback inhibition on the pituitary gland. This would then lead to a rise in secreted TSH and stimulation of the thyroid. The persistent induction of UGT by TCDD and the subsequent prolonged stimulation of the thyroid could result in thyroid follicular cell hyperplasia and hypertrophy of the thyroid, thereby increasing the risk of progression to neoplasia. Increases in blood TSH levels are consistent with prolonged stimulation of the thyroid and may represent an early stage in the induction of thyroid tumors identified in animal bioassays. Statistically significant increases in neonatal blood TSH levels have been recently been reported in children born to TCDD-exposed mothers in the Seveso cohort (Baccarelli et al., 2008, 197059, discussed in Section 2.4.1.1.1.4.4). Support for this hypothesis comes from several studies showing that TCDD decreases serum total thyroxine and free thyroxine concentrations in rats following both single dose and repeated dose exposures (Bastomsky, 1977, 548760; Brouwer et al., 1998, 201801; Pohjanvirta et al., 1989, 548766; Potter et al., 1983, 548769; Potter et al., 1986, 548771; Sewall et al., 1995, 198145; Van Birgelen et al., 1995, 198052). Further support comes from studies of transgenic animals in which TCDD exposure resulted in a marked reduction of total thyroxin and free T4 levels in the serum of AhR+/- mice but not AhR-/- mice (Nishimura et al., 2005, 197860). Additionally, gene expression of UGT1A6, CYP1A1, and CYP1A2 in the liver was markedly induced by TCDD in AhR+/- but not AhR-/- mice (Nishimura et al., 2005, 197860).

5.1.2.3.4. *Summary of TCDD mode of action in rodents.*

Overall, there are inadequate data to support the conclusion that any of the particular mode of action hypotheses described above is operant in TCDD-induced carcinogenesis. However, the wealth of scientific evidence available indicates that most, if not all, of the biological and toxic effects of TCDD are mediated by the AhR. Although the receptor may be necessary for the occurrence of these events, it is not sufficient because other proteins and conditions are known to affect the activity of the receptor and its ability to alter gene expression or to induce other effects. Certain studies could be interpreted to indicate AhR-independent mechanisms, although these studies have not clearly ruled out involvement of the AhR. The

only consistent, but limited, evidence for TCDD-induced effects that do not involve the AhR comes from studies using AhR-deficient transgenic animals. Here however, only minor effects occurred following treatment with extremely high doses of TCDD. Thus, a toxic response to TCDD has AhR interaction as a key event, but there are various species-, cell-, development-, gender-, and disease-dependent differences in the cellular milieu that can affect the nature and extent of the response observed.

The findings that many AhR-modulated effects are regulated with distinct specificity supports the understanding that the molecular and cellular pathways leading to any particular toxic event are extremely complex. Precise dissection of these events represents a considerable challenge, especially in that a toxic response may depend on timely modulation of several genes rather than of just one particular gene, and possibly modulation of these genes in several rather than just one cell type or tissue.

While a defined mechanism at the molecular level or a defined mode of action for TCDD-induced carcinogenicity is lacking, EPA concludes the following

- interaction with the AhR is a necessary early event in TCDD carcinogenicity in experimental animals.
- through interaction with the AhR, TCDD modifies one or more of a number of cellular processes, such as induction of enzymes, changes in growth factor and/or hormone regulation, and/or alterations in cellular proliferation and differentiation.
- AhR activation is anticipated to occur in humans and may progress to tumors. AhR is present in human cells and tissues, studies using human cells are consistent with the hypothesis that the AhR mediates TCDD toxicity and no data exist to suggest that the biological effects of AhR activation by TCDD are precluded in humans.
- non-AhR mediated carcinogenic effects of TCDD are possible.

5.1.3. Summary of the Qualitative Weight of Evidence Classification for TCDD

Under the 2005 Cancer Guidelines (U.S. EPA, 2005, 086237), TCDD is characterized as carcinogenic to humans, based on the available data as of 2009. This conclusion is based on

- Multiple occupational epidemiologic studies showing strong evidence of an association between TCDD exposure and increased mortality from all cancers.
- Epidemiological studies showing an association between TCDD exposure and certain cancers in individuals accidentally exposed to TCDD in Seveso, Italy.

- Extensive evidence of carcinogenicity at multiple tumor sites in both sexes of multiple species of experimental animals.
- General scientific consensus that the mode of TCDD's carcinogenic action in animals involves AhR-dependent key precursor events and proceeds through modification of one or more of a number of cellular processes, such as induction of enzymes, changes in growth factor and/or hormone regulation, and/or alterations in cellular proliferation and differentiation.
- The human AhR and rodent AhR are similar in structure and function and human and rodent tissue and organ cultures respond to TCDD in a similar manner and at similar concentrations.
- General scientific consensus that AhR activation is anticipated to occur in humans and may progress to cancers.

5.2. QUANTITATIVE CANCER ASSESSMENT

5.2.1. Summary of NAS Comments on Cancer Dose-Response Modeling

5.2.1.1. *Choice of Response Level and Characterization of the Statistical Confidence Around Low Dose Model Predictions*

The NAS commented on the low dose model predictions in the 2003 Reassessment, including EPA's development of ED_{01} (effective dose eliciting x percent response) estimates for numerous study/endpoint combinations. The committee also suggested that EPA had not appropriately characterized the statistical confidence around such model predictions in the low-response region of the model.

> The committee concludes that EPA did not adequately justify the use of the 1% response level (the ED_{01}) as the POD for analyzing epidemiological or animal bioassay data for both cancer and noncancer effects. The committee recommends that EPA more explicitly address the importance of the selection of the POD and its impact on risk estimates by calculating risk estimates using alternative assumptions (e.g., the ED_{05}) (NAS, 2006, 198441, p. 18)
>
> It is critical that the model used for determining a POD fits the data well, especially at the lower end of the observed responses. Whenever feasible, mechanistic and statistical information should be used to estimate the shape of the dose-response curve at lower doses. At a minimum, EPA should use rigorous statistical methods to assess model fit, and to control and reduce the uncertainty of the POD caused by a poorly fitted model. The overall quality of the study design is also a critical element in deciding which data sets to use for quantitative modeling (NAS, 2006, 198441, p. 18).
>
> EPA should … assess goodness-of-fit of dose-response models for data sets and provide both upper and lower bounds on central estimates for all statistical

estimates. When quantitation is not possible, EPA should clearly state it and explain what would be required to achieve quantitation (NAS, 2006, 198441, p. 10).

The NAS also suggested that EPA report information describing the adequacy of dose-response model fits, particularly in the low-response region. For those cases where biostatistical modeling was not possible, the NAS recommended that EPA identify the reasons.

> The Reassessment should also explicitly address the importance of statistical assessment of model fit at the lower end and the difficulties in such assessments, particularly when using summary data from the literature instead of the raw data, although estimates of the impacts of different choices of models would provide valuable information about the role of this uncertainty in driving the risk estimates (NAS, 2006, 198441, p. 73).

5.2.1.2. *Model Forms for Predicting Cancer Risks Below the Point of Departure (POD)*

The NAS focused much of its review on EPA's derivation of a cancer slope factor. Specifically, the NAS commented extensively on the selection of the appropriate point of departure (POD) and the extrapolation of dose response modeling below the POD.

The NAS questioned EPA's choice of a linear, nonthreshold model for extrapolating risk associated with exposure levels below the POD, concluding that the current scientific evidence was sufficient to justify the use of nonlinear methods when extrapolating below the POD for TCDD carcinogenicity. The committee further recommended that EPA include a nonlinear model for low dose cancer risk estimates as a comparison to the results from the linear model.

> The committee concludes that EPA's decision to rely solely on a default linear model lacked adequate scientific support. The report recommends that EPA provide risk estimates using both nonlinear and linear methods to extrapolate below PODs(NAS, 2006, 198441, p. 5).
> After reviewing EPA's 2003 Reassessment and additional scientific data published since completion of the Reassessment, the committee unanimously agreed that the current weight of scientific evidence on the carcinogenicity of dioxin is adequate to justify the use of nonlinear methods consistent with a receptor-mediated response to extrapolate below the POD. The committee points out that data from NTP released after EPA generated the 2003 Reassessment provide the most extensive information collected to date about TCDD carcinogenicity in test animals, and the committee found the NTP results to be

> compelling. The committee concludes that EPA should reevaluate how it models the dose-response relationships for TCDD… (NAS, 2006, 198441, p. 16).
>
> Because EPA's assumption of linearity at doses below the 1% excess risk level for carcinogenic effects of TCDD, other dioxins, and DLCs is central to the ultimate determination of regulatory values, it is important to critically address the available scientific evidence on the most plausible shape of the dose-response relationship at doses below the POD (LED_{01}). On the basis of a review of the literature, including the detailed review prepared by EPA and presented in Part II of EPA's Dioxin Risk Assessment and new literature available since the last EPA review, the committee concludes that, although it is not possible to scientifically prove the absence of linearity at low doses, the scientific evidence, based largely on mode of action, is adequate to favor the use of a nonlinear model that would include a threshold response over the use of the default linear assumption (NAS, 2006, 198441, p. 122).
>
> On the whole, the committee concluded that the empirical evidence supports a nonlinear dose-response below the ED_{01}, while acknowledging that the possibility of a linear response cannot be completely ruled out. The Reassessment emphasizes the lack of such nonlinear models, hence its adoption of the approach of linear extrapolation below the POD level. Although this approach remains consistent with the cancer guidelines (U.S. EPA, 2005, 086237; see also Appendix B), EPA should acknowledge the qualitative evidence of nonlinear dose response in a more balanced way, continue to fill in the quantitative data gaps, and look for opportunities to incorporate mechanistic information as it becomes available. The committee recommends adopting both linear and nonlinear methods of risk characterization to account for the uncertainty of dose-response relationship shape below ED_{01} (NAS, 2006, 198441, p. 72).

5.2.2. Overview of EPA Response to NAS Comments on Cancer Dose-Response Modeling

EPA agrees with the NAS that the approaches to cancer dose-response modeling for TCDD should be clearly communicated and justified. Furthermore, due to the abundance of new information on TCDD carcinogenicity published since the 2003 Reassessment, EPA has reevaluated the cancer dose-response modeling for TCDD presented in the 2003 Reassessment. As detailed below in Section 5.2.3, EPA has conducted an updated cancer dose-response assessment for TCDD that incorporates key NAS recommendations discussed in this document, reflects the current state-of-the science in cancer dose-response modeling and integrates new TCDD carcinogenic information. Detailed responses to the NAS comments summarized above are found in Section 5.2.3.3.

The 2003 Reassessment presents an extensive dose-response assessment of TCDD and provides a comprehensive summary of dose-response relationships. The analyses and discussions synthesized a considerable breadth of data and model types, highlighting the strengths and weaknesses of the then-available scientific information. Modeling included both administered dose and steady state body burden dose metrics, taking into account variation in half-lives of TCDD across species. These body burden calculations used a simple one-compartment kinetic model based on the assumption of a first-order decrease in the levels of administered dose as a function of time. An excess risk of 1% was chosen to model the cancer data, but comparative results were also shown for 5% and 10% excess risk (see Table 8-2 of the 2003 Reassessment). Dose response was also explored thoroughly for a number of in vitro and biochemical endpoints in addition to the in vivo data analyses, and ranges of these values were presented (see Figures 8-1, 8-2 and 8-3 of the 2003 Reassessment). Thus, the 2003 Reassessment provides an initial evaluation of the carcinogenic database for TCDD and serves as the foundation for the analyses presented below.

5.2.3. Updated Cancer Dose-Response Modeling for Derivation of Oral Slope Factor

The following sections describe the dose-response analysis of the cancer data from epidemiologic cohort studies (see Section 2.4.1 and Table 2-4) and rodent bioassays (see Section 2.4.2 and Table 2-6), concluding with the derivation of oral slope factors for TCDD based on epidemiologic data (see Section 5.2.3.1) and rodent bioassay data (see Section 5.2.3.2).

5.2.3.1. *Dose-Response Modeling Based on Epidemiologic Cohort Data*

The 2003 Reassessment included dose-response analyses and the development of oral slope factors from the following three occupational cohorts: the NIOSH cohort, the Hamburg cohort, and the BASF cohort. In this document, EPA determined that specific studies from each of these cohorts (Becher et al., 1998, 197173; Ott and Zober, 1996, 198408; Steenland et al., 2001, 198589) met the epidemiologic study inclusion criteria (see Section 2.3.1 and Section 2.4.1). In Section 5.2.3.1.1, the oral slope factors derived from these studies in the 2003 Reassessment are reviewed. Another study that met the current epidemiologic study inclusion criteria (Warner et al., 2002, 197489) was also briefly discussed in the 2003 Reassessment, but an oral slope factor was not derived from that study. In Section 5.2.3.1.2.2, EPA discusses its

unsuccessful attempt to use the categorical results published by (Warner et al., 2002, 197489) to develop an oral cancer risk estimate.

Since the publication of the 2003 Reassessment, additional cancer epidemiologic studies based on these cohorts have been published in the peer-reviewed literature. Of these, Collins et al. (2009, 197627) and Cheng et al. (2006, 523122) met the epidemiologic study inclusion criteria (see Section 2.3.1 and Section 2.4.1). In Section 5.2.3.1.2, EPA evaluates the suitability of deriving an oral slope factor from the Cheng et al. (2006, 523122) study and derives oral slope factor estimates. Although the Collins et al. (2009, 197627) study met the study inclusion criteria, EPA could not derive an oral slope factor from that study. In Section 5.2.3.1.2.3, EPA discusses why an oral cancer risk estimate was not developed using the positive results for the soft-tissue sarcoma mortality published by Collins et al. (2009, 197627).

5.2.3.1.1. *Evaluation of Epidemiologic Studies Used in the 2003 Reassessment for OSF Derivation.*

In the 2003 Reassessment, EPA reported dose-response modeling results for three epidemiologic studies of human occupational cohorts: the NIOSH cohort with data published by Steenland et al. (2001, 198589); the Hamburg cohort with data published by Becher et al. (1998, 197173); and the BASF cohort with data published by Ott and Zober (1996, 198408). Each of these studies is summarized in Section 2.4.1 of this document and in the 2003 Reassessment (Part II, Chapter 8; Part III, Chapter 5). Furthermore, EPA has evaluated the suitability of these studies for use in TCDD dose-response modeling and concluded that each of these studies meet the inclusion criteria for epidemiology studies presented in Section 2.3.1.

Each of these studies reports all cancer mortality as an outcome. Steenland et al. (2001, 198589) and Becher et al. (1998, 197173) analyzed cohorts of primarily male workers who experienced occupational exposures to TCDD over long periods of time, while Ott and Zober (1996, 198408) studied a cohort of primarily male workers who were exposed to high TCDD concentrations at a single point in time due to an industrial accident.

The authors of all three of these studies measured, and then back-extrapolated, TCDD levels in a subset of workers to estimate exposures during employment and then the authors used this information to estimate exposures in the remainder of the cohort. These measured TCDD samples generally were collected decades after the last known occupational exposure. In each

study, the authors relied on TCDD measures in the cohort to back-calculate serum lipid or body fat levels of TCDD using a simple one-compartment kinetic model based on the assumption of a first-order decrease in the levels of exposure dose as a function of time. The assumed half-life of TCDD used in the models varied from study to study. None of the studies sampled TCDD levels from the entire cohort; for example, Ott and Zober collected samples from 138/243 workers (57% of the cohort), which was the highest percentage of workers sampled among the three studies. Steenland et al. (2001, 198589) and Becher et al. (1998, 197173) used the measured and back-extrapolated TCDD concentrations to estimate the exposures that were associated with various occupations within the cohort, and subsequently used this information to develop exposure matrices (i.e., the TCDD load per unit time for an occupation) that then could be used to estimate the cumulative dioxin dose for each cohort member. Ott and Zober (1996, 198408) used regression procedures with data on time spent at various occupational tasks to estimate TCDD levels for all members of the cohort. Following the estimation of worker exposures in each of these three studies, the studies' authors divided these cohorts into exposure subgroups based on the estimated TCDD levels.

In the 2003 Reassessment, EPA identified a POD based on a 1% response in cancer mortality (ED_{01}) for the Steenland et al. (2001, 198589), and the Ott and Zober (1996, 198408) studies. EPA extrapolated from this POD to lower doses using a straight line drawn from the POD to the origin—zero incremental dose, zero incremental response—to give a probability of extra risk. Because there was insufficient evidence to support an assumption of nonlinearity, EPA chose to develop these models using a linear model.

5.2.3.1.1.1. *Steenland et al. (2001, 198589).*

Steenland et al. (2001, 198589) developed dose-response models based on TCDD exposures and all cancer mortalities from eight plants in the NIOSH cohort (see Section 2.4.1.1.1.1.3 for study details). Serum lipid levels of TCDD in 1988 were measured in 193 workers at one of these plants. Steenland and coauthors relied on a first-order kinetic model (assuming a constant 8.7 year half-life) to back-extrapolate to serum TCDD levels at the time of the last occupational exposure. The study authors assigned exposure estimates to each of the 3,538 workers in the cohort based on a job-exposure matrix. This matrix was based on (1) an estimated level of contact with TCDD, (2) the degree of TCDD contamination of the products

the workers produced, and (3) the fraction of a workday during which the worker likely contacted the TCDD-contaminated products. They then estimated each worker's serum TCDD levels as an area under the concentration curve (AUC) for lipid-adjusted serum levels over time. The mortality analysis was conducted on 256 cancer decedents.

Several different dose-response models were fit to these data to provide estimates of fatal cancer risk. The best-fitting model was a Cox regression exposure-response model using the log(AUC) of TCDD lipid concentration (ppt-year) lagged by 15 years as the exposure metric. Steenland and colleagues also developed a piecewise linear regression model with no lag, in which two separate linear slopes were estimated. This analysis assumed a background exposure of 0.5 pg/kg-day. The lipid concentrations were converted to body burdens by dividing by 4. The central tendency estimate and lower bound ED_{01}s from the piecewise linear model and their associated cancer slope factors for the most sensitive endpoint (male cancer mortality) are presented in Table 5-1.

5.2.3.1.1.2. ***Becher et al. (1998, 197173).***

Based on the Hamburg cohort, Becher et al. (1998, 197173) reported a dose-response analysis for all fatal cancers combined (see Section 2.4.1.1.1.3.4 for study details). The mortality analysis was conducted in 1992 on 124 cancer decedents. The exposure variable in the study was the integrated blood levels for TCDD concentration over time (AUC, ng/kg-years), as estimated by Flesch-Janys et al. (1998, 197339); these were converted to body burdens by dividing by 4. Estimates of the half-life of TCDD, based on the sample of 48 individuals with repeated measures, were incorporated into a model that back-extrapolated TCDD exposures to the end of the employment after accounting for the workers' ages and body fat percentages. These estimated exposure measures were then applied to the entire cohort, which consisted of all 1,189 regular male employees who were employed for at least 3 months between 1952 and 1984 at the Boehringer chemical plant in Hamburg, Germany.

Becher et al. (1998, 197173) used a Cox regression approach for the dose-response modeling and developed three models: a multiplicative model, an additive model, and a power

model.[32] The response variable in each model was the SMR for total cancer mortality. The models were calculated with lag times of up to 20 years. The multiplicative model provided the best fit; however, the study authors judged the fits for all three models to be acceptable. The model results were used to calculate unit risk estimates derived as the risk of cancer death through age 70 given a daily dose of 1 pg/kg body weight of TCDD minus the risk given no exposure to TCDD. These calculations were based on background German cancer mortality rates. The model results were used to calculate cancer risk estimates. The lower bound estimates on the dose were not available for models published by Becher et al. due to the absence of statistical parameter measures. The central tendency estimate ED_{01}s from the three statistical models and their associated cancer slope factors are presented in Table 5-1.

5.2.3.1.1.3. *Ott and Zober (1996, 198408).*

In the 2003 Reassessment, EPA also developed a dose-response analysis based on a study reported by Ott and Zober (1996, 198408) for cancer incidence and mortality experienced by 243 men, who were exposed to TCDD in 1953 during an accident at the BASF plant in Germany (see Section 2.4.1.2.1.2.1 for study details). The cohort was followed through 1992. TCDD blood lipid levels were available for 138 of these men 30 years after the accident. These levels were back-extrapolated and used to estimate the AUC for TCDD. Body burdens (ng/kg) were estimated by dividing AUC by 4, and steady-state body burdens were estimated assuming a constant half-life of approximately 7.1 years.[33] Ott and Zober (1996, 198408) used Cox proportional hazard approaches to estimate both cancer incidence and cancer mortality risk per

[32]The "multiplicative model" set relative risk (RR) equal to exp(βd), where the dose d is the AUC. The "additive model" set RR = 1+βd, and the "power model" set RR = exp(β log (kd+1)). The values β and k are estimated parameters.

[33]Based on the initial body burden (B_0) EPA estimated the body burden at time t using the following formula: $B(t) = B_0 e^{-k_e t}$, where k_e is an elimination constant equal to ln(2)/(half-life in years). This implies that the AUC at time T after initial exposure is $AUC = \frac{B_0}{k_e}(1 - e^{-k_e T})$. T in this case was 39 years (time from the accident in 1953 to the follow-up in 1992). Dividing by a lifetime of 71 years (mean age in 1954, 33 years, plus 38 years from 1954 to the followup in 1992) yields the lifetime mean body burden as: $B_{mean} = \frac{B_0}{71 k_e}(1 - e^{-k_e T})$. In the 2003 Reassessment, EPA converted the steady-state body burden to units of equivalent initial dose by dividing by the constant $\frac{1}{71 k_e}(1 - e^{-k_e T})$. With the given values for half-life and T, that constant is 0.1411 and 1/(the constant) is 7.09.

unit TCDD dose.[34] Ott and Zober reported conditional risk ratios for cancer mortality that were slightly larger than the conditional risk ratios for cancer incidence, which is counter-intuitive. The risk of cancer mortality would be expected to be greater than the risk of cancer incidence. The conditional risk ratio (and 95%CI) for all cancer mortality (1.22; 1.00−1.50) exceeded the conditional risk ratio for all incident cancer cases (1.11; 0.91−1.35). Similarly, the conditional risk ratios for digestive cancer mortality (1.46; 1.13-1.89) and respiratory cancer mortality (1.09; 0.70−1.68) were also both larger than the conditional risk ratios for all digestive cancers (1.39; 1.07−1.69) and all respiratory cancers (1.02; 0.65−1.59). As expected, in this cohort, incident cases exceeded cancer mortality for total cancers (47 vs. 31), digestive cancers (12 vs. 11) and respiratory cancers (13 vs. 11). Ott and Zober also reported that conditional risks for mortality for all cancer and lung cancer associated with cigarette smoking were also higher than the respective incidence risks. In their Cox regression analysis, Becher et al. (1998, 197173) also report that the regression coefficient for total cancer mortality (0.0096) was slightly larger than the regression coefficient for total cancer incidence (0.0089). The finding of Ott and Zober and Becher et al. that the risk of cancer mortality is greater than cancer incidence is possibly due to a systematic difference in the reference population for incidence vs. the reference population for mortality. The central tendency estimate and lower bound ED_{01}s from the modeling and their associated cancer slope factors are presented in Table 5-1.

5.2.3.1.2. *Evaluation of Other Epidemiologic Studies Considered for OSF Derivation.*

Three additional epidemiologic studies that met the study inclusion criteria (see Section 2.3) for use in dose response modeling as set forth in this document are evaluated in this section for the estimation of cancer risk estimates. These studies were either published after the Reassessment (Cheng et al. (2006, 523122) and Collins et al., (1996, 197637)), or not used to derive an OSF in the Reassessment (Warner et al., 2002, 197489). Each study is summarized in Section 2.4.1.

[34]The model from Ott and Zober has risk proportional to $e^{\beta \times dose}$ with $\beta = \ln(1.22)$. The corresponding slope for the mean (steady-state) body burden is 7.0851 * log(1.22) * 0.001 (the 0.001 converts nanograms to micrograms).

5.2.3.1.2.1. *Cheng et al. (2006, 523122).*

As discussed in Section 2.4.1.1.1.1.4, Cheng et al. (2006, 523122)) analyzed the relationship between TCDD dose and all cancer mortality for the same subset of NIOSH workers as analyzed previously by Steenland et al. (2001, 198589). In contrast to Steenland et al., Cheng et al. (2006, 523122) used the "concentration- and age-dependent elimination model" (concentration- and age-dependent elimination [CADM], discussed in Section 3.3; see also Aylward et al. (2005, 197114)), rather than a constant 8.7-year half-life, and calculated serum-derived TCDD estimates for use in dose-response analysis. An important feature of CADM is that it incorporates concentration- and age-dependent elimination of TCDD from the body, meaning that the effective half-life of TCDD elimination varies based on exposure history, body burden, and age of the exposed individuals. As discussed in Section 3.3, the use of the CADM model to simulate TCDD kinetics in the NIOSH cohort results in time-integrated body burden estimates four to five times greater than those obtained with the simple first-order model, with smaller differences between the two methods at lower exposures.

Following the estimation of dose using the CADM-derived AUC values, Cheng and colleagues (Cheng et al., (2006, 523122); the "Cheng analysis") derived dose-response estimates for the NIOSH cohort using linear Cox regression for both lagged and un-lagged exposure on various subsets of the data (high-exposures trimmed). The results for the lagged-exposure analysis are summarized in Table 5-2. For comparison, the Cox regression coefficient from the analysis conducted by Steenland et al. (2001, 198589), which relied on a first-order elimination rate model assuming a constant 8.7-year half-life, is also shown in the table. As in Steenland et al. (2001, 198589),[35] Cheng et al. (2006, 523122) found a much stronger relationship between cancer mortality and exposure metrics lagged 15 years compared to the relationships for unlagged exposures. Cheng et al. (2006, 523122) also noted that the dose-response relationship plateaued above the 95th percentile of exposure. For exposures lagged 15 years, the regression coefficient (β) of the linear slope derived by Cheng et al. (2006, 523122) was 3.3×10^{-6} per ppt-year lipid-adjusted serum TCDD, with a standard error of 1.4×10^{-6} (Table III of Cheng et al. (2006, 523122)). The upper 5% of the exposure range (individuals $\geq$252,950 ppt-year lipid adjusted serum TCDD) was excluded in estimating this slope. Because this exclusion reduces

[35] Lagged exposures modeled only for log-transformed serum concentrations, not for untransformed serum concentrations in the piece-wise linear model.

the upper portion of the response where the slope is shallow[36], this likely better represents the slope in the region of the curve where the fatal cancer risk is increasing with dose, which is the equivalent of dropping the highest dose in an animal bioassay or using a piece-wise linear model as in Steenland et al. (2001, 198589).

To develop cancer risks for TCDD, EPA used the modeling results of the Cheng analysis, with conversion to oral intake using the Emond human PBPK model as follows. The slope (β) from the Cheng analysis is the slope of the linear relationship between the natural logarithm of the rate ratio (RR) and the cumulative fat TCDD concentration (fat-AUC). Conceptually, the slope (β) is similar to an OSF, except that it is expressed in terms of fat-AUC rather than intake. Also, the slope represents the incremental increase in cancer mortality (expressed as an RR) above the background TCDD exposure experienced by the NIOSH cohort rather than above zero. Using the upper 95% bound on β and assuming that the slope is the same below the NIOSH cohort background exposure level (approximately 5 ppt/yr TCDD fat concentration), EPA calculated risk-specific doses (as daily oral intakes) for TCDD for risk levels of concern to EPA. The risk-specific doses were estimated from the Emond human PBPK model for the lifetime-average TCDD fat concentrations corresponding to the fat-AUC predicted by the Cheng et al. model for each of the risk levels of concern. The steps in this computation are as follows:

- Background cancer mortality risk estimate (R_0). EPA used an R_0 of 0.112 as reported by Cheng et al. (2006, 523122)[37]
- Total cancer mortality risk in the exposed group associated with a specified (extra) risk level (RL) of fatal cancer (TR_{RL}). A TR_{RL} associated with any given extra risk level (e.g., 0.01, 1×10^{-6}) can be calculated using the following relationship for extra risk:

$$ER = \frac{TR_{RL} - R_0}{1 - R_0} \qquad \text{(Eq. 5-1)}$$

[36] Steenland et al. (2001, 198589); Steenland and Deddens (2003, 198587) found a slightly negative slope for the higher exposures, stating that the phenomenon could be a result of exposure misclassification, depletion of susceptible individuals or saturation of receptor-mediated processes.

[37] In Table IV, Cheng et al. (2006, 523122) report two estimates of background fatal cancer risk, R_0, for males aged 75 years: 0.112 and 0.124. A R_0 estimate of 12.4% was used by Steenland et al. (2001, 198589), and 11.2%, as estimated for all males in the 1999–2001 Surveillance Epidemiology and End Result data set. EPA chose to use the more recent estimate of 11.2% for the purpose of predicting risk in the current U.S. population.

- Incremental cancer mortality risk in the exposed population based on a given extra risk (R_D). R_D, is calculated as the difference between the total risk and background risk and expressed in terms of RL and R_0 by combining Equations 5-2 and 5-1.

$$R_D = TR_{RL} - R_0 \quad \text{(Eq. 5-2)}$$

$$R_D = RL \times (1 - R_0) \quad \text{(Eq. 5-3)}$$

- Cumulative TCDD concentration in the fat compartment for a given extra risk (AUC_{RL}). AUC_{RL} is then calculated by taking the natural logarithm of Equation 3 from Cheng et al. (2006, 523122), rearranging and substituting for RR[38] (RR = $[R_D + R_0]/R_0$):

$$AUC_{RL} = \ln((R_D + R_0)/R_0)/\beta^* \quad \text{(Eq. 5-4)}$$

where β^* is the central-tendency regression slope or the 95% upper bound (β_{95}) determined by summing the regression coefficient (β) and the product of 1.96 and the standard error of the regression coefficient, yielding an estimate of 6.0×10^{-6} per ppt-year lipid adjusted serum TCDD, as follows:

$$\beta_{95} = \beta + 1.96 * SE \quad \text{(Eq. 5-5)}$$

- Continuous daily TCDD intake associated with a given extra risk [D_{RL}]. Because the fat concentrations generated by CADM are not linear with oral exposure at higher doses, a single oral slope factor to be used for all risk levels cannot be obtained; the response is approximately linear with fat concentrations and oral intake at lower doses. Instead, a risk-specific D_{RL} must be estimated by converting the respective AUC_{RL} to the corresponding lifetime daily intake, using an appropriate human toxicokinetic model. EPA has chosen to use the Emond human PBPK model for this purpose because the CADM configuration does not facilitate this process and so that the dose conversions are consistent with those used in the derivation of the RfD. A D_{RL} is obtained from the Emond model by finding the average lifetime daily intake corresponding to the AUC_{RL} in the fat compartment.[39]

Note that there are two nonlinear steps in the estimation of risk-specific doses from the Cheng et al. model. First, fat-AUC (AUC_{RL}) and the incremental cancer mortality risk (R_D) do

[38] As defined by Cheng et al. (2006, 523122, p. 1063).

[39] Although the NIOSH cohort exposures are reported as LASC, they are treated as fat concentrations in the Cheng analysis because fat in all tissues are modeled as one compartment (hence equal) in CADM. The translation to oral intake in the Emond model is from the fat compartment, rather than the serum compartment, even though the serum and fat compartments are not equivalent, because the regression slope (β) in the Cheng analysis is in terms of the (equivalent) fat compartment.

not have a linear relationship (see Eq. 5-5); however, the relationship becomes virtually linear below an incremental risk of 10^{-3} (see Table 5-3). Second, TCDD fat concentration is not linear with oral intake in the Emond human PBPK model (see Section 3); this relationship also is close to linear below the 10^{-5} risk level. The resulting predicted cancer-mortality risk is approximately linear with daily oral intake at low doses. Table 5-3 shows the AUC_{RL} and D_{RL} based on the 95% upper-bound regression slope (β_{95}) from the Cheng analysis for a number of risk levels of interest to the EPA. For comparative purposes, EPA has also shown the equivalent oral slope factor ($RL \div D_{RL}$) for those same risk levels. Table 5-4 also shows analogous results based on the best estimate of regression coefficient ($\beta = 3.3 \times 10^{-6}$) for total fatal cancers from the Cheng analysis.

5.2.3.1.2.2. *Warner et al. (2002, 197489).*

Warner et al.(2002, 197489) is a study of 981 females exposed to elevated TCDD levels following the Seveso accident of 1976 (see Section 2.4.1.1.1.4.2 for study details). The TCDD exposure pattern involving a single period of elevated TCDD exposures followed by an extended period of lower ambient level TCDD exposures and elimination is similar to that of the BASF cohort (Ott and Zober, 1996, 198408). TCDD levels, measured or estimated in blood lipids shortly after the time of the accident, were available for all women. These women were divided into four exposure groups of <20, 20−44, 44.1−100, and >100 ppt. In this cohort, 21 total cancers have been observed; 15 of these were breast cancer cases and 3 were thyroid cancer cases. Cox proportional hazards modeling showed that the hazard ratio for breast cancer associated with a 10-fold increase in serum TCDD levels ($\log_{10}$ (TCDD)) was significantly increased to 2.1 (95% CI = 1.0−4.6). Rate ratios (95% CI) for cancer incidence in these 4 groups were 1.0, 1.0 (0.2−5.5), 2.2 (0.5−10.8) and 2.5 (0.5−11.8). Using a Cox proportional hazards model and assuming continuous exposure, the rate ratio was 1.7 (0.9−3.4) for each 10-fold increase in serum TCDD; that is, a $\log_{10}$ transformation of the exposure estimates in their analysis was presented. They reported a test for trend of $p = 0.09$.

EPA attempted to estimate an ED_{01} from the modeled results of Warner et al. (2002, 197489) from the statistically significant hazard ratio for breast cancer. However, EPA had to estimate the slope of the tangent to the log-linear relationship. Because the exponentiated slope of a log-dose linear relationship is not constant but varies with dose, and because the lowest

exposure measure was well-above the 1% response region of interest, EPA could not confidently estimate the tangent to the log-dose linear relationship. The transformation of the $\log_{10}$ dose units to linear units of TCDD yielded an implausibly low ED_{01} and correspondingly high cancer risk that was inconsistent with a visual inspection of the untransformed plot. EPA was not confident in these values for health risk assessment because of uncertainties in the transformation in the low response region of the original model. Thus, EPA did not derive an ED_{01} or oral slope factor for this study.

5.2.3.1.2.3. *Collins et al. (2009, 197627).*

Collins et al. (2009, 197627) investigated the relationship between serum TCDD levels and mortality rates in the NIOSH cohort (see Section 2.4.1.1.1.5 for study details). The investigators completed an extensive dioxin serum evaluation of workers employed by the Dow Chemical plant in Midland, Michigan, that made 2,4,5-trichlorophenol (TCP) from 1942 to 1979 and 2,4,5-T from 1948 to 1982. Collins et al. (2009, 197627) developed historical TCDD exposure estimates for all 1,615 workers using serum samples from 280 former workers that were collected during 2004−2005. Investigators calculated a cumulative measure of exposure using a simple one-compartment first-order pharmacokinetic model and elimination rates as estimated from the BASF cohort (Flesch-Janys et al., 1996, 197351). The follow-up interval for these workers covered the period between 1942 and 2003. Thus, the study included 10 more years of follow-up than earlier investigations of the entire NIOSH cohort. A key limitation of this study is that the derivation of the SMRs and slope parameters did not include a lag period, unlike other analyses of the NIOSH cohort (e.g., Cheng et al., 2006, 523122; Steenland et al., 2001, 198589).

Although results were largely negative, statistically significant mortality in the cohort was found for soft-tissue sarcoma (SMR = 4.1, 95% CI = 1.1−10.5), based on only four deaths. A regression coefficient of 0.05872 (standard error not reported), and a hazard ratio of 1.060 (95% CI = 1.017 to 1.106) were reported by Collins et al. (2009, 197627) for soft-tissue sarcoma. Although it met the dose-response study criteria, EPA could not calculate an upper bound on the regression coefficient because the standard error was not given. In addition, EPA was unable to estimate an extra-risk value because the reference population response was not specified. Thus, EPA did not derive an ED_{01} or oral slope factor for this study.

5.2.3.2. *Dose-Response Modeling Based on Animal Bioassay Data*

Figure 5-3 provides a summary of the process EPA has utilized to select and identify candidate TCDD OSFs from key animal bioassays that were identified in Section 2.4.3 of this document. For each in vivo animal cancer study that qualified for TCDD dose-response assessment using the study inclusion criteria specified in Section 2.3.2, EPA first selected the species/sex/tumor data set combinations that had been characterized as having statistically significant increases in tumor incidence by either a pair-wise test between the treated group and the controls or by a trend test showing increases in tumors with increases in dose. Next, EPA used the Emond animal kinetic model discussed in Section 3 to estimate blood concentrations corresponding to each study's average daily administered doses for use in dose response modeling. Benchmark dose lower confidence bounds ($BMDL_{01}$s) were then estimated for the blood concentrations by (1) using the multistage cancer model for each species/sex/tumor combination within each study and (2) using a Bayesian Markov Chain Monte Carlo framework that assumes independence of tumors, modeling all tumors together for each species/sex combination within each study. The final selected models were subjected to goodness-of-fit tests and visual inspections of fit to the raw data. Thus, for each sex/species combination within each study, this process generated a $BMDL_{01}$ for each single tumor type and another $BMDL_{01}$ for the combined tumors. Finally, using the Emond human kinetic model discussed in Section 3, human equivalent doses ($BMDL_{HED}$s) were then estimated for each of the $BMDL_{01}$s and, using a linear extrapolation, OSFs were calculated by $OSF = 0.01/BMDL_{HED}$. The highest OSF for a species/sex combination for either a single tumor type or all combined tumors was selected as a candidate OSF for TCDD cancer assessment. These steps in Figure 5-3 are further described in detail in the following sections.

5.2.3.2.1. *Selection of key data sets.*

Based on the study selection criteria outlined in Section 2.3.2 (see Figure 2-3), EPA selected five animal bioassays for use in the cancer dose-response assessment for TCDD (see Table 2-6 and Section 2.4.2 for detailed study descriptions). Four of these studies (Della et al., 1987, 197405; Kociba et al., 1978, 001818; NTP, 1982, 594255; Toth et al., 1979, 197109), were evaluated in the 2003 Reassessment, while one study (NTP, 2006, 543749) was published after the 2003 Reassessment was released. The NTP (2006, 543749) study was specifically called out

by the NAS (2006, 198441)) report as cancer bioassay data that EPA should evaluate prior to completing its TCDD dose-response assessment. As discussed below, EPA has chosen to conduct dose-response modeling for a number of tumor types from each of the sex/species combinations in these studies in order to maximize the amount of information available to support OSF derivation. Because tumors occurred in multiple sites in the exposed animals, each tumor type was considered separately (individual tumor models) and were also combined into composite tumor incidence dose estimates (multiple tumor models).

The tumor incidence tables for these five bioassays are shown in Tables 5-5 through 5-14 (see Section 2.4.2 for details of these studies). The data in these tables are summarized from each study's reference publication and are the species/sex/tumor incidence data used for TCDD dose-response modeling in this report. EPA selected the animal bioassay data sets in Tables 5-5 through 5-14 because they had been characterized by the study authors as having statistically significant increases in tumor incidence by either a pair-wise test between at least one treated group and the controls or by a trend test showing increases in tumors with increases in dose. An exception was made for cases where statistical significance was found in only one dose group that was not the highest dose group, and there were zero responses in every other dose group including controls; these datasets were not modeled. For example, in NTP (2006, 543749), EPA notes that while the uterine tumors were statistically significant at 46 ng/kg using a pair-wise test, there were no uterine tumors in any other dose group, including the control and high dose groups, and the trend test was not significant; EPA excluded this tumor type from the analysis. In addition, datasets with combined tumors for the same site were given priority over subsets of tumors for that site. For example, in the NTP (1982, 594255) study on female mice, data on combined hepatocellular adenomas or carcinomas were modeled, but not data on hepatocellular adenomas alone (not statistically significant) or on hepatocellular carcinomas alone (statistically significant trend and high dose group). In the case of the Kociba et al. (1978, 001818) female rat combined hepatocellular adenomas and carcinomas only, EPA used data from a reanalysis of the pathology slides that was published by Goodman and Sauer (1992, 197667); because the study authors did not statistically analyze the revised tumor incidence data from their reanalysis, EPA applied a Fischer's Exact Test to evaluate the statistical significance of those data. In the case of the NTP (2006, 543749) study only, information was available regarding the length of time that the animals stayed on test (105 weeks); animals who died within the first year were censored

from analysis in this document because animals who died within the first year were not considered to have been alive long enough to develop tumors. Therefore, those animals were not included in the denominators in Table 5-11. These adjusted incidence data were used in the analysis of tumor dose-response for NTP (2006, 543749) in this document. The tumor incidence data in Tables 5-5 through 5-14 include

- nasal, tongue and adrenal tumors in males (Table 5-5), and liver, nasal and lung tumors in females from the Kociba et al. (1978, 001818) 2-year study of Sprague-Dawley rats (Table 5-6),
- subcutaneous tissue, liver, adrenal and thyroid tumors in females (Table 5-7) and liver, thyroid and adrenal tumors in males (Table 5-8), from the NTP (1982, 594255) 2-year study of Osborne-Mendel rats,
- subcutaneous tissue, hematopoietic system, liver and thyroid tumors in females (Table 5-9), and lung and liver tumors in males, from the NTP (1982, 594255) 2-year study of B6C3F$_1$ mice (Table 5-10),
- liver, oral mucosa, pancreas and lung tumors in females from the NTP (2006, 543749) 2-year study of Sprague-Dawley rats (Table 5-11),
- liver tumors in males from the Toth et al. (1979, 197109) 1-year study of Swiss/H/Riop mice (Table 5-12), and
- liver tumors in males (Table 5-13) and females from the Della Porta et al. (1987, 197405) 52-week study of B6C3F$_1$mice (Table 5-14).

For each cancer endpoint, the reported (administered) doses from each study were converted, where necessary, to average daily doses in ng/kg-day (e.g., doses administered 5 days/week were adjusted by multiplying by 5 and dividing by 7 to get average daily doses). These doses were then subjected to kinetic modeling to generate blood concentrations for use in TCDD dose-response modeling.

5.2.3.2.2. *Dose adjustment and extrapolation methods for selected data sets.*

5.2.3.2.2.1. ***Dose metric estimation for dose-response modeling.***

Tables 5-5 through 5-14 show the blood concentrations that were used in TCDD dose-response modeling of the animal bioassay data. Based on kinetic analysis (see Section 3), a choice for whole blood concentration of TCDD was made for the purpose of dose extrapolation between animals and humans. In order to estimate blood concentrations for each study selected,

the Emond PBPK model was run using ACSLX® software, version 2.5.0.6 (see Section 3). Depending on the selected study, either rat or mouse versions of the model were used. In each case, the simulation was performed using the exposure and observation durations, the body weights, and the adjusted doses from the original studies. Details of PBPK model input parameters are given for each study's m-file in Appendix C.2. In the case of Toth et al. (1979, 197109) study, which dosed the animals for a year and then followed up for the lifetime of the animal, only the one-year simulation was performed. The m-files were used to run the appropriate PBPK model to estimate time-averaged, maximum, and terminal (end of exposure) blood concentration (see Appendix C.3). Other model simulated dose metrics such as concentrations for liver, fat, Ah-receptor bound in liver, body burden, and the time at which the maximum concentration was reached for each dose metric are also reported for illustrative purposes in Appendix C.3. The complete results for each study modeled are shown in Appendix C.3.

5.2.3.2.2.2. ***Calculation of human equivalent doses (HEDs).***

Human equivalent doses (ng/kg-day), corresponding to each BMDL (ng/kg) were calculated using the Emond human PBPK model (see Section 3) and are denoted as $BMDL_{HED}$s. The Emond human PBPK model was run for 70 years assuming a constant daily dose starting from birth. The model concentrations were averaged over both the entire 70 year lifetime (lifetime average) and over the five years surrounding the peak concentration (five-year average) (see Section 3.3.1, describing first order body burden estimation). The human equivalent doses were estimated by adjusting the daily dose model input until the time-averaged whole blood concentration matched the associated alternative dose BMDL (derived earlier from animal PBPK model). For animal studies which lasted longer than 540 days, the lifetime average was used; for studies lasting less than 540 days, the five year average was used. The process was iterative and continued until the modeled human concentration was within 1% of the BMDL. In general, however, the concentrations matched to within 0.1%.

5.2.3.2.3. *Dose-response modeling approaches for rodent bioassays.*

5.2.3.2.3.1. ***Modeling of individual tumors.***

EPA's BMDS Software, version 2.1 was used to estimate the $BMDL_{01}$s for each of the species/sex/tumor combinations, using the blood concentrations and incidence data shown in Tables 5-5 through 5-14. Each data set was modeled using the multistage cancer model, and a $BMDL_{01}$ in blood concentration was estimated. The multistage model has been used by EPA in the majority of its quantitative cancer assessments because it is statistically robust and able to provide good fits to a wide range of dose-response patterns. It is also consistent with the multistage nature of the carcinogenic process. The mathematical form of the multistage model is

$$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)] \qquad \text{(Eq. 5-6)}$$

where

$P(d)$ = lifetime excess risk (probability) of cancer at dose d
q_i = parameters estimated in fitting the model, $i = 1, \ldots, k$.

To estimate the BMD_{01}s and $BMDL_{01}$s, BMDS was run with all parameters set to their defaults; up to three degrees of freedom were specified for the dichotomous, multistage cancer model; and a 95% confidence level. A 1% extra risk benchmark response (BMR) was used for each tumor type, as this response level was judged to be sufficiently close to the observed responses (see Section 5.2.3.2.6.11 for an expanded discussion). The $BMDL_{01}$ (ng/kg) was then converted to a $BMDL_{HED}$ (ng/kg-day) using the Emond human model, and an OSF in units of $(mg/kg\text{-}day)^{-1}$ was calculated by, $OSF = 0.01/BMDL_{HED} \times 10^6$. Because of the nonlinearity of blood concentration and ingested dose in the Emond Human PBPK model, the cancer risk is only approximately linear with the TCDD blood concentration and low TCDD oral ingestion doses, but is not linear with ingested TCDD at higher doses.[40] Thus, to use these estimates in human health risk assessment, risk-specific TCDD oral intake levels corresponding to the target risk levels should be calculated, using a procedure similar to that for the slope factors based on epidemiologic data (see Table 5-3). In the following sections, results are presented for the

[40] This situation is analogous to that for the cancer risk modeling of epidemiologic data from the Cheng et al. (2006) analysis in Section 5.2.3.1.2.1.

models that provided the best overall fit to the data as judged by comparison of likelihood ratios for models that had an acceptable fit (chi-squared goodness of fit statistic $p > 0.05$).

5.2.3.2.3.2. ***Multiple tumor (Bayesian) models.***

Statistically significant increased tumor incidences were observed at multiple sites in male and/or female rats (Kociba et al., 1978, 001818; NTP, 1982, 594255; NTP, 2006, 543749) and male and female mice (NTP, 1982, 594255) following oral exposures to TCDD. With this multiplicity of tumors, the concern is that a potency or risk estimate based solely on one tumor site (e.g., the most sensitive site) may underestimate the overall cancer risk associated with exposure to this chemical. Relevant approaches in the 2005 Cancer Guidelines (U.S. EPA, 2005, 086237) for characterizing total risk include the following: (1) analyze the incidence of tumor-bearing animals, or (2) combine the potencies associated with significantly elevated tumors at each site. The NRC (1994, 006424) concluded that an approach based on counts of animals with one or more tumors (tumor-bearing animals) would tend to underestimate overall risk when tumor types occur independently, and thus an approach based on combining the risk estimates from each separate tumor type should be used. On independence of tumors, NRC (1994, 006424) stated "…a general assumption of statistical independence of tumor-type occurrences within animals is not likely to introduce substantial error in assessing carcinogenic potency."

Because potencies are typically upper bound estimates, summing such upper bound estimates across tumor sites is likely to overstate the overall risk. Therefore, following the recommendations of the NRC (1994, 006424) and the 2005 Cancer Guidelines (U.S. EPA, 2005, 086237), a statistically valid upper bound on combined risk was derived, assuming independence, in order to gain some understanding of the overall risk resulting from tumors occurring at multiple sites. In the case of TCDD, tumors are thought to be independent across the sites found in these three studies because: (1) they are in different organs and tissues, specifically liver, lung, thyroid, subcutaneous tissue, oral cavity, tongue, pancreas, adrenal cortex and the hematopoietic system; (2) different kinds of tumors were found, even within the same organ (e.g., both cholangiocarcinomas and hepatocellular adenomas were found in female rat livers in NTP (2006, 543749); and (3) the tumors found in these studies were not progressive (i.e., they did not metastasize to other sites in the body). It is important to note that this estimate

of overall potency describes the risk of developing tumors at any combination of the sites and is not the risk of developing tumors at all sites simultaneously.

For modeling individual tumor data, the multistage model is specified as shown in the previous section (see Eq. 5-6). Under the assumption of independence, the model for the combined (or composite) tumor risk is still multistage, with a functional form that has the sum of stage-specific multistage coefficients as the corresponding multistage coefficient.

$$P_c(d) = 1 - exp[-(\Sigma q_{0i} + d\Sigma q_{1i} + d^2\Sigma q_{2i} + \ldots + d^m\Sigma q_{mi})], \text{ for } i = 1,\ldots, k, \qquad \text{(Eq. 5-7)}$$

where k = total number of sites.

The resulting equation for fixed extra risk (BMR) is polynomial in dose (when logarithms of both sides are taken) and can be solved in a straightforward manner for the combined BMD. However, the current version of BMDS cannot estimate confidence bounds for this combined BMD.

Therefore, a Bayesian approach to finding confidence bounds on the combined BMD was implemented using WinBUGS (Spiegelhalter et al., 2003, 594261). WinBUGS software is freely available and implements Markov Chain Monte Carlo (MCMC) computations. Use of WinBUGS has been demonstrated for derivation of a distribution of BMDs for a single multistage model (Kopylev et al., 2007, 194860) and is easily generalized (Kopylev et al., 2009, 198071) to derive the distribution of BMDs for the combined tumor load, following the NRC (1994, 006424) methodology described above. The advantage of a Bayesian approach is that it produces a distribution of BMDs that allows better characterization of statistical uncertainty. For the current analysis, a diffuse (high variance or low tolerance) Gaussian prior restricted to be nonnegative was used. The posterior distribution was based on three simulation chains with 50,000 burn-in (i.e., the initial 50,000 iterations were dropped) and a thinning rate of 20, resulting in 150,000 interactions total. The median and 5th percentile of the posterior distribution provided the BMD_{01} (central estimate) and $BMDL_{01}$ (lower bound) for combined tumor load, respectively.

The methodology above was applied to the statistically significant dose-response data from Kociba et al. (1978, 001818), NTP (1982, 594255), and NTP (2006, 543749) (see

Section 2.3.2 for data set selection criteria).[41] As with the risk estimates generated for individual tumor sites, the combined analysis used the internal dose metric, whole blood concentration (see Section 3). For the combined tumors for each sex/species combination, a $BMDL_{01}$ in blood concentrations was estimated. The $BMDL_{01}$ (ng/kg) was then converted to a $BMDL_{HED}$ (ng/kg-day) using the Emond human model, and an OSF in units of $(mg/kg\text{-}day)^{-1}$ was calculated by, $OSF = 0.01/BMDL_{HED} \times 10^6$. Because of the nonlinearity of blood concentration and ingested dose in the Emond Human PBPK model, the cancer risk is linear only with the TCDD blood concentration and low TCDD oral ingestion doses, but is not linear with ingested TCDD at higher doses; a single OSF cannot represent the entire range of risks for oral ingestion. Thus, to use these estimates in human health risk assessment, risk-specific TCDD oral intake levels corresponding to the target risk levels should be calculated using a procedure similar to that for the slope factors based on epidemiologic data (see Table 5-3).

5.2.3.2.4. ***Results of dose-response modeling for rodent bioassays.***

Table 5-15 presents the benchmark dose modeling results for both the individual tumors and the combined tumors based on TCDD blood concentrations. The *p*-values in the table are for a chi-square goodness of fit statistic with significance of $p > 0.05$. Goodness of fit was acceptable at $p > 0.05$ for all models. The difference in log likelihood (dLL) statistic documents the difference in log likelihoods between stages of the models in cases where the stage is above 1; it shows the difference between the stage in the table and the lower stage. For example, for the NTP (2006, 543749) liver cholangiocarcinomas, twice the difference of 2.92 would be >3.84, the test statistic from the assumed chi-square distribution,[42] with $p = 0.95$, justifying the choice of 3 stages over 2 stages. The best fitting multistage models include: a 1-stage (linear) model for all of the individual tumor data sets from Kociba et al. (1978, 001818), NTP (1982, 594255), and Toth et al. (1979, 197109), for liver carcinomas in females in Della Porta et al. (1987, 197405), as well as for the pancreatic and oral mucosa tumors in NTP (2006, 543749); a

[41] Because only one turmor site was statistically significantly elevated in both the Della Porta et al. (1987, 197405) and Toth et al. (1979, 197109) (i.e., only increased incideces of liver tumors were statistically significant elevated in both studies), a multi-tumor analysis was not conducted.

[42]The chi-square distribution with 1 degree of freedom is the correct distribution only under standard conditions (e.g., no boundary parameters in null hypothesis). Thus, the correct distribution for the situation where the parameter of interest is on the boundary, as happens with testing for the order of the multistage model, and, possibly nuisance parameters (estimated parameters of the model), is very difficult to derive (Self and Liang, 1987, 594398). Therefore the *p*-value of chi-square with one degree of freedom is used as the best available choice.

2-stage model for the lung tumors in NTP (2006, 543749) and for liver carcinomas in males from Della Porta et al. (1987, 197405); and a 3-stage model for the liver cholangiocarcinoma and liver adenoma data sets from NTP (2006, 543749). The multi-stage model fit was not significant ($p > 0.1$) in the NTP (1982, 594255) study for lung tumors in the male mouse ($p = 0.09$), adrenal cortex ($p = 0.06$)and thyroid follicular cell adenomas ($p = 0.06$) in male rats, and subcutaneous tissue in female mice ($p = 0.09$), and was also not significant for liver carcinomas ($p = 0.019$) in female mice in Della Porta et al. (1987, 197405). For the Toth et al. (1979, 197109) liver tumors, the model fit to all of the data was poor, and the highest dose group was dropped in order to achieve an acceptable fit ($p = 0.29$). The BMD_{01}s and $BMDL_{01}$s (ng/kg) were estimated from these multistage models for the individual tumors. BMD_{01}s and $BMDL_{01}$s (ng/kg) were also provided in Table 5-15 for the combined tumors for each sex/species combination within a study. These were estimated from the distributions of BMD_{01}s produced by the Bayesian MCMC simulation (see Section 5.2.3.1.2.3.2). The BMD_{01}s and $BMDL_{01}$s (ng/kg) for the combined tumors in Table 5-15 are the mean and lower 95% percentile values from these distributions, respectively.

5.2.3.2.4.1. *Individual tumor models.*

Table 5-16 shows the $BMDL_{HED}$s (ng/kg-day) that were estimated from the $BMDL_{01}$s in Table 5-15 using the Emond human model (see Section 5.2.3.1.2.2.2) and the OSFs calculated by, $OSF = 0.01/BMDL_{HED} \times 10^6$ to convert the OSF to $(mg/kg\text{-}day)^{-1}$ units. BMDS results, details of the model fits and dose-response graphics for all endpoints are shown in Appendix F. Although only the blood concentration results are presented in this section, for comparison purposes, Appendix F also provides modeling results for the studies' administered average daily doses. Table 5-16 lists the OSFs in decreasing value. It can be seen that liver tumors in male mice yield the highest slope factors; OSF values are 5.9×10^6 and 5.2×10^6 per mg/kg-day in NTP (1982, 594255) and Toth et al. (1979, 197109), respectively. The OSFs for the new NTP (2006, 543749) study in female rats are two orders of magnitude lower, ranging from 1.8×10^4 to 1.8×10^5 per mg/kg-day, representing the lowest OSFs for TCDD from the individual tumor models.

5.2.3.2.4.2. ***Multiple tumor (Bayesian) models.***

Table 5-17 shows the $BMDL_{HED}$s (mg/kg-day) that were estimated from the $BMDL_{01}$s in Table 5-15 using the Emond human model (see Section 5.2.3.1.2.2.2) and the OSFs calculated by, $OSF = 0.01/BMDL_{HED} \times 10^6$ to convert the OSF to $(mg/kg\text{-}day)^{-1}$ units. Table 5-17 lists the OSFs in decreasing value. It can be seen that the combined liver and lung tumors in male mice yield the highest OSF value of 9.4×10^6 per mg/kg-day from NTP (1982, 594255), and the combined adrenal, tongue and nasal tumors in male rats yield the lowest OSF value of 3.2×10^5 from Kociba et al. (1978, 001818). The OSF for the combined liver, oral mucosa, lung, and pancreatic tumors in female rats from the newer NTP (2006, 543749) study is 4.4×10^5.

5.2.3.2.5. *Summary evaluation of slope factor estimates from rodent bioassays.*

To estimate a range of candidate TCDD OSFs from the animal data, dose-response modeling of the five chronic rodent bioassays identified in Section 2.4.3 was conducted. Dose-response modeling was performed using whole blood concentrations, and $BMDL_{HED}$ values (ng/kg-day) were derived for the 28 species/sex/endpoint data sets individually (see Table 5-16) and for seven species/sex combined tumor data sets (see Table 5-17).

The highest OSFs that have been derived for these animal cancer bioassays using the multistage models are from the multiple tumor analyses for NTP (1982, 594255; 2006, 543749) and Kociba et al. (1978, 001818), presented in Table 5-17, and from the individual tumor analyses for Toth et al. (1979, 197109) liver tumors and Della Porta et al. (1987, 197405) liver carcinomas in male mice, presented in Table 5-16. The most sensitive species and sex is male mice, for which the estimated $BMDL_{HED}$ for combined tumors is 1.1×10^{-3} ng/kg-day. This result, which is derived under the assumption that multiple tumor types occur independently in the exposed animals, is, as expected, lower than the $BMDL_{HED}$ for the most sensitive individual tumor.

Based on these results, EPA believes that a credible value for the $BMDL_{HED}$ derived from the animal studies lies in the range shown in Table 5-17 between 3.1×10^{-2} and 1.1×10^{-3} ng/kg-day. These values, which correspond to oral slope factor values of 3.2×10^5 and 9.4×10^6 per mg/kg-day, respectively, encompass the range at which elevated cancer risks can be detected for the most sensitive species, sex, and endpoints in the animal bioassay data.

As noted above in Sections 5.2.3.1.2.2 and 5.2.3.1.2.3, the cancer mortality risk is strictly linear only with TCDD blood concentration, such that a single OSF cannot represent the entire range of risks for oral ingestion. The OSFs shown in Tables 5-16 and 5-17 are based on HEDs corresponding to the $BMDL_{01}$, which are most representative of lower human exposure levels, including ambient exposures. For higher exposures, the risks increase at a slower rate with increasing dose and the corresponding OSFs are lower; in those cases, risk-specific doses can be calculated as previously described (see Section 5.2.3.2.3.2).

5.2.3.2.6. *Qualitative uncertainties in slope factor estimates from rodent bioassays.*

This section presents a qualitative discussion of the uncertainties associated with calculating the OSF for TCDD from chronic animal bioassay data. Discussions on the feasibility of conducting a quantitative uncertainty analysis for TCDD using dose-response methods are provided in Section 6.4.2 of this document.

5.2.3.2.6.1. ***Quality of studies relied upon for determining POD.***

EPA considers the overall quality and breadth of the studies used for the cancer dose-response analysis to be excellent. All of the studies were published in the peer-reviewed literature, and two of them were conducted by NTP (1982, 594255; 2006, 543749). Kociba et al. (1978, 001818), Della Porta et al. (1987, 197405) and Toth et al. (1979, 197109) are older studies, but appear to have been conducted according to good laboratory practice standards. The control and dose group sample sizes were relatively large, ~40−50 animals or more per group for all of the studies. All five studies exposed the test animals via the oral route to TCDD alone, as was stipulated in EPA's study inclusion criteria. Collectively, these five studies reported development of numerous cancer endpoints (tumors) in both sexes in two strains of rats (Sprague-Dawley and Osborne-Mendel) and two strains of mice (i.e., $B6C3F_1$, Swiss/H/Riop). The overall high quality of these studies and the strong, positive association between TCDD exposure and cancer suggests that study quality is not a major contributing factor to uncertainty in the risk estimates.

5.2.3.2.6.2. *Interpretation of results from studies relied upon for determining POD.*

As discussed in Section 3.4.3.2.1, questions arose about the interpretation of liver tumor responses in female rats in the Kociba et al. (1978, 001818) study. Three re-evaluations of the slides have been reported (Goodman and Sauer, 1992, 197667; Kociba et al., 1978, 001818; Squire, 1980, 594272). The decision to use the Goodman and Sauer (1992, 197667) evaluation was based on their use of the most current tumor classification procedures. The incidence of hepatocellular adenomas and carcinomas (individually and combined), however, did vary (sometimes widely) for each dose group across the three evaluations. Although the state-of-the-science is reflected in the Goodman and Sauer analysis, there is some uncertainty in the interpretation of any post-hoc analysis. No issues have arisen with regard to the interpretation of the NTP (1982, 594255; 2006, 543749), Della Porta et al. (1987, 197405) or Toth et al. (1979, 197109) tumor identification and classification.

5.2.3.2.6.3. *Consistency of results across chronic rodent bioassays.*

The existence of five high-quality chronic bioassays for TCDD increases confidence and reduces uncertainty in the cancer OSFs. Considered together, these studies tested two species and both sexes of mice and rats, and a wide range of well-characterized tumor types. All five studies were consistent in observing increases (at some dose level) in rates of liver tumors (in both species and sexes). While tumors at other sites were observed (and those sites varied across study, species, and sex), the liver tumors were consistently the most sensitive indicators of carcinogenic response (with respect to $BMDL_{HED}$ estimates). Lung tumors were also consistently observed across three of the studies, in male mice in the NTP (1982, 594255) study and in female rats in Kociba et al. (1978, 001818) and NTP (2006, 543749). As discussed above, the two most sensitive single-tumor endpoints as judged by $BMDL_{01}$ values were associated with elevated liver tumor risks, followed by lung, lymphoma or leukemia, thyroid and adrenal cancers. The consistency of tumor types and sensitivities across endpoints and studies lends confidence to the multistage modeling results.

5.2.3.2.6.4. *Human relevance of rodent tumor data.*

There is some concordance in the tumor responses observed in the rodent test species and humans, however, the most sensitive tumor site in the animals, the liver, has not been associated

with cancer from TCDD exposures in humans. On the other hand, lung cancer and leukemia are found both in the animal studies and in epidemiologic studies of exposed workers. The consistency across sex, species, and strains in the animal studies suggests that the occurrence of several of these tumors, in particular, liver and lung tumors is not an idiosyncratic response of a particular combination of species, strain, or sex. As discussed in Section 5.2.1, the likely AhR related carcinogenic mechanism is credible for humans as well as for rodent species.

5.2.3.2.6.5. ***Relevance of rodent exposure scenario.***

Three of the five chronic rodent bioassays exposed the test animals for ~2 years, the majority of their lifespans. Toth et al. (1979, 197109) exposed the animals only for one year, but they were kept on the study for a second year before they were evaluated for cancer. The Della Porta et al. (1987, 197405) study also exposed the test animals for one year, and a dosing error occurred during the study. At ages 31 to 39 weeks, 41 male mice and 32 female mice in the 2,500 ng/kg BW dose group were mistakenly administered a single dose of 25,000 ng/kg BW TCDD. TCDD treatment for the 2,500 ng/kg BW dose group was halted for 5 weeks (beginning the week after the 25,000 ng/kg BW dose was administered in error) and resumed until exposure was terminated at 57 weeks. Thus, the large single dose and subsequent period without TCDD exposure confounds the dose-response relationship for this study. In general, these lifetime bioassays in animals have long been used by EPA to assess potential lifetime exposures and effects in humans. However, in the case of TCDD, the half life of TCDD in the body for rats, mice, and humans is very different (see Section 3). Thus, there is a significant amount of uncertainty in the use of rat and mouse data to develop OSFs for human cancer risk assessment of TCDD.

5.2.3.2.6.6. ***Impact of background TCDD exposures.***

It is known that TCDD has been found in the feed used in animal bioassays, and that this is a confounding factor, particularly in older studies. The effect of TCDD in the diets of test species has the potential to be quite significant given the low levels of TCDD at which adverse effects have been observed. Insofar as that is an issue, the risks associated with TCDD exposures in the animal bioassays, and therefore the OSFs, would be biased high, which could be the case for the NTP (1982, 594255), Della Porta et al. (1987, 197405), Kociba et al. (1978,

001818) and Toth et al. (1979, 197109) studies. The impact of this issue is that the newer study, NTP (2006, 543749), accounted for TCDD exposures in the animal feed. Thus, there is likely to be less uncertainty in the TCDD dose-response information presented in NTP (1982, 594255; 2006, 543749) than in the other four studies conducted before 1990.

5.2.3.2.6.7. ***Choice of endpoint for POD derivation.***

As noted above, the liver tumor PODs represent the most sensitive single-tumor endpoint across the five cancer bioassays. Thus, the liver cancer endpoints must be seriously considered for derivation of a TCDD OSF. As discussed in the previous section, EPA has also developed Bayesian dose-response estimates for combined tumors, which yield $BMDL_{01}$ values slightly lower than those for any individual tumor type. Although it is the most conservative choice to select the lowest combined tumor POD for OSF derivation, there are uncertainties associated with the multiple tumor analysis. The assumption of independence of tumors across sites is reasonable, particularly since the tumors from TCDD do not metastasize. However, the independence assumption lacks hard evidence and needs further laboratory confirmation.

5.2.3.2.6.8. ***Choice of animal-to-human extrapolation method.***

The analyses presented here have used the Emond human kinetic model for extrapolating dose from animals to humans (as discussed in Section 3.4.2). The rationale for this choice is that the blood concentration metric most accurately reflects the concentration of TCDD in the various tissues. As discussed in Section 3.4.3.2.4, use of the blood concentration dose metric results in critical dose estimates (HEDs) that are considerably lower (10- to more than 100-fold) than those derived based on administered dose. This does not reflect bias in the blood-based measure; rather it is a reflection of the nonlinear biokinetics of TCDD in the body. EPA has also explored the impacts of using other dose metrics, including AhR-bound TCDD concentration calculated based on the Emond model. As discussed in Section 3.4.3.2.6.2, this also results in HED estimates much lower than those obtained based on administered dose.

5.2.3.2.6.9. ***Choice of model for POD and model uncertainty for POD derivation.***

The bioassay-based cancer dose-response assessment in this section has used the multistage model which is the standard model choice for such assessments and has been the basis

for most of EPA's cancer risk assessments. The multistage model is the standard because it is the only available model form that allows for low-dose linearity while accommodating curvilinearity at higher doses and can be readily implemented.

There is some model choice uncertainty associated with instances of lack of fit. When the multistage model does not adequately describe the observed pattern of responses (typically determined by examining the *p*-value for lack of fit), a decision must be made about possible adjustments, including the dropping of higher dose groups thought to be less relevant to the estimation of low-dose slopes. In this analysis, poorer fits (*p*-values less than 0.10) were observed in five cases, four from NTP (1982, 594255) and one from Della Porta et al. (1987, 197405) (see Table 5-15). The lowest $BMDL_{01}$ associated a low *p*-value ($p = 0.09$) was for the lung tumors in the NTP (1982, 594255) male mouse, the third lowest POD behind the liver PODs in the individual tumor data sets. The other instances were for adrenal cortex and thyroid follicular cell adenomas in male rats and for subcutaneous tissue in female mice in the NTP (1982, 594255) study and for liver carcinomas in female mice in Della Porta et al. (1987, 197405). In those instances, the *p*-values were 0.06, 0.06, 0.09, and 0.019, respectively. These poorly fit data sets provide OSF estimates that are uncertain and also contribute to uncertainty in the combined tumor PODs from NTP (1982, 594255). The lowest $BMDL_{01}$ in the combined tumors is for the male mice combined liver and lung tumors, thus estimates from this sex/species combination from NTP (1982, 594255) is highly uncertain and impacts its choice as a POD.

5.2.3.2.6.10. ***Statistical uncertainty in model fits.***

Every model fit to a data set is associated with some inherent statistical uncertainty. For this reason, bounds were calculated and used for OSF derivation (e.g., lower bounds on benchmark doses, in this case the $BMDL_{01}$s). Those bounds account for uncertainties associated with finite samples of test animals, both in terms of the number of dose groups and of the number of animals per dose group. Valid and accepted statistical procedures have been applied to ascertain the impact of those limitations on the estimates of interest. That being the case, the statistical uncertainties associated with finite samples have been adequately addressed.

5.2.3.2.6.11. *Choice of risk level for POD derivation.*

The BMR level that has been used for the POD in deriving the cancer OSF is one percent extra risk. A single BMR was chosen for consistency across studies. Also, a BMR of 1% was judged to be near the range of the observations. For the TCDD animal cancer bioassay data, although many of the first positive tumor incidence responses (relative to controls) are closer to 10% (some higher), some are as low as 2%. Furthermore, most of the BMD_{01} values are within a factor of 3 of the lowest tested dose, and the $BMDL_{01}$ values are generally less than a factor of 2 below the BMD. Table 5-18 presents a comparison of BMDs, BMDLs and slope factors for 1%, 5% and 10% BMRs from the multi-tumor analyses of NTP (1982, 594255; 2006, 543749) and Kociba et al. (1978, 001818) and for selected single tumor data sets from Toth et al. (1979, 197109) and Della Porta et al. (1987, 197405). In Table 5-18, the choice of BMR has little or no impact on the slope factors based on TCDD blood concentration for the combined or single tumor incidences selected as representative of each study.[43] In contrast, Table 5-19 presents a comparison of Human Equivalent Dose BMDs, BMDLs and slope factors for 1, 5, and 10% BMRs from these same datasets. Table 5-19 shows that, when converting the blood concentration to the equivalent HED, a 2-fold to 4-fold decrease in the OSF is obtained when using a BMR of 10% rather than 1%. This result is a consequence of the nonlinearity in the Emond PBPK model at higher doses, where dose-dependent elimination of TCDD in the liver results in a less-than-proportional increase in blood concentration relative to oral intake. At lower exposure levels, blood concentration is proportional to oral intake. Therefore, EPA has chosen the lower BMR of 1% as more representative of the low-dose risk.

5.2.3.3. *EPA's Response to the NAS Comments on Choice of Response Level and Characterization of the Statistical Confidence Around Low Dose Model Predictions*

The NAS was concerned with the statistical power to determine the shape of the dose response curve at low doses, well below observed dose-response information. EPA shares this concern in that the shape of the dose-response curve in the low-dose region cannot be determined with confidence when based on higher dose information.

[43] This will generally be the case for multistage model fits with 1st-degree coefficients greater than zero because the response at the BMDL is virtually linear at BMRs of 10% or less. For model fits dominated by higher-order coefficients, linearity of response at the BMDL begins at lower BMRs.

When tumor data are used for dose-response modeling, a POD is obtained from the modeled tumor incidences. When assessing carcinogenicity using a linear extrapolation approach from a POD, a balance must be struck between staying within the range of the observations and obtaining a representative estimate of the low-dose slope. Traditional cancer bioassays, with approximately 50 animals per group, can typically support modeling down to an increased incidence of 1–10%; epidemiologic studies, with larger sample sizes, below 1%. For the TCDD animal cancer bioassay data, most of the low-dose tumor incidence responses are under 10% (relative to controls), with some as low as 2%. For comparison purposes, BMDs, BMDLs and OSFs from the animal cancer bioassay benchmark dose modeling assuming 1, 5, and 10% extra risk are shown in units of blood concentrations and human equivalent doses in Tables 5-18 and 5-19, respectively. After evaluating the magnitude of the uncertainty in $BMDL_{01}$s against the impact of using $BMDL_{10}$s, EPA has chosen to use a 1% BMR in all cases, determining that the uncertainty bounds on the $BMDL_{01}$ values are reasonable.

In the analysis of the animal cancer bioassays presented in this document, the multistage cancer model was applied with a linear dose extrapolation to zero. EPA used a 1% excess risk estimate, i.e., a $BMDL_{01}$, as the POD for development of candidate TCDD cancer oral slope factors using a Bayesian multitumor approach (see Section 5.2.3.2. The advantage of a Bayesian approach is that it produces a distribution of BMDs that allows better characterization of statistical uncertainty.

Central tendency slope estimates and upper bound oral slope factor estimates are part of the standard BMDS multistage cancer model and are included in each output file for the animal bioassay single tumor analyses in Appendix F. Central tendency BMDs are also reported for the results of the animal bioassay multitumor analysis (see Table 5-15). Central tendency slope estimates are given for all the qualifying epidemiological studies as well (see Tables 5-1 and 5-4), where possible.

5.2.3.4. *EPA's Response to the NAS Comments on Model Forms for Predicting Cancer Risks Below the POD*

The NAS offered extensive comments on the cancer dose-response modeling in the 2003 Reassessment. Although epidemiologic and rodent bioassay data are useful for the evaluation of the dose-response curve within the range of the observed response data, they have traditionally

not been useful sources of information for identifying a threshold or for estimating the shape of the dose-response curve below the POD. Rather, mechanistic toxicological data have been the evidentiary sources of choice for those types of analyses. As noted above, any quantitative estimation of carcinogenic risk associated with TCDD exposure requires low-dose extrapolation of experimental data. Unfortunately, the shape of the dose-response curve in the low dose region is unknown.

Several of the analyses of epidemiological cohort data evaluated the fit of different dose-response models to the data. Log-dose models accentuate the importance of low-dose low-magnitude responses and can yield implausible results. The most relevant models used in these studies are the untransformed-dose Cox regression models. Better results have been obtained in the cohort analyses when the flattening of the hazard-ratio curve is taken into account. The latter has been modeled explicitly by Steenland et al. (2001, 198589), who use a piecewise linear model and implicitly by Cheng et al. (2006, 523122), who drop out a percentage of the high-dose response data and fit a linear model to the remainder. Importantly, the analyses of the epidemiologic cohorts presented in Section 5.2.3.1 are limited to evaluation and reanalyses of published data as reported by the study authors. EPA does not have access to the raw data from these epidemiologic studies and, therefore, could not conduct *de novo* analyses.

5.2.3.4.1. *Choice of extrapolation approach*

5.2.3.4.1.1. ***TCDD and receptor theory.***

TCDD is considered to be a receptor-mediated carcinogen in animals. Nearly all TCDD experimental data are consistent with the hypothesis that the binding of TCDD to the AhR is the first step in a series of biochemical, cellular, and tissue changes that ultimately lead to toxic responses observed in both experimental animals and humans (Part II, Chapter 2 of the 2003 Reassessment). Ligand-receptor binding, like any bimolecular interaction, obeys the law of mass action as originally formulated by A.J. Clark (Limbird, 1996, 594276). The law of mass action predicts the fractional receptor occupancy at equilibrium as a function of ligand concentration. Fractional occupancy (Y) is defined as the fraction of all receptors that are bound to ligand:

$$Y = \frac{[TCDD - AhR]}{[AhR]_{TOT}} = \frac{[TCDD - AhR]}{[AhR] + [TCDD - AhR]} = \frac{[TCDD]}{[TCDD] + K_d} \quad \text{(Eq. 5-8)}$$

where [TCDD] is the concentration of the ligand, [AhR] is the concentration of the receptor and [TCDD-AhR] is the amount of liganded receptor. The equilibrium dissociation constant K_d describes the affinity of the interaction and is the concentration of TCDD that results in 50% receptor occupancy. This simple equation defines a rectangular hyperbola, which is the characteristic shape of the vast majority of biological dose-response relationships.

In certain cases, no response occurs even when there is some receptor occupancy. This suggests that there may be a threshold phenomenon that reflects the biological "inertia" of the response (Ariens et al., 1960, 594279). In other cases, a maximal response occurs well before all receptors are occupied, a phenomenon that reflects receptor "reserve" (Stephenson, 1956, 594280). Therefore, the law of mass action cannot by itself fully explain the effect or response observed after TCDD interacts with AhR. The ligand-receptor complex is associated with a signal transduction or effector system. In the case of the AhR, this effector system can be considered to be the transcriptional machinery itself. The key feature of this formulation is that a response is proportional, or a function of, the number of receptors occupied.

Furthermore, for a ligand such as TCDD that elicits multiple receptor-mediated effects, one cannot assume that the binding-response relationship for a simple effect (such as enzyme induction) will necessarily be identical to that for a different and more complex effect (such as cancer). The cellular cascades of events leading to different complex responses (e.g., altered immune function, developmental effects, or cancer) are different, and other rate-limiting events likely influence the final biological outcome resulting in different dose-response curves. Thus, even though TCDD binding to AhR is assumed to be the initial event leading to a spectrum of biological responses, TCDD-AhR binding data may not always correlate with the dose-response relationship observed for particular effects.

A receptor-based mechanism would predict that, except in cases where the concentration of TCDD is already high (i.e., [TCDD]~K_d), incremental exposure to TCDD will lead to some increase in the fractional occupancy of AhR. However, as discussed above, it cannot be assumed that an increase in receptor occupancy will necessarily elicit a proportional increase in all biological response(s), because numerous molecular events contributing to the biological endpoint are integrated into the overall response. That is, the final biological response could be considered as an integration of a series of interdependent dose-response curves with each curve dependent on the molecular dosimetry for each particular step. Dose-response relationships that

will be specific for each endpoint must be considered when using mathematical models to estimate the risk associated with exposure to TCDD. It remains a challenge to develop models that incorporate all the complexities associated with each biological response as the modes of action for various toxicological endpoints appear to vary greatly. For TCDD, extensive experimental data from studies using animal and human tissues indicate that cell- or tissue-specific factors determine the quantitative relationship between receptor occupancy and the ultimate biological response. This would suggest that the parameters for each mathematical model might only apply to a single biological response within a given tissue and species, making extrapolation to other systems challenging.

5.2.3.4.1.2. ***Low-dose extrapolation: threshold or no threshold?***

As indicated in the 2005 Cancer Guidelines,[44] toxicity reference values for human noncancer endpoints have historically been estimated based on a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) from animal bioassay studies. This terminology suggests a biological population threshold beneath which no harm is anticipated. Reference values such as the oral reference dose (RfD) or inhalation reference concentration (RfC) are derived by applying uncertainty factors (UFs) to a POD. Depending on the nature of available data and modeling choice, a POD can be selected from values other than an NOAEL or LOAEL, such as an ED_x, or a benchmark dose (BMD) or its BMDL. An RfD is described as "likely to be without appreciable risk" but the probabilistic language has not as yet been operationalized. There is no quantitative definition of "appreciable" and no mechanism to compute risk as a function of dose, so as to ascertain that the risk is indeed not appreciable. The risk at the RfD is not calculated, and it cannot be calculated within the current UF framework. Instead, a hazard quotient is computed as the ratio of a given exposure to the RfD, or a margin of exposure is estimated as the ratio of the POD to the human exposure level.

Cancer endpoints are predominantly thought to have no population biological threshold. Although the terminology "threshold/nonthreshold" is still common in cancer dose-response

[44]As stated in the 2005 Cancer Guidelines (U.S. EPA, 2005, 086237): "For effects other than cancer, reference values have been described as being based on the assumption of biological thresholds. The Agency's more current guidelines for these effects (U.S. EPA, 1996, 594399; U.S. EPA, 1998, 030021) however, do not use this assumption, citing the difficulty of empirically distinguishing a true threshold from a dose-response curve that is nonlinear at low doses."

discussions, the 2005 Cancer Guidelines propose a different terminology, whereby "nonlinear models" are those whose dose-response *slope* is zero at or above zero. In the natural language, and indeed in data analysis, it is difficult to distinguish the following situations:

- The response approaches zero as dose goes to zero, versus
- The response *slope* goes to zero as dose goes to zero (nonlinear model).

This use of "nonlinear" is acknowledged to be idiosyncratic.[45] The NAS review (NAS, 2006, 198441) does not consistently apply the terminology from the 2005 Cancer Guidelines, nor does it consistently distinguish the above two circumstances: "…the observed data are more consistent with a sublinear response that approaches zero at low doses rather than a linear dose response" (NAS, 2006, 198441). The point of a nonlinear model in the sense of the 2005 Cancer Guidelines is that the response *slope* approaches zero. Both linear and nonlinear *responses* approach zero at low dose (in the absence of background). Since the terms "linear," "sublinear," and "nonlinear" invite confusion in this context, the following terminology is used in this document:

Threshold Model: There is some threshold dose T > 0 such that the probability of response for any dose less than or equal to T is zero, and the probability is nonzero for any dose greater than T.

Linear/ Linear above Threshold Model: For the linear model, the probability of response is proportional to the dose. For the linear over threshold model, the probability of response is zero for a dose below the threshold, and it is proportional to the excess dose over the threshold otherwise. Note that under the EPA cancer guidelines, the linear above threshold model is classified as a nonlinear model.

Nonlinear Model: Any model that is not linear.

Supralinear/ Supralinear above Threshold Model: For the supralinear model, the slope of the probability of response decreases as dose increases; in other words, the second derivative of the response curve is negative. For the supralinear above threshold model,

[45]From the 2005 Cancer Guidelines (U.S. EPA, 2005, 086237): "The term '*nonlinear*' is used here in a narrower sense than its usual meaning in the field of mathematical modeling. In these cancer guidelines, the term '*nonlinear*' refers to threshold models (which show no response over a range of low doses that include zero) and some nonthreshold models (e.g., a quadratic model, which shows some response at all doses above zero). In these cancer guidelines, a nonlinear model is one whose slope is zero at (and perhaps above) a dose of zero. …. Use of nonlinear approaches does not imply a biological threshold dose below which the response is zero."

the second derivative is negative above the threshold, and the response probability is zero below the threshold.

Sublinear/Sublinear above Threshold Model: For the sublinear model, the slope of the probability of response increases as dose increases; in other words, the second derivative of the response curve is positive. For the sublinear above threshold model, the second derivative is positive above the threshold, and the response probability is zero below the threshold.

Zero Slope at Zero Model: The slope of the response curve is zero at or above dose zero.

All of these models may be understood in an individual or population sense. According to the 2005 Cancer Guidelines, the trigger for applying the basic RfD methodology for cancer endpoints is sufficient evidence for the "zero slope at zero" model for the population. By definition, any sublinear, supralinear, or linear model *above the threshold* is a zero slope at zero ("ZS@Z") model.

The relation between individual and population models is not immediately evident. Figure 5-4 shows dose-response curves of the probability of response vs. dose for different models dose-response shapes. The left panel in Figure 5-4 shows a supralinear dose-response curve; the rate of increase of the response probability goes down as dose increases, or in the strict mathematical sense, the second derivative is negative. The middle panel shows a sublinear dose-response curve; the second derivative is positive. In this case the slope at zero is zero (ZS@Z). However, sublinearity, in the strict mathematical sense, by itself does not imply that the slope at zero is zero. The probit dose-response model shown in the right graph is sublinear and has positive slope at zero (the log-probit model is zero slope at zero).

If individuals in a population have different dose-response curves, then the population dose-response curve is obtained by averaging all these dose-response curves over the population. The shape of the population dose-response curve will generally be quite different from the individual curves. Figure 5-5 is a simple depiction of the relationship of individual vs. population dose response. The left panel in Figure 5-5 shows dose-response curves for seven individuals, each with a supralinear dose-response curve above individual-specific thresholds. Averaging these curves gives the dashed dose-response curve, which is nearly linear. The graph on the right is similar, except that the individual dose-response curves are linear above individual thresholds. The population curve is quadratic and zero slope at zero applies.

Of course these are not the only possibilities; in general, the population dose-response curve depends on (1) the distribution of individual thresholds in the neighborhood of zero, (2) the dose-response curve for each individual, and (3) the dose metric. Under EPA's Cancer Guidelines, the zero-slope-at-zero criterion applies strictly to ingested dose, but the other two factors (distribution of individual thresholds and dose-response curve for each individual) need to be established before a zero slope at zero dose can be established. Otherwise the default linear extrapolation to zero approach applies.

On the nature or the distribution of individual thresholds, often referred to as the population tolerance distribution, there is ongoing debate as to how receptor kinetics influence the shape of that distribution. Even within an individual, there is a lack of consensus as to whether receptor kinetics confer linear or sublinear attributes to downstream events, or whether receptor kinetics, themselves, are linear, sublinear, or supralinear. Whatever the nature of the form of receptor kinetics, it may have little or no influence on the ultimate population response. The kinetics of receptors is in the domain of the individual, rather than the population. As described previously, receptor kinetics are governed by the law of mass action, which leads to a low-dose proportional response model, generally modeled by some form of Hill function, the low-dose linear form being Michaelis-Menten kinetics. There is no *a priori* reason to believe that the shape of the dose-response curve in an individual has any relationship to the shape of the population response, particularly for quantal endpoints. Lutz and Gaylor (2008, 594297) present an argument for considering the population response in terms of the more traditional tolerance distribution, which is likely the result of more variable factors than the shape of receptor kinetics. Perhaps more to the point, receptor activation is only the first of many events in the path to the apical event (a tumor in this example). Because there are undoubtedly numerous additional downstream events that must occur before the apical effect is observed, there are many opportunities for interindividual variability to become manifest in the tolerance distribution. Even at the first step, a more likely contributor to interindividual variability than the shape of the response is the dose resulting in the response, as measured by the ED_{50} (K_m in the Michaelis-Menten formulation), which shifts the response curve. Factors that influence shifts in response curves are generally modeled as normal or log-normal distributions and may confer a log-normal shape on the population tolerance distribution, particularly if there are a number of dependent sequential steps or distinct subpopulations (Hattis and Burmaster, 1994, 594301; Hattis et al.,

1999, 594299; Lutz, 1999, 594298), although other distributions could be equally likely (Crump et al., 2010, 380192).

To see how the discussion over threshold/nonthreshold might play out for TCDD, consider the equilibrium dissociation constant K_d for TCDD, which measures the binding affinity of TCDD to the AhR. Lower values indicate higher binding affinity and (other things being equal) greater risk. For Han/Wistar rats, the value $K_d = 3.9$ is reported (standard deviation not given); human values are reported as $K_d = 9.6 \pm 7.8$ *(0.3 – 38.8 with 15 of 67 donors without detectable binding)* (Connor and Aylward, 2006, 197632). If AhR binding is the rate-limiting step for carcinogenesis, then the majority of a human population may be less susceptible than Han/Wistar rats, whereas a population threshold, if it exists, might still be well below the Han/Wistar rat threshold, given the large variability in the human K_d estimate (see also Section 6.4.2.9). The NAS contends that an AhR-mediated mode of action indicates a threshold dose-response relation (NAS, 2006, 198441). Presumably, the value of the threshold, if it exists, depends on the AhR binding affinity. Arguing for a population threshold in this case requires two types of information:

1. The distribution of the individual thresholds induced by, among other things, the individual K_d values; and
2. The dose-response function for values above the threshold induced by K_d.

Without this information, the shape of the population dose-response curve cannot be determined with any confidence and the default linear relationship applies; response probability is modeled as a linear function of dose, for dose near zero. However, from the 2005 Cancer Guidelines: "When adequate data on mode of action provide sufficient evidence to support a nonlinear mode of action *for the general population* (emphasis added) and/or any subpopulations of concern, a different approach—a reference dose/reference concentration that assumes that nonlinearity—is used." In current terminology, the reference dose methodology applies if there is sufficient evidence supporting a "zero slope at zero" model; otherwise, the linear nonthreshold model applies by default.

In principle, the choice between the above models could fall within the purview of dose-response modeling. However, standard statistical methods encounter well-known difficulties in

detecting thresholds. Without going into detail, suffice to say that the maximum likelihood estimate of response probability when no responses are observed in a finite sample is always zero. That said, some researchers have attempted to identify thresholds (Aylward et al., 2003, 594305; Mackie et al., 2003, 594303) or nonlinearity (Hoel and Portier, 1994, 198741) by means of parameter estimation of appropriate models. A review of 344 rodent bioassays on 315 chemicals led to the following conclusion by Hoel and Portier (1994, 198741):

> We have also found that the oft-held belief that genotoxic compounds typically follow a linear dose-response pattern and that nongenotoxic compounds follow a nonlinear or threshold dose response pattern is not supported by the data. In fact we find the opposite with genotoxic compounds differing from linearity more often than nongenotoxic compounds.

The choice between a linear and "zero slope at zero" model in current practice does not fall under dose-response model fitting, it is made on the basis of a structured narrative as set forth in the 2005 Cancer Guidelines (U.S. EPA, 2005, 086237):

> In the absence of sufficiently, scientifically justifiable mode of action information, EPA generally takes public health-protective, default positions regarding the interpretation of toxicologic and epidemiologic data: animal tumor findings are judged to be relevant to humans, and cancer risks are assumed to conform with low dose linearity. … The linear approach is used when: (1) there is an absence of sufficient information on modes of action or (2) the mode of action information indicates that the dose-response curve at low dose is or is expected to be linear. Where alternative approaches have significant biological support, and no scientific consensus favors a single approach, an assessment may present results using alternative approaches. A nonlinear approach can be used to develop a reference dose or a reference concentration.

5.2.3.4.1.3. ***Extrapolation method.***

The 2005 Cancer Guidelines (U.S. EPA, 2005, 086237) emphasize that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve.

The NAS was critical of EPA's decision to apply linear low-dose extrapolation for TCDD cancer assessment in the 2003 Reassessment and encouraged the use of a nonlinear approach. The 2005 Cancer Guidelines state that a nonlinear approach should be used when

"there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses."

Receptor modeling theory (as outlined in the 2003 Reassessment, Part II, Chapter 8) indicates that exogenous compounds which operate through receptor binding mechanisms, such as TCDD, will follow a linear dose-response binding in the 1−10% receptor occupancy region. This theory has been supported by empirical findings and suggests that the proximal biochemical effects (such as enzyme induction) and transcriptional reactions for TCDD may also follow linear dose-response kinetics. More distal toxic effects could take any one of multiple forms (i.e., linear, sublinear, supralinear or threshold) depending on (1) the toxic mechanism; (2) location on the dose-response curve; and (3) interactions with other processes such as intracellular protein binding and cofactor induction/repression.

In the case of TCDD, many adverse effects experienced at low exposure levels have too much data variability to distinguish on a statistical basis (goodness-of-fit) between dose-response curve options, and whether the dose-response is linear, sublinear or supralinear. For tumor responses, with the exception of squamous cell carcinoma of the oral mucosa and adenomas or carcinomas of the pancreas, which were fit with a linear multistage model, the tumor endpoints in the NTP (2006, 543749) study using female Sprague-Dawley (S-D) rats are all best fit with a sublinear model (i.e., the multistage model fits to tumor incidence data were second or third degree; see Table 5-15 and Appendix F). For all tumor incidence data from three of the other cancer bioassays that met the study inclusion criteria (Kociba et al., 1978, 001818; NTP, 1982, 594255; Toth et al., 1979, 197109), the multistage model fit was linear (first degree), when based on either administered dose or modeled blood concentrations (see Appendix F). For Della Porta et al. (1987, 197405), the female liver carcinomas were linear (first degree), but the female liver adenomas and the male liver carcinomas were best modeled using a second degree model (see Table 5-15).

Another issue of potential importance when evaluating the shape of the dose-response curve for low dose effects is the concept of "interacting background." The concept of interacting background refers to a pathological process in the exposed population that shares a causal intermediate with the toxicant being evaluated. On this issue, a recent NAS committee (NAS, 2009, 594307) contended that

> …the current EPA practice of determining "nonlinear" MOAs does not account for mechanistic factors that can create linearity at low dose. The dose-response relationship can be linear at a low dose when an exposure contributes to an existing disease process Crump et al., 1976, 003192; Lutz, 1990, 000399. Effects of exposures that add to background processes and background endogenous and exogenous exposures can lack a threshold if a baseline level of dysfunction occurs without the toxicant and the toxicant adds to or augments the background process. Thus, even small doses may have a relevant biologic effect. That may be difficult to measure because of background noise in the system but may be addressed through dose-response modeling procedures. Human variability with respect to the individual thresholds for a nongenotoxic cancer mechanism can result in linear dose-response relationships in the population (Lutz, 2001, 053426; NAS, 2009, 594307.

AhR activation could be considered a causal intermediate in several disease processes. Recent studies have linked AhR activation in the absence of exogenous ligand to a multitude of biological effects, ranging from control of mammary tumorigenesis to regulation of autoimmunity (reviewed in Hahn et al., 2009, 548725). While the level of background activation of AhR by endogenous compounds (or exogenous compounds other than TCDD) in the human population is unknown, given the ubiquitous nature of several of the known endogenous and exogenous AhR ligands, it is reasonable to assume that a certain baseline level of AhR activation exists in the population. The degree to which TCDD exposure augments this baseline level of AhR activation is unknown.

The 2005 Cancer Guidelines (U.S. EPA, 2005, 086237) recommend that the method used to characterize and quantify cancer risk from a chemical be determined by what is known about the mode of action of the compound and the shape of the cancer dose-response curve. The linear approach is used if there is sufficient evidence supporting linearity or if the mode of action is not understood (U.S. EPA, 2005, 086237). In the case of TCDD, (1) the mode of action of TCDD-induced carcinogenesis beyond potential AhR activation is unknown; (2) information is lacking to determine the shape of the dose-response curves at low doses for various adverse endpoints (including cancer) in humans or experimental animals; (3) there is undoubtedly a certain level of interacting background (i.e., AhR activation by endogenous ligands) in the human population; (4) many of the rodent cancer dose-response relationships (Kociba et al., 1978, 001818; NTP, 1982, 594255; Toth et al., 1979, 197109) are consistent with low-dose linearity (first degree multistage model fit) when based on either administered dose or modeled blood concentrations;

and (5) higher human interindividual variability compared to experimental rodents will tend to shift the shape of the dose-response towards linear (relative to rodents). None of these suggestions of linearity, however, is conclusive (see next section for additional detail). The true shape of the dose-response curve remains unknown. Therefore, in the absence of sufficient evidence to the contrary or evidence to support nonlinearity, to estimate human carcinogenic risk associated with TCDD exposure EPA assumed a linear low-dose extrapolation approach.

5.2.3.4.1.4. *Discussion of low-dose linearity.*

Any quantitative estimation of carcinogenic risk associated with TCDD exposure requires low-dose extrapolation of high dose experimental and epidemiologic data. Unfortunately, despite the availability of the extensive database on the biological effects of TCDD, the shape of the dose-response curve in the low-dose region is not known. This situation is not unique to TCDD. For most carcinogens the available biological data do not provide sufficient mechanistic information to determine the shape of the dose-response relationship at doses below the levels where direct experimental or epidemiologic data are available. EPA's Guidelines for Carcinogen Risk Assessment (2005, 086237) recognize this situation and describe approaches the Agency uses for dose response assessment in cancer risk assessments depending on the available scientific database. EPA's basic approach makes a distinction between "low-dose linear" and "nonlinear" dose response patterns. This distinction is important to understand as it addresses the potential response at low dose, not the empirical pattern of response seen in the available (often high dose) tumor data. To put matters simply, under a low-dose-linear model, the estimated risk due to the carcinogen exposure is approximately proportional to the dose received (at low dose). In mathematical terms, a low-dose-linear model is one whose slope is greater than zero at a dose of zero (U.S. EPA, 2005, 086237; footnote, p. 1-11). Importantly, a low-dose-linear model need not be linear at higher doses, and this is consistent with upward curving responses (e.g., linear-quadratic) and downward curving (plateauing) responses that may be seen various cancer studies. In EPA's terminology a "nonlinear" dose-response, refers to situations where there is not a linear component in the response at low-dose. In this context, a "nonlinear model" is one whose slope is zero at (and perhaps above) a dose of zero (ibid). Nonlinear response patterns can include threshold models where there is no response below a defined dose

level, or other patterns where response at low dose otherwise decreases rapidly as compared to a low-dose-linear model.

As stated in the previous section, the low-dose linear approach for the TCDD carcinogenicity assessment in this document is based on EPA's scientific baseline inference ("default") regarding dose-response modeling. EPA believes that the mode of action is not known, so is using the default linear extrapolation approach specified by EPA's cancer guidelines.

Nonetheless, there are biological data on TCDD that help inform the appropriateness of low-dose-linear risk extrapolation for this compound. Furthermore, there is utility in summarizing scientific reasoning that supports the approach of low-dose linearity as an appropriate scientific baseline inference ("default") for carcinogen risk assessment.

The issues pertaining to low-dose linearity were discussed in the report of a recent state-of-the-science workshop on issues in low-dose risk extrapolation held by U.S. EPA and Johns Hopkins Risk Science and Public Policy Institute in 2007 (White et al., 2009, 622764). This report states:

> The complex molecular and cellular events that underlie the actions of agents that lead to cancer and noncancer outcomes are likely to be both linear and nonlinear. At the human population level, however, biological and statistical attributes tend to smooth and linearize the dose-response relationship, obscuring thresholds that might exist for individuals. Most notable of these attributes are population variability, additivity to preexisting disease or disease processes, and background exposure–induced disease processes; measurement error also undoubtedly contributes to this phenomenon. The linear appearance of the population-level dose-response function does not presume that the dose-response relationship is necessarily linear for individuals (Lutz, 1990, 000399; 2001, 053426; Lutz et al., 2005, 087763), but may reflect a distribution of individual thresholds. These attributes are likely to explain, at least in part, why exposure-response models of the relationship between cancer or noncancer health effects and exposure to environmental toxicants with relatively robust human health effects databases at ambient concentrations (e.g., ozone and particulate matter air pollution, lead, secondhand tobacco smoke, radiation) do not exhibit evident thresholds, even though the MOAs include nonlinear processes for key events NRC (2005); U.S. EPA (2006, 088089; 2006, 157071; 2006, 090110); U.S. DHHS (2004, 056384).

Original arguments in favor of low-dose linearity for carcinogen risk assessment (including for ionizing radiation, as developed from human data) are based on the occurrence of damage (often termed "hits") to DNA and the inference that resulting mutations would contribute to cancer development. These arguments envisioned direct damage to DNA; however, based on subsequent advances in mechanistic understanding, damage to DNA by "secondary" reactive molecules (not just direct hits to DNA by radiation or other agents) is also considered to play a major role. TCDD is not thought to produce DNA damage directly. However, DNA damage may result subsequent to increased formation of reactive molecules (reactive oxygen species (ROS) and metabolites of endogenous compounds). Thus, the presence of low-dose linearity by this pathway would depend on whether such reactive molecules were produced at low dose and whether that increased formation was proportional to dose. If that were the case for TCDD, which is still unknown, arguments in favor of low-dose linearity remain similar to those for direct-acting agents.

The kinetics of ligand receptor binding, and then the attachment of a receptor/ligand complex to a promoter region of DNA are biochemical processes where low-dose linearity can occur. Simple receptor binding interactions are often modeled using Michaelis-Menten relationships which are linear at low dose. Thus, the *early* key events in a process of a receptor-mediated toxicity pathway may often be expected to be low-dose linear. However, as in any toxicity process, the ultimate shape of the dose-response relationship for an apical[46] toxicity endpoint will depend on all the processes involved, not just receptor kinetics. These issues were considered by NRC (NAS, 2009, 594307) which included as an indication for non-threshold dose response: "The fact that in receptor-mediated events, even at very low doses a chemical can occupy receptor sites and theoretically perturb cell functions (such as signal transduction or gene expression) or predispose the cell to other toxicants that bind to or modulate the receptor systems (such as organochlorines and the aryl hydrocarbon receptor or endocrine disruptors and hormonal binding sites)." The role of these factors for TCDD has not been fully elucidated.

Two other factors supporting low-dose linearity discussed in the workshop described by White et al. (2009, 622764) are additivity to background processes (dose additivity) and the magnitude of human heterogeneity.

[46] An apical endpoint is an observable outcome in a whole organism, such as a clinical sign or pathologic state, that is indicative of a disease state that can result from exposure to a toxicant (NAS, 2007).

Concerning dose additivity, Crump et al. (1976, 003192) argued in the context of a carcinogenic response that if the carcinogenic process resulting from exposure to an exogenous agent (e.g., TCDD) is already operant in causing background responses, then the effect of the exposure is to augment this process in a dose-additive fashion. The additional response caused by the exposure is expected to increase approximately linearly with exposure at low exposures (i.e., be low-dose linear). The NRC Science and Decisions report (NAS, 2009, 594307) examined the issue of additivity to background, in particular calling attention to a need for systematic consideration of endogenous processes related to disease development as well as additivity to other exogenous exposures.[47] While the baseline activity (unexposed to exogenous agents) of AhR is not well understood, the effects of exogenous agents need to be considered in terms of how they add on to or modulate baseline physiological processes instead of considering TCDD or other exogenous ligands to be "acting in a vacuum."

The issue of human heterogeneity relative to the rodents used in bioassays has been discussed at length in the literature and will not be repeated here (see also relevant text in Section 5.2.3.4.1.3). However, as discussed by NAS (2009, 594307), even in situations where processes thought to be nonlinear are precursors to the development of cancer in test animals, a different situation may result in humans: "However, given the high prevalence of those background processes, and given the multitude of chemical exposure and high variability in human susceptibility, the results may still be manifested as low-dose linear dose-response relationships in the human population." The population dose-response will be influenced by heterogeneities in the population that affect internal dose as well as response. First, even if there is strong curvilinearity in the dose-response curve in the dose range of relevance to human exposures, there may be large differences across individuals in the doses at which transitions in the shape of the dose-response curve occur. Greater variability in response to exposures would be anticipated in heterogeneous populations than in inbred laboratory species under controlled conditions (due to, e.g., genetic variability, disease status, age, and nutrition). The effect of increased heterogeneity will be a broadening of the dose-response curve (i.e., less rapid fall-off of response with decreasing dose) in diverse human populations and, accordingly, a greater

[47] It may be noted that when there are multiple exogenous exposures, it may be difficult to ascertain which exposure came first. However, the point is that if a combination of endogenous and exogenous factors is operative in causing biological response, then an additional small, dose additive, exposure can be predicted to cause a proportionate change in response.

potential for risks from low-dose exposures (Lutz et al., 2005, 087763; Zeise et al., 1987, 060867). The degree to which heterogeneity must be increased to "linearize" sublinear responses of varying degrees has not yet been established.

Interpreting the shape of animal bioassay dose-response model fits always involves assumptions about the shape of the response in the unobserved range (i.e., low dose). Cancer bioassays can provide relatively little information on actual dose-response patterns below the point of departure. However, it is generally not possible to either exclude or affirm low-dose linear components statistically based upon empirical modeling of the dose-response data.[48] Dose-response modeling can, however, be useful in describing the size of a linear component in the response that is compatible with study data. As an example, NRC (NAS, 2006, 198441) advised EPA to examine the results of the NTP (2006, 543749) study as indicating nonlinearity of the observed tumor response. Among the tumors seen in the NTP bioassay, the dose-response shape for cholangiosarcoma is notably curvilinear in the dose range of the observed tumor response. Figure 5-6 shows the multistage modeling of the cholangiosarcoma data from the NTP bioassay. The BMDL is calculated at an extra risk of 0.01. Even though the MLE dose response is nonlinear (1st-degree coefficient is zero), the dose-response curve pertaining to the statistical upper bound on risk (calculated here as the 95% lower confidence bound on dose) is approximately linear below the 0.01 benchmark level and roughly superposes on the EPA default linear extrapolation (see Figure 5-6B). For the oral squamous cell carcinoma (SCC) tumor data (plot not shown), the MLE dose-response curve itself displays low-dose linearity (1st-degree coefficient is greater than zero) and the EPA low-dose linear extrapolation is indistinguishable from the upper bound curve. These observations are consistent with the findings of Subramaniam et al. (2006), that for the large majority of chemicals, straight line extrapolation of risk from the BMDL provides slope factor values very similar to those obtained by using an upper bound on the multistage model risk estimate. Furthermore, in this assessment, EPA has chosen to derive oral slope factors based on combined tumor incidence whenever possible, modeling them under an assumption of independence. A Bayesian analysis is used in this document to develop PODs based on combined tumor risk across the significantly elevated tumor types observed in this bioassay (see Section 5.2.3.2.3.2). As a result of this analysis, the

[48] EPA policy is to allow for low-dose linearity in the modeling of tumors if a non-linear MOA has not been established.

central estimate for the composite dose-response curve shows little curvilinearity and the MLE dose-response curve is substantially linear below a 0.1 extra risk level (see Figure 5-7A and 5-7B; see also Section 5.2.3.2.6.11).

The results here provide a comparison of EPA's linear (straight line) dose-response estimates with the degree of linearity seen in the fitted dose-response curves and the statistical upper bounds on these curves. To do this the fitted model needs to allow for the possibility of both curvilinearity at high dose and linearity at low dose. The multistage model has these properties, which is among its advantages for application in carcinogen risk assessment. Most other models commonly used to fit data in the observed range do not have this property.[49]

One other issue relative to the determination of linearity arises in the visual interpretation of dose-response plots. The common practice of plotting receptor kinetics data on semi-logarithmic plots for scale convenience has unfortunately led to difficulties in the interpretation of the shape of these relationships. An example is presented using the modeling study of Kohn and Melnick (2002, 199104), which was cited by NRC (NAS, 2006, 198441) in its review of EPA's dioxin assessment as an example of nonlinear behavior at low dose: "Response is a function of the number of occupied and activated receptors, which typically exhibit steep dose-response relationships. For example, Kohn and Melnick (2002, 199104) modeled the shape of the dose-response relationship for receptor-mediated responses, using the estrogen receptor and various xenoestrogens as a model receptor and ligands, respectively. The model included a variety of assumptions with regard to receptor number, ligand binding affinity, and partial agonist activities, yet in every instance clear sublinear responses were observed at low doses." However, as shown in Figure 5-8, the apparent strong upward curvature of the low-dose relationship is no longer seen when the results are plotted on an arithmetic scale. Instead, the system may be seen as providing an example of close to linear behavior in the low-dose region.

[49] The standard Hill models do not: A Hill model is only linear at low dose when the Hill parameter is equal to 1 (and in that case the Hill model is linear over the full dose range until the high dose region of "saturation" where the km parameter results in downward curvature). Thus, while the Hill model is a valuable tool for fitting data in the observed experimental range, it is not helpful in illustrating the potential for low-dose response. However, some have considered a dose-additive version of the Hill model which would allow for low-dose linearity.

5.2.3.4.1.5. ***Consideration of nonlinear methods.***

While the 2005 Cancer Guidelines deem linear extrapolation to be most appropriate for TCDD, EPA has carefully considered the NAS recommendation to provide risk estimates using both linear and nonlinear methods.

The 2005 Cancer Guidelines state

> For cases where the tumors arise through a nonlinear mode of action, an oral reference dose or an inhalation reference concentration, or both, should be developed in accordance with EPA's established practice for developing such values ... This approach expands the past focus of such reference values (previously reserved for effects other than cancer) to include carcinogenic effects determined to have a nonlinear mode of action.

In this section, EPA presents two illustrative examples of RfD development for carcinogenic effects of TCDD. Each of these examples focuses on data derived from animal bioassays as described in Section 2.4.2.

5.2.3.4.1.5.1. Illustrative RfDs based on tumorigenesis in experimental animals.

TCDD has been shown to be a multisite carcinogen in both sexes of several species of experimental animals. It also has been shown to be carcinogenic to humans. Most of the available quantitative human epidemiologic data related to TCDD carcinogenesis are for all cancer mortality. Mortality is a frank effect and is generally considered to be inappropriate for RfD development, therefore, the illustrative example below utilizes available evidence from experimental animals. Table 5-20 presents candidate PODs and RfDs for TCDD carcinogenicity based on combined tumor responses from the animal bioassays described in Section 2.4.2. The PODs from the NTP (2006, 549255; 2006, 543749) and Kociba et al. (1978, 001818) animal studies were derived from Bayesian multitumor dose-response modeling (as described in Section 5.2.3.2, Table 5-17) using a BMR of 1%. Because only TCDD-induced liver tumors were reported by Toth et al. (1979, 197109), the BMR of 1% (POD) from that study was generated using a first degree linear multistage model (see Table 5-15). TCDD-induced liver tumors were reported by Della Porta et al. (1987, 197405), with the male mouse producing the lowest BMR of 1% (POD) using a second degree linear multistage model (see Table 5-15). Following BMD modeling, $BMDL_{HED}$s were then estimated (see Tables 5-16 and 5-17) using the

TCDD whole-blood-concentration dose metric from the Emond model as described in Section 3. The illustrative RfDs were derived by dividing the $BMDL_{HED}$s by appropriate uncertainty factors. In each instance, a total UF of 30 was applied, comprising factors of 3 for the toxicodynamic component of the interspecies extrapolation factor (UF_A) and a factor of 10 for human interindividual variability (UF_H).

As shown in Table 5-20, the illustrative RfDs for TCDD-induced tumors range from 3.6E-11 for liver and lung tumors in male mice (NTP, 1982, 594255) to 1.0E-9 for adrenal cortex, tongue and nasal/palate tumors in male rats (Kociba et al., 1978, 001818). This illustrative RfD range for TCDD tumorigenesis falls within the range of candidate RfDs for noncancer TCDD effects presented in Table 4-5.

5.2.3.4.1.5.2. Illustrative RfDs based on hypothesized key events in TCDD's MOAs for liver and lung tumors.

As described in Section 5.1, most evidence suggests that the majority of toxic effects of TCDD are mediated by interaction with the AhR. EPA considers interaction with the AhR to be a necessary, but not sufficient, event in TCDD carcinogenesis. The sequence of key events following binding of TCDD to the AhR and that ultimately leads to the development of cancer is unknown. While the mode of action of TCDD in producing cancer has not been elucidated for any tumor type, the best characterized carcinogenic actions of TCDD are in rodent liver, lung, and thyroid. The hypothesized sequence of events following TCDD interaction with the AhR is markedly different for each of these three tumor types. Additionally, no detailed hypothesized mode of action information exists for any of the other reported tumor types.

The endpoints selected for this illustration were evaluated to provide insight into the quantitative relationships between tumor development and precursor events in TCDD-induced carcinogenesis. The endpoints described below may or may not be biologically adverse in themselves; the intent herein was to consider TCDD-induced biochemical and cellular changes that could lead to subsequent tumor development.

In the following exercise, illustrative RfDs were derived for key events in TCDD's hypothesized modes of action in the liver and lung. No appropriate dose-response data were identified for key events in TCDD's hypothesized MOA for thyroid tumors in a

sex/species/strain that has been shown to develop thyroid tumors (i.e., female B6C3F1 mice and male and female Osborne-Mendel rats (NTP, 1982, 594255)).

As this is an illustrative exercise only, only studies that were originally identified in Section 2 for potential noncancer dose-response modeling were evaluated here (see Section 2.4.2 for study details). There may be additional studies available in the literature that would further inform the dose-response assessment of these endpoints.

Additionally, for animal model consistency, only results from studies conducted in female S-D rats are presented here. The majority of the available information on TCDD carcinogenicity (and TCDD carcinogenic precursor events) comes from studies conducted in female S-D rats and the most recent TCDD carcinogenicity study was conducted in female S-D rats (NTP, 2006, 197605). While both Kociba et al. (1978, 001818) and NTP (2006, 543749) have conducted TCDD carcinogenicity studies in female S-D rats, different substrains were used; this difference in substrain may have resulted in the different carcinogenic responses reported from the two studies. While the carcinogenicity of TCDD in female S-D rats has been well characterized, this animal model does not exhibit the full suite of tumor responses reported for TCDD (for instance, female S-D rats have not been shown to develop thyroid tumors). Additionally, the most sensitive single tumor response in female S-D rats from NTP (2006, 543749) is squamous cell carcinoma of the oral mucosa (see Section 5.2.3.2), a tumor type for which no mode of action information exists. Therefore, the illustrative RfDs described below may not be protective against all tumor types.

For each endpoint, PODs for illustrative cancer RfD development were identified as described for the noncancer RfD derivation in Section 4. Briefly, for the endpoints identified below, the $NOAEL_{HED}$s and/or $LOAEL_{HED}$s were determined based on EPA analysis of the original data presented by the study author (see Section 2.4.2 for details) and by application of the Emond PBPK models as described in Section 3.3.4. $BMDL_{HED}$s were determined as described in Section 4.2 for all data sets amenable to BMD modeling. Modeling outputs for the endpoints are presented in Appendices E and G as noted in Table 5-21. The illustrative RfDs were derived by dividing the POD by appropriate uncertainty factors as indicated in Table 5-21.

5.2.3.4.1.5.2.1. *Liver tumors.*

Figure 5-9 presents one hypothesized mode of action for TCDD-induced liver tumors in rats. TCDD activation of the AhR leads to a variety of changes in gene expression, including

increased CYP1A1 mRNA and subsequent increases in CYP1A1 activity. These alterations in gene expression are hypothesized to lead to hepatotoxicity, followed by compensatory regenerative cellular proliferation and subsequent tumor development. The details of the mechanism of TCDD-induced hepatotoxicity have not been fully determined but both CYP induction and oxidative stress have been postulated to be involved (Maronpot et al., 1993, 198386; Viluksela et al., 2000, 198968). Additionally, oxidative DNA damage has been implicated in liver tumor promotion (Umemura et al., 1999, 198001). The enhanced cell proliferation arising from either altered gene expression or hepatotoxicity, or both, could be the principal factor leading to promotion of hepatocellular tumors (Whysner and Williams, 1996, 197556).

A dose-response relationship exists for TCDD-mediated hepatotoxicity, and this parallels the dose-response relationship for tumor formation (or formation of foci of cellular alteration as a surrogate of tumor formation). However, the dose-response relationship for other TCCD-induced responses such as enhanced gene expression is different from the dose-response for tumor formation in terms of both efficacy and potency (see Popp et al. (2006, 197074) for review).

A representative endpoint for each of the hypothesized key events following AhR activation for TCDD-induced liver tumors was identified and is shown in Figure 5-9. Illustrative RfDs based on each representative endpoint are shown in Table 5-21.

5.2.3.4.1.5.2.2. *Lung tumors.*

Far less is known about TCDD's mode of action in the lung. Figure 5-10 presents two hypothesized modes of action for TCDD-induced lung tumors in rats. The first hypothesized mode of action of TCDD in the lung involves disruption of retinoid homeostasis in the liver. Retinoic acids and their corresponding nuclear receptors, the RARs and the RXRs, work together to regulate cell growth, differentiation, and apoptosis. It is hypothesized that TCDD, through activation of the AhR, can affect parts of the complex retinoid system and/or other signaling systems regulated by, and/or cross-talking with, the retinoid system (reviewed in (Nilsson and Håkansson, 2002, 548746)). These effects are then hypothesized to lead to lung tumor development, however the mechanisms underlying this hypothesis are not well-defined. The second hypothesized mechanism for the carcinogenic action of TCDD in the lung is through

induction of metabolic enzymes. Through activation of AhR and subsequent induction of metabolizing enzymes (such as CYP1A1), TCDD may enhance bioactivation of other carcinogens in lung (Tritscher et al., 2000, 197265). However, there are few studies to support this hypothesis.

Representative endpoints could only be identified for two of the hypothesized key events following AhR activation for TCDD-induced lung tumors. These endpoints are presented in Figure 5-10. Illustrative RfDs based on each of these two representative endpoints are shown in Table 5-21. There is insufficient information to form any conclusions on the quantitative progression to tumorigenicity or on the relative protection afforded by preventing the key events shown.

5.2.3.4.1.5.2.3. *Limitations of illustrative RfDs based on hypothesized key events in TCDD's MOAs for liver and lung tumors.*

A trend for increasing RfD values that follows the progression of endpoints towards the production of tumors is evident. However, there are a number of factors that prevent making strong conclusions based on this exercise. These limitations include the following

- This example addresses only two tumor types in one species, strain and sex (female S-D rats), with little information available on the hypothesized mode of action for lung tumors. No mode of action information is available for the most sensitive tumor type in this animal model (squamous cell carcinoma of the oral mucosa). Therefore, it is possible that the illustrative RfDs presented in this example would not be protective against all tumor types in female S-D rats. Importantly, other animal models have been shown to be more sensitive to TCDD-induced carcinogenesis based on combined tumor analysis (see Section 5.2.3.2); an RfD based on tumorigenesis in this animal model may not be protective against tumorigenesis in other, more sensitive, animal models (or, by extension, in humans).
- Several of the BMDLs are based on poorly-fitting models, such that the RfD is based on a LOAEL (or LOEL), which is not a particularly good measure for comparison across endpoints (e.g., LOAELs are dependent on dose spacing in bioassays). Furthermore, the hepatotoxicity BMDL based on a dichotomous 10% BMR, is not directly comparable to all the other BMDLs based on a continuous 1 standard-deviation BMR (Crump, 2002, 035681). In addition, as the earlier effects (CYP induction, cellular proliferation) are not considered to be necessarily adverse in themselves, the BMR of 1 standard-deviation from the mean may not be the best choice for determining a POD based on biological signficance. The use of the 1 standard-deviation BMR for the illustrative examples is primarily for comparison on an equal-magnitude-of-response basis across endpoints.

- The endpoints selected as representative of each hypothesized key event may not be the most appropriate choices. These particular endpoints were chosen because they were the most sensitive indicator (i.e., lowest POD) from the available data or were the only available choice based on a lack of data for other effects related to the hypothesized key event.
- The optimum timing of these events may not be reflected in the endpoints selected. Almost certainly, changes in gene expression are early events, such that a single exposure should be relevant, as in the mRNA changes reported after a single TCDD exposure (Vanden Heuvel et al., 1994, 594318), although it is not known whether the magnitude of these changes would be altered after longer-term exposure, or whether longer-term exposure would be more relevant to downstream events. Similarly, single exposures for induction of CYP enzymes would seem to relevant as a measure of the immediate effect, but it may be longer-term repeated CYP activity that is important for longer-term downstream events; Table 5-21 shows a nominal order-of-magnitude difference in effect levels for similar effect magnitudes (ca. 20-fold) from single exposures (Kitchin and Woods, 1979, 198750) and long-term exposures (53-weeks; NTP, 2006, 543749). The relevant exposure durations for oxidative stress and later effects are longer term, so a measurement of oxidative stress at 90-days in a rodent may be appropriate; Wyde et al. (2001, 198575) suggest that induction of 8-oxo-dG DNA adducts are a result of longer-term oxidative stress because of the lack of effect of single exposures. Hepatotoxicity and hepatocellular proliferation events would appear at successively later times, but the effective exposure levels would depend heavily on the endpoints chosen to represent those events and the time at which they were measured. The toxic hepatopathy endpoint reported in NTP (2006, 543749), is a general measure of mild to moderate liver toxicity, but is measured only at the end of the study when tumors have already appeared. Hepatocyte hypertrophy, measured at 31weeks may be more duration-relevant, but may not indicate actual hepatocellular toxicity.
- The lowest of the tested doses may well be much higher, given that all animal diets are contaminated to a certain extent by TCDD, resulting in initial TCDD body burdens in all animals. Vanden Heuvel et al. (1994, 594318) reported TCDD liver concentrations in control animals almost as high as for the low-dose group, which could equate to a significant increase in the actual exposure experienced by the low-dose group. A similar effect on the low-dose group (0.45 ng/kg) in Kitchin and Woods (1979, 198750) is possible, although they did not report control animal tissue concentrations. Higher exposure levels or longer-term exposures would not be affected to the same degree, as administered TCDD levels would likely be large compared to initial body burden or low-level feed stock exposure.

Given the limitations described above, establishing an unambiguous progression of effects is extremely problematic given the lack of sufficient data. Identifying a RfD that could be considered to be protective against tumorigenesis in humans based on these data and models is subject not only to the determination of effective low doses for the RfDs in Table 5-21 but also

to the determination of effective exposures that could be considered to be protective of all other tumor types in female S-D rats as well as all other animal models. The latter would entail identifying precursors that are sufficient in themselves for progression to tumorigenesis for all tumor types. Given the disparate sequence of hypothesized key events following TCDD-induced AhR activation for the tumor types for which some information is available, AhR binding/activation is the only key event that is likely to be shared across tumor types. No appropriate quantitative data on AhR binding/activation by TCDD in relevant animal models were located; therefore, an illustrative RfD based on TCDD AhR activation could not be developed.

Simon et al. (2009, 594321) present a similar analysis for the liver tumors observed in the NTP (2006, 543749) study, showing a progression of effects from early biochemical events to irreversible liver toxicity, culminating in tumorigenesis. While illustrative of the putative tumor-promoting MOA for TCDD, the limitations of using such an approach within the context of an assessment of the overall carcinogenic risk of TCDD as detailed above still apply. Simon and colleagues also present RfDs for liver tumors and several precursor endpoints. All the RfDs presented in Simon et al. (2009, 594321) are essentially equivalent and are 1 to 3 orders of magnitude higher than the RfDs for equivalent endpoints presented in Table 5-21. These discrepancies are partly due to the fact that the Emond PBPK models (Emond et al., 2004, 197315; Emond et al., 2005, 197317; Emond et al., 2006, 197316; see also Section 3.3.4) used in this document predicts lower TCDD intakes for similar tissue concentrations than the CADM kinetic model (Aylward et al., 2005, 197014; Carrier et al., 1995, 197618) used by Simon and colleagues. However, a larger contributor to these discrepancies is the use of a chemical-specific adjustment factor (CSAF) of 0.1 for the toxicodynamic component of the interspecies uncertainty factor by Simon et al. (2009, 594321), while EPA used an uncertainty factor of 3. EPA does not find that the *in vitro* evidence presented by Simon et al. in support of a CSAF of 0.1 for interspecies toxicodynamics meets the burden of proof necessary for a reduction in this uncertainty factor.

5.3. DERIVATION OF THE TCDD ORAL SLOPE FACTOR AND CANCER RISK ESTIMATES

EPA was able to derive candidate OSFs for all cancer mortality from human epidemiologic studies as well as for individual and combined tumor incidence from rodent cancer bioassays. Each of these studies was selected for TCDD dose-response modeling using the study inclusion criteria outlined in Section 2. The derivation of these OSFs can be found for the epidemiologic data in Section 5.2.3.1 and for the rodent bioassay data in Section 5.2.3.2.

The OSFs based on epidemiologic studies from three cohorts ranged from 3.75×10^5 to 2.5×10^6 per mg/kg-day (see Tables 5-1 and 5-3). For the animal data, OSFs based on individual tumors were developed for 28 study/sex/endpoint combinations, and the results ranged from 1.8×10^4 to 5.8×10^6 per mg/kg-day (see Table 5-16). The OSFs based on combined tumors were developed for 7 study/sex combinations, and the results ranged from 3.2×10^5 to 9.4×10^6 per mg/kg-day (see Table 5-17). Figure 5-11 demonstrates the range of these OSFs in units of per mg/kg-day. The human study OSFs are shown at the far left of the figure, and the rodent endpoints are arranged by species to the right. For comparison with the other studies, the OSF from Cheng et al. (2006, 523122) is based on a 1×10^{-6} risk level (Table 5-3).

As recommended by expert panelists at EPA's 2009 Dioxin Workshop (U.S. EPA, 2009, 522927) and in the 2005 Cancer Guidelines (U.S. EPA, 2005, 086237), EPA has chosen to give higher consideration to the human epidemiologic data rather than the animal bioassay data in developing an OSF for TCDD. Candidate OSFs derived from the human data are consistent with the animal bioassay OSFs; specifically, the human OSFs fall within the same range as the animal bioassay OSFs. Because all the human and animal studies were considered to be of high quality and yielded similar ranges of OSFs, EPA has chosen to rely on the epidemiologic data for OSF derivation.

The strengths and limitations of the five epidemiological studies meeting the inclusion criteria for cancer dose-response modeling are summarized in Table 5-22. Among the human studies, the occupational TCDD exposures in the NIOSH and Hamburg cohorts are assumed to be reasonably constant over the duration of occupational exposure. In contrast, the TCDD exposure patterns in the Seveso and BASF cohorts are associated with industrial accidents; as a consequence, the exposure patterns are acute, high dose followed by low-level background exposure. Such exposure patterns similar to those experienced by the BASF and Seveso cohorts

have been shown to yield higher estimates of risk when compared to constant exposure scenarios with similar total exposure magnitudes (Kim et al., 2003, 199146; Murdoch and Krewski, 1988, 548718; Murdoch et al., 1992, 548719). Thus, EPA has judged that the NIOSH and Hamburg cohort response data are more relevant than the BASF and Seveso data for assessing cancer risks from continuous ambient TCDD exposure in the general population.

The NIOSH (Cheng et al., 2006, 523122; Steenland et al., 2001, 198589) and Hamburg (Becher et al., 1998, 197173) cohort studies report cumulative TCDD levels in the serum for cohort members. The most significant difference among the Cheng et al. (2006, 523122) analysis and those of Steenland et al. (2001, 198589) and Becher et al. (1998, 197173) is the method used to back-extrapolate exposure concentrations based on serum TCDD measurements. Steenland et al. (2001, 198589) and Becher et al. (1998, 197173) back-extrapolated exposures and body burdens using a first-order model with a constant half-life. In contrast, Cheng et al. (2006, 523122) back-extrapolated body burdens using a kinetic modeling approach that incorporated concentration- and age-dependent elimination kinetics.

Although all three of these are high-quality studies, the kinetic modeling used by Cheng et al. (2006, 523122) is judged to better reflect TCDD pharmacokinetics, as currently understood, than the first-order models used by Steenland et al. (2001, 198589) and Becher et al. (1998, 197173). EPA believes that the representation of physiological processes provided by Cheng et al. (2006, 523122) is more realistic than the assumption of simple first-order kinetics and this outweighs the attendant modeling uncertainties. Furthermore, the use of kinetic modeling is consistent with recommendations both by the NAS and the Dioxin Workshop panel.

However, as discussed in Section 3.3.2, the kinetic model that they employed does have certain limitations, including the fact that it has been calibrated based on a relatively small number of human subjects. In addition, their kinetic model does not allow body mass index (BMI; and hence fat content) to vary with age, which may bias the model results. Nonetheless, EPA prefers the increased technical sophistication of the dose estimates used in the cancer mortality risk estimates derived from Cheng et al. (2006, 523122) to those derived from Steenland et al. (2001, 198589).

EPA, therefore, has decided to use the results of the Cheng et al. (2006, 523122) study for derivation of the TCDD OSF based on total cancer mortality as calculated by EPA using data and models from the Cheng et al. (2006, 523122) study as described in

Section 5.2.3.1.2. Although the OSF is only strictly defined for exposures above the background exposure experienced by the NIOSH cohort, which was assumed to be 0.5 pg/kg-day TCDD, or 5 pg/kg-day total TEQ, EPA assumes that the slope (risk vs. blood concentration) is the same below those background exposure levels as it is above. Table 5-3 shows the oral slope factors at specific target risk levels ($OSF_{RL}s$) which range from 1.1×10^5 to 1.3×10^6 per (mg/kg-day). EPA recommends the use of an OSF of 1×10^6 per (mg/kg-day) when the target risk range is 10^{-5} to 10^{-7}. Although EPA prefers the human data, EPA also presents a number of OSFs derived from rodent bioassays. Most of these animal studies are of note, because in general they were well-designed and conducted. In particular, the NTP (2006, 543749) study was recently conducted and represents the most comprehensive evaluation of TCDD chronic rodent toxicity to date.

5.3.1. Uncertainty in Estimation of Oral Slope Factors from Human Studies

A fair amount of uncertainty is associated with the estimation of slope factor values and cancer risk specific doses for TCDD based on the epidemiological studies. In some instances, the influence of a given factor is theoretically amenable to analysis, but such investigation is limited by the availability of sufficiently detailed data to support such an analysis. In other cases, only very broad ranges can be placed on the uncertainty associated with a given feature of the analysis, or uncertainties must be discussed qualitatively.

The following four sources of uncertainty are addressed in this section: uncertainty in exposure estimates in the epidemiologic studies (see Section 5.3.1.1), uncertainty in the shape of the dose-response curve (see Section 5.3.1.2), uncertainty in extrapolating risks below exposure levels in the reference population (see Section 5.3.1.3), uncertainty in cancer risk estimates arising from background DLC exposure (see Section 5.3.1.4) and uncertainty in cancer risk estimates arising from occupational coexposures to DLCs (see Section 5.3.1.5). Section 5.3.2 explores other sources of uncertainty in the epidemiologic risk estimates including the use of cancer mortality rather than cancer incidence data in the derivation of the oral slope factor, possible influences of inter-individual variability in TCDD kinetics, and exposures to other occupational carcinogens.

5.3.1.1. *Uncertainty in Exposure Estimation*

The major technical challenge within each of the epidemiological studies was developing relevant and precise estimates of exposure. While Warner et al.(2002, 197489) collected blood samples relatively close to the time of the Sevesso accident and could reasonably estimate peak exposures based on these collected samples, in the case of the Becher et al. (1998, 197173), Ott and Zober (1996, 198408), Steenland et al. (2001, 198589), and Cheng et al. (2006, 523122) studies, the major exposure issues included the following

- Selecting (an) appropriate dose metric(s) for dose-response modeling,
- Estimating serum TCDD levels for the entire cohort based on measurements from a smaller number of the subjects in the cohort collected long after the occupational exposures had occurred, and then assigning exposures to the remaining members of the cohort based on qualitative job classifications.
- Estimating time-weighted average tissue doses (e.g., lipid-average serum concentration over time) based on single samples taken at one point in time. (Except for the Becher et al. (1998, 197173) analysis where one of the study strengths was their estimate of TCDD half life, which utilized repeated measurements from a subset of their cohort).

In the Becher et al. (1998, 197173), Steenland et al. (2001, 198589), and Cheng et al. (2006, 523122) studies, dose-response modeling was performed using ppt-years lipid-adjusted serum concentration as the primary dose metric for TCDD; serum TCDD was the only direct measurement of exposure or dose that was available. In addition, as discussed in Section 3.3.4, serum concentration is a reasonable index of total tissue concentration (target organ dose), and lipid-adjusted serum concentration provides a reasonable index of TCDD in the fatty components of tissues. Ott and Zober (1996, 198408) used ng/kg body weight at the time of the accident as the primary dose metric, and U.S. EPA (2003, 537122) later converted these to units of ppt-years lipid-adjusted serum concentration.

The decision to use cumulative serum concentrations (ppt-years) as the primary dose metric for carcinogenicity is based on the understanding that time weighted concentrations (over a chronic exposure period) are the most appropriate dose measures for cancer risk assessment. This may not be strictly true if cancer induction by TCDD is considered to be a "threshold process." However, as discussed in Section 5.2, there are reasonable grounds to believe that the

assumption of low-dose linearity is reasonable for TCDD, especially when calculating population risks where the effects of interindividual variability must be taken into account.

In addition to the issue of low-dose thresholds, the rationale for using cumulative dose metrics also can fail at high doses if the adverse response in question involves a step that is saturable (e.g., where there is a maximum level of response that cannot be exceeded owing to a rate-limited process). There is some evidence for such a phenomenon in the NIOSH cohort where cancer risks in the highest exposure group (>50,000 ppt-years) appear to saturate, and the response decreases at this level (Steenland et al., 2001, 198589). Steenland et al. (2001, 198589) suggest that the apparent saturation of dose-response in this cohort may be due, at least partially, to exposure misclassification among the highest exposed individuals, rather than to an actual reduction in response per unit exposure.

The uncertainty associated with differences in the exposure patterns is important to consider across the five epidemiologic studies. Steenland et al. (2001, 198589), Cheng et al. (2006, 523122), and Becher et al. (1998, 197173) studied cohorts exposed to elevated TCDD levels over a long period of time, while Ott and Zober (1996, 198408) and Warner et al.(2002, 197489) studied cohorts exposed to TCDD levels significantly above background at one point in time but the exposures and likely the TCDD body burdens declined significantly following these periods of elevated exposure. Both these chronic and acute exposures can be analyzed in terms of cumulative exposure to TCDD. Use of such a metric requires an assumption that the "actual" cancer potency associated with a cumulative dose where much of the dose is received at a single point in time and then gradually eliminated would be similar to the cancer potency of the same cumulative dose received over a longer period of time and also gradually eliminated. While EPA believes that such an assumption is not unreasonable, the experiment of Kim et al. (2003, 199146), which showed statistically significant increase in liver effects due to a peak TCDD dose when compared to chronically-dosed Sprague-Dawley rats administered the same levels of TCDD when measured as a cumulative dose, suggests that additional analyses of cumulative and peak TCDD dose measures may need to be conducted.

There are uncertainties associated with the approaches used to estimate TCDD exposures in the members of the occupational epidemiologic studies for which no measurement data were available. To impute TCDD levels for workers without measured samples, all four occupational epidemiologic studies matched workers for whom measured TCDD samples had never been

reported to workers with measured TCDD levels based on job histories. The NIOSH cohort is used to illustrate some of the uncertainties. In the NIOSH cohort, the subset of workers (roughly 5% of the total cohort) with blood serum data comprised surviving members of the cohort (in 1988), and therefore, their age distribution likely differed from the rest of the cohort. For each worker in this subset, the following data were available: (1) job classification information, (2) employment history, and (3) serum TCDD measures. All of the workers in this subset were employed at a single plant where the work histories were less detailed than at other plants, and many of the workers at this plant had the same job title and were employed during the same calendar period. There is an assumption that workers with same job title and work history were exposed to the same TCDD levels within a plant and across plants; this obviously does not account for exposure heterogeneity.

Both Steenland et al. (2001, 198589) and Cheng et al. (2006, 523122) addressed the potential for exposure measurement error in TCDD estimates and possible exposure misclassification. For the highest exposure workers, Steenland et al. (2001, 198589) and Cheng et al. (2006, 523122) found weak, "noisy," and/or negative exposure-response relationships. Steenland et al. (2001, 198589) suggests that possible explanations for this observation include the saturation of effects at the upper end of the dose-response curve, instability of the TCDD exposure estimates based on the limited number of highly exposed individuals, and the increased probability of exposure misclassification for workers whose job histories indicate the highest exposures. As Steenland et al. (2001, 198589) reported, some of the highest exposures might have been inaccurately estimated because they occurred in workers exposed to short-term, high-dose exposures during spill clean-up. Cheng et al. (2006, 523122) used sensitivity analyses to examine this measurement error issue and evaluated the potential for exposure misclassification by using ln-transformed TCDD ppt-years. The authors removed all observations with exposures within the lower and upper 1, 2.5, or 5th percentiles of the TCDD ppt-year distribution and also removed observations within just the upper 1, 2.5, or 5th percentile of TCDD ppt-years. These sensitivity analyses yielded results similar to those reported in the primary analysis. An additional concern is that exposure errors might distort the exposure distribution in the population, which generally spreads the response out over a wider dose range. This serves to increase the variance of the regression model, altering both the POD and the corresponding OSF.

Becher et al. (1998, 197173) only considered workers from a single plant but their analysis included workers employed in five different job locations within the plant. The influence of worker location on slope factor estimates does not appear to be further explored and may represent a source of uncertainty.

To estimate long-term body burden metrics from the serum TCDD measurements, Steenland et al. (2001, 198589) employed simple first order kinetic elimination rate model with a half-life of 8.7 years. Limitations of this approach include (1) the average elimination half-life among the study subjects may not be 8.7 years given differences between the study population and the Ranch Hand population from which the value was estimated, (2) use of a single-value estimate fails to take into account the inherent variability in elimination half life among the individual workers, and (3) it fails to take into account variations in elimination kinetics throughout the lifetime of the exposed worker due to change in body fat, age, etc. The impact of these potential sources of bias on the estimates of time-integrated body burden cannot be quantitatively assessed. However, Steenland et al. (2001, 198589) noted that modest changes in elimination half-life (to 7.1 years) had only a very small impact on risk estimates.

Cheng et al. (2006, 523122) estimated past body burdens using the CADM approach (described in Section 3) (Aylward et al., 2005a, b) rather than a half-life estimate. As noted above, the incorporation of concentration- and age-dependent elimination into this approach has significant advantages over the use of a constant elimination half-life. However, as discussed in Section 3.3, the CADM has only been subject to limited testing against human validation data sets, so the degree to which its advantages are realized in practice cannot be easily assessed. There are no available human data in the low dose region, the region of interest to this assessment, to compare with the CADM (or Emond) model predictions.

Becher et al. (1998, 197173) developed half life estimates based on multiple TCDD blood measures in 48 individuals from this cohort. These half life estimates were then used to back calculate TCDD concentrations at the end of each worker's employment, accounting for age and percentage of body fat. This cohort-specific information may provide a better exposure estimate than Steenland et al. (2001, 198589) or Ott and Zober (1996, 198408) who used similar kinetic approaches. However, the comparison of the accuracy of the exposure estimates across the cohorts is not easily assessed. There are several assumptions and important uncertainties involved in modeling TCDD exposures in these cohorts. The study authors have invoked

different kinetic assumptions when extrapolating measured levels of TCDD in sera backward in time to estimate higher chronic or peak dosage (i.e., there is uncertainty in these back-calculations that includes assumptions regarding elimination kinetics). There is also uncertainty in applying such estimates to other members of the cohort based on similar characteristics (e.g., job category).

5.3.1.2. *Uncertainty in Shape of the Dose-Response Curve*

Another source of uncertainty is the nature of the dose-response curve in the low dose region of interest for risk assessment for environmental exposures (e.g., <1 pg/kg-day). The epidemiologic data are based on occupational studies in which exposures were often several orders of magnitude higher than environmental exposures. In these studies, data from the low dose region are quite sparse, and only one study examined uncertainty due to the low dose region. Steenland and Deddens (2003, 198587) attempted to analyze this region specifically by fitting threshold curves to the NIOSH data in which there was no extra risk from exposure until some specific level. However, this model did not fit as well as models without a threshold. In general, the usual assumption of linearity in the low dose region seems reasonable when using epidemiologic data given the lack of data in this region that precludes the rejection of linearity.

There is uncertainty in the extrapolation of the OSF to the low dose region (e.g., <5 pg/kg-day). EPA developed the cancer assessment in this document assuming the slope in the low-dose region of the dose-response curve is linear; the decision was made due to the lack of sufficient evidence to support an assumption of nonlinearity as outlined in the EPA's Cancer Guidelines (U.S. EPA, 2005, 086237). Similarly, there is uncertainty as to whether a threshold exists for TCDD-induced toxicity leading to tumorigenesis and the dose associated with such a threshold, if it exists, is unknown. EPA chose to model this dose-response without a threshold because there is insufficient evidence to support an assumption of a threshold.

It also is noteworthy that the shapes of the exposure-response in several of these studies, based on the published statistical models, is indicative of a response that tends to tail off or "plateau" at high cumulative exposures to TCDD. This phenomenon has been seen in many studies of occupational carcinogens, and may reflect a number of things including exhaustion of people susceptible to cancer, saturation of biological pathways which are part of the pathway to

cancer, and increased error measurement of dose at high levels biasing dose-response towards the null (Stayner et al., 2003, 054922).

5.3.1.3. *Uncertainty in Extrapolating Risks below Reference Population Exposure Levels*

Another source of uncertainty in using human epidemiologic data is due to the lack of completely unexposed populations; there are no human populations that have zero dioxin exposure. The cancer exposure responses modeled in all epidemiologic cohorts, whether primarily exposed via occupational or environmental exposures, can be evaluated with confidence only above the lowest exposed group (i.e., the reference population). There are substantial uncertainties associated with estimating cancer risks from background exposures of TCDD and DLCs because these risks are aggregated in the overall background risk of the referent population, to which outcomes of cohort subjects experiencing higher dioxin exposures are compared. Therefore, the risk modeled from the epidemiologic data is unavoidably the incremental risk above a background exposure to dioxins in the general environment (assumed to be primarily from food intake). Typically, serum TCDD levels in the general populations in the geographic locations and times at which the epidemiologic studies were undertaken have been reported to be on the order of 5 to 20 ppt (Mocarelli et al., 1991, 199600)(WHO, 1998; Pinsky and Lorber, 1998). Hence, the extra risks should be considered as those incurred by added exposure above these background exposures, which then introduces uncertainty associated in the cancer slope factor estimate at exposures below background levels. EPA assumes that the slope of the risk curve below the background exposure experienced by the epidemiologic study cohorts is the same as the (modeled) slope above those background exposure levels; data do not exist to test this assumption.

Also, background TCDD/DLC exposures experienced by the epidemiologic study cohorts have been estimated to be much larger (5 to 10-fold) than current background levels. Lorber et al. (2009, 543766) estimate that current U.S. intake rates are roughly 0.58 pg TEQ/kg-day at the 50th percentile and suggest that human TEQ ingestion exposures likely peaked in the 1970's. Steenland et al. (2001, 198589), presumably based in part on WHO (1998), estimated background intake rates to be 5 pg TEQ/kg-day for the NIOSH cohort. As a result, the assessment of cancer mortality risk at current background exposure levels is also subject to extrapolation uncertainty.

5.3.1.4. *Uncertainty in Cancer Risk Estimates Arising from Background DLC Exposure*

None of the slope factors presented in this document, whether based on epidemiologic studies or animal bioassays, takes into account the impact of background exposure to DLCs. Background DLC exposure can be estimated for only one of the animal cancer bioassays NTP (2006, 543749). Background TCDD and DLC exposure for the rats in the NTP (2006, 543749) does not appear to have been significant, with respect to the magnitude of administered doses (see Section 5.3.2.1). However, given the trend towards lower exposures to TCDD in recent years, the TCDD/DLC exposure may have been much higher in the older studies (e.g., Kociba et al., 1978, 001818; NTP, 1982, 543764; Toth et al., 1979, 197109). The impact of background TCDD/DLC exposure on the cancer risk modeling of any of the bioassay data would be to increase the dose term associated with each response; consequently, increasing the magnitude of the BMDL, with a proportional reduction in the magnitude of the slope factor, although the effect would probably be small (see Section 5.3.2.1). Note that the shift in dose increases the estimated low doses proportionately more than the higher doses, potentially obscuring the relationship between dose and response in the low dose region.

Background dioxin exposure for the epidemiologic cohorts, however, could have been substantial with respect to the TCDD exposures in the reference populations used in the modeling. As an example, the background dioxin intake the NIOSH cohort, which is the basis for the oral slope factor described previously in this section (5.3), was estimated to be 0.5 pg/kg-day for TCDD and 10 times higher (5 pg/kg-day) for total TEQ (Steenland et al., 2001, 197433)(WHO, 1998). WHO (1998) estimated that TCDD comprised only about 5 to 10% of total TEQ from exposure to DLCs in food, based on DLC exposure estimates and TEFs available at that time. Eskenazi et al. (2004, 197160) estimated that TCDD was 20% of total TEQ in the serum of the reference population in the Seveso Women's Health Study from measurements taken in 1976. Based on more recent estimates (Lorber et al., 2009, 543766), TCDD is about 10% of total TEQ in human serum in the United States. Steenland et al. (2001, 198589) assumed a (cumulating) background exposure of 5-6 ppt TCDD and 50 ppt total TEQ per year in serum for their analysis of the NIOSH cohort cancer mortality response. The resulting cumulative background exposures, particularly for total TEQ, are large compared to the lower cumulative occupational exposures over the life-time of the cohort (birth to death or end of follow-up).

Crump et al. (2003, 197384), based on Steenland et al. (2001, 198589), assumed a cumulative background serum concentration of 3,000 ppt-year for total TEQ (50 ppt/year × 60 years), which is much larger than the lower NIOSH cohort occupational TCDD exposures. The latter, when grouped in cumulative TCDD serum-concentration septiles Steenland et al. (2001, 197433), range from 260 to 850 ppt-yr in the first few septiles. Conceivably, the much larger background exposure could have a somewhat larger effect on the slope factor than for the relatively lower background exposure in the animal bioassays. Because the Cheng et al. (2006, 523122) modeling does not account for background TEQ, the resulting slope factor is biased high. None of the published analyses of the NIOSH cohort data (Cheng et al., 2006, 523122; Crump et al., 2003, 197384; Steenland et al., 2001, 198589) present an analysis that addresses the effect of background TEQ exposure on the modeled risk.[50] Given the data and modeling results currently available, the EPA could not find an approach for expressing the quantitative impact with any accuracy or confidence.

5.3.1.5. *Uncertainty in Cancer Risk Estimates Arising from Occupational DLC Coexposures*

The slope factor estimates are based on an assumption that occupational exposure was entirely to TCDD, with no explicit consideration of the risk attributable to occupational DLCs. Because TCDD typically occurs as a component of a mixture with other DLCs that are assumed to affect cancer risk through dose addition, the assumption that the exposures are entirely TCDD could lead to a positive bias in the slope factor estimates derived from these epidemiologic studies, if the estimates are confounded by other exposures to DLCs and the TEQ dose is larger than the fraction accounted for by TCDD alone. The magnitude of the potential bias can be estimated in a general way through the estimation of risks for plausible mixtures of DLCs and TCDD exposures in the cohort with the same composition as the Steenland et al. (2001, 198589) and Cheng et al. (2006, 523122) studies, but the detailed data required to perform such an analysis on the NIOSH cohort are not available. In addition to the slope factor estimated for TCDD, Becher et al. (1998, 197173) also evaluated the slope based on TEQs. They found a dose-response effect for TCDD but not for TEQ (excluding TCDD) which suggests that confounding by DLCs did not occur.

[50] Steenland et al. (2001, 197433) present a TEQ analysis but for a scenario where total TEQ is 10 times the TCDD exposure for both background and occupational exposure.

5.3.2. Other Sources of Uncertainty in Risk Estimates from the Epidemiological Studies

Other aspects of the Steenland et al. (2001, 198589), Cheng et al. (2006, 523122), Becher et al. (1998, 197173), and Ott and Zober (1996, 198408) studies that are not directly associated with TCDD or DLCs may contribute uncertainty to the cancer slope factor estimates. This section lists several of these and discusses their potential directional bias in slope. General issues associated with potential confounding effects also were discussed in the 2003 Reassessment (U.S. EPA, 2003, 537122).

All of the studies that meet the criteria (with the exception of Warner et al., 2002, 197489) measure cancer mortality rather than cancer incidence. This likely biases the slope factor downward relative to a slope calculated for cancer incidence, the typical basis of EPA cancer slope factors. In the NIOSH cohort, roughly one-third of the fatal cancers were identified as lung cancer. Because of the high case mortality rate associated with lung cancer during the period of cohort evaluation (e.g., the 5-year relative survival rates for lung cancer were less than 10% before 1973 and were less than15% before 1995 (Horner et al., 2009), the slope factor estimated for cancer mortality might not be much lower than that calculated for cancer incidence. This assumes that the outcome of a cancer incident (i.e., cancer mortality) is independent of occupational TCDD exposure levels. Estimation of cancer incidence in the general population associated with TCDD exposure would require assumptions related to the relative survival and age-specific cancer risks in the exposed population compared to the NIOSH cohort or the Hamburg cohort; insufficient data are available to support such an analysis.

The routes of TCDD exposures in the occupational cohorts include dermal and inhalation exposures (Steenland et al., 1999, 197437), the U.S. population is assumed to be primarily exposed through the intake of TCDD and DLCs in foods). Given the persitence of TCDD in the body, differences in exposure routes may not be significant, but route-specific effects can not be precluded. The directional bias on the slope factor that is associated with this uncertainty is not known.

Occupational exposures to other carcinogens could lead to uncertainty in the slope factor. For example, in addition to TCDD, the Hamburg cohort was also exposed to hexachlorocyclohexane (HCH), which IARC classified as possibly carcinogenic to humans, and lindane, which EPA (2001) stated had "suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential." While such co-exposures would not bias the exposure

metric (i.e., not dose additive), to the extent that they were correlated with TCDD exposure, the cancer mortality risk attributed to TCDD would be overestimated,biasing the slope factor high because all cancers are attributed to TCDD. To examine this, Cheng et al. (2006, 523122) assessed the impact of possible confounding by conducting excluding individual plants in the modeling. If the estimated cancer risks as a function of exposure did not change too much when specific facilities were left out, then confounding was deemed unlikely. Cheng et al. (2006, 523122) likewise found little variation in risks based on these analyses.

There is adequate evidence to believe age, gender, and body fat content all can have a significant impact on elimination kinetics and consequent cancer risks associated with TCDD exposure (U.S. EPA, 2003, 537122). While the authors evaluating the Hamburg cohort accounted for such impacts in their kinetic analysis, interindividual kinetic differences were not considered in evaluations of other cohorts.

There may be gender differences that affect susceptibility to TCDD exposure. The cohorts analyzed by Steenland et al. (2001, 198589), Cheng et al. (2006, 523122), Ott and Zober (1996, 198408) and Becher et al. (1998, 197173) were comprised almost exclusively of men. This precluded systematically addressing differences between males and females in these studies. Further, because EPA could not develop an estimate from the Warner et al. (2002, 197489) cohort, none of the studies analyzed here for cancer dose-response contained a significant percentage of women. Thus, the generalizability of the slope factor estimates to women is uncertain.

Finally, of these cancer cohorts only the Seveso cohort included children. The unique sensitivities of infants, toddlers, and children cannot be addressed based on information in the occupational cohorts, although the increases in cancer risk in the Seveso cohort, to date, appear to be modest. Aside from differences in exposure patterns and body fat content, the unique developmental status of children may result in a substantially different profile of cancer risks (and magnitudes of those risks) than can be addressed by simply compensating on the basis of differences in body weight, food intake, etc. Further, because EPA could not develop an estimate from the Warner et al. (2002, 197489) cohort, none of the studies for cancer dose-response analyzed contained a significant percentage of women. Thus, the generalizability of the slope factor estimates to women and children is uncertain.

A number of other factors are routinely evaluated in cancer epidemiology studies, but appear likely to have little impact on the direction of the slope factor; however, they likely increase overall variability either in the dose or response. These include smoking and lifestyle factors. Intraindividual variation in TCDD kinetics and susceptibility also could affect the relationship between exposure and cancer risk. In each of these cases, it is difficult to determine the directional bias these factors introduce into the derivation of the slope factor, unless somehow they are correlated with with occupational dioxin exposures.

5.3.2.1. *Effect of Added Background TEQ on TCDD Dose-Response*

A source of uncertainty for TCDD dose-response modeling is the impact that background exposures of TCDD and other DLCs might have on the modeling output. As mentioned previously in Text Box 4-1, NTP (2006, 543749) presented measurements of TCDD in the fat of control animals. To study the potential impact of background TCDD and total TEQ on the cancer dose-response modeling for the NTP (2006, 197605) study, EPA has estimated background levels of TCDD and TEQ (based on total TCDD, PeCDF and PCB126) from the mixture study to serve as surrogates for background exposures in the TCDD-only study (limit of detection too high for control level measurements). Background doses were estimated as:

$$Chemical_i(B) = \frac{Chemical_i(fat_{MC}) \times TEF_i}{TCDD(fat_{TCDD})} \times Dose_{TCDD} \qquad \text{(Eq. 5-9)}$$

where

- $Chemical_i(B)$ = estimate of background exposure to Chemical i in ng/kg units of TCDD blood concentrations at 105 weeks, for *i* = TCDD, PeCDF and PCB126.
- $Chemical_i(fat_{MC})$ = mean pg/g of Chemical i in the fat tissues of the control animals at 105 weeks in mixtures study (NTP, 2006, 543749).
- $TCDD(fat_{TCDD})$ = mean pg/g of TCDD in the fat tissues of the 3 ng/kg dose group at 105 weeks in the TCDD study (NTP, 2006, 197605).
- $Dose_{TCDD}$ = 2.56 ng/kg TCDD blood concentration for the 3 ng/kg dose group in the TCDD study (from the Emond rat PBPK modeling of NTP, 2006, 197605)
- TEF_i = Toxicity Equivalence Factor for Chemical i (from Van den berg et al. (2006, 543769)).

Assuming simple proportionality of blood TCDD concentrations between controls and low-dose (3 ng/kg) animals, the TEF-adjusted ratio of each congener (Chemical *i*) in control-animal fat to low-dose-animal fat is multiplied by the modeled TCDD blood concentration for the low-dose animals to obtain an equivalent background exposure in the dose metric (ng/kg whole blood) used to calculate all the OSFs in this assessment. For total TEQ, the estimates across the three congeners are summed. The total TEQ estimates are biased somewhat high because they are based on terminal (2-year) measurements rather than representing lifetime averages. Background exposures are then added to the modeled TCDD blood concentrations for several different background exposure scenarios (see Table 5-23) prior to conducting Benchmark-Dose (BMD) modeling.

BMD modeling was conducted for the cholangiocarcinoma endpoint in the TCDD study (NTP, 2006, 197605). This was done for scenarios that added the following estimated TCDD or TEQ background doses to the TCDD study doses: background TCDD only, total estimated TEQ, twice the total TEQ and ten times the background TCDD (see Table 5-23). These doses may bound the potential background exposures as TCDD has been thought to represent about 10% of all TEQs at environmental levels (WHO, 1998). Table 5-24 shows that, as expected, adding to the exposure term increases the BMDL (and decreases the OSF) and also shifts the shape of the dose-response slope slightly towards sublinear (see Appendix I). However, at these background exposure levels relative to the administered dose levels, there is very little quantitative impact on the cancer dose-response modeling for the NTP (2006, 197605) study. Even with the most extreme assumption that background TCDD is only 10% of total background TEQ, the BMDL changes by only 12%. Assuming that background exposures were higher for older studies (e.g., Kociba et al., 1978, 001818; NTP, 1982, 594255), the impact would be somewhat higher, but unless the background exposures were substantially higher than the lower tested doses (ca. 1−10 ng/kg-day), a significant change in the dose-response modeling results would not be expected.[51]

However, as discussed previously, background TEQ exposures were likely very high with respect to the lower occupational TCDD exposure levels as reported in the epidemiologic studies. Table 5-25 shows the relative increase in exposure levels (as cumulative serum TCDD

[51] Note that the situation is different for single-exposure studies where accumulated body burden from background exposures could be higher than the lowest administered dose (see Tex Box 4.1 in Section 4.4).

concentrations) for the NIOSH cohort septiles assuming that total background TEQ is 10 times background TCDD and that 50 ppt TEQ per year is accumulated in serum (Crump et al., 2003, 197384; Steenland et al., 2001, 198589). Although definitive quantitative analyses have not yet been published or designed, the impact on modeled TCDD risk from these studies could be substantial. The expectation for the direction of the effect would be the same as for the animal bioassays; adding to the exposure magnitude without changing the response would decrease the unit risk.

5.3.3. Approaches to Combining Estimates from Different Epidemiologic Studies

Meta-analyses and pooled analyses are two common approaches for combining epidemiologic study data. Meta-analyses are a useful way to combine epidemiologic data from different studies and derive a common estimate of effect, particularly when there are a large number of comparable studies that are fairly homogenous as to make them possible to combine. A meta-analysis often involves a weighted average of effect measures, dose-response coefficients, or ED_{01}s.

Unlike a meta-analysis, a pooled analysis combines the original exposure and health outcome data across multiple studies, enabling a fit of new models to the data which were not used in the original publications. Whereas a pooled analysis of the four different cohorts considered here would be useful to explore the functional form and fit of models (either statistical or multistage) across all four cohorts, this would entail a lengthy undertaking and is not being contemplated here, due in part to concerns about the confidence in the results of such an undertaking.

5.3.3.1. *The Crump et al. (2003, 197384) Meta-analysis*

Crump et al. (2003, 197384) published a meta-analysis that incorporated data from the three studies EPA used in the quantitative dose-response modeling presented in the 2003 Reassessment (U.S. EPA, 2003, 537122). These three study populations were the NIOSH (Steenland et al., 2001, 197433), the Hamburg (Becher et al., 1998, 197173), and the BASF (Ott and Zober, 1996, 198408) cohorts. The data for the NIOSH study included six additional years of follow-up and improved TCDD exposure estimates that had not been applied to EPA's dose-response modeling in the 2003 Reassessment. This study examined the relationship between

TCDD exposure and all-cancer mortality. SMR statistics that had been used in all three studies were applied.

The Crump et al. (2003, 197384) analysis was based on published data, and therefore, selection of the dose metric was limited to how aggregated data had been presented in the publications. For the NIOSH component of the analysis, the exposure data were based on worker-specific data and specific processes performed at each plant (Steenland et al., 2001, 197433). The previous approach assigned workers that had broad categories of exposure duration with the same cumulative serum level, and did not take into account the particular plant or the job assignment within the plant. The Crump et al. (2003, 197384) approach did take into account when exposure occurred in relation to the follow-up interval. The TCDD exposure metric used was a cumulative serum lipid concentration (CSLC). For the Hamburg cohort, Crump et al. (2003, 197384) used an average value from the exposure ranges provided in Flesch-Janys et al. (1998, 197339). For the BASF cohort, arithmetic averages for the dose categories were converted to TCDD CSLC intakes by dividing them by 0.25 (average body fat of 25%) and a decay rate that corresponded to a half-life of 7 years.

The outcome variable for the dose-response modeling was all cancer mortality, and CSLC was the independent variable. Crump et al. (2003, 197384) performed a series of trend tests to determine the lowest dose for which a statistically significant trend in SMR could be shown and all other lower doses. These tests also examined the highest dose in which there was no statistically significant trend using data from this dose and all other lower doses. Estimates of ED_{10}, ED_{05}, and ED_{01} for TEQ with respect to the lifetime probability of dying from cancer were calculated. This calculation assumed a first-order elimination process with a half-life of 7.6 years, a 50% systemic uptake of ingested dioxin, that dioxin concentration in serum lipid is a suitable measure for dioxin concentration in all lipid, and that all dioxin is sequestered in lipid (which comprises 25% of body weight). Age-specific mortality rates in the presence of dioxin exposure were then generated. Life-table methodology was used to calculate lifetime risks of cancer mortality.

Based on the modeling results, the hypothesis of a baseline SMR of 1.0 was rejected, and the linear model produced an SMR estimate of 1.17 (95% CI = 1.04−1.30) from these studies. The dose-response curves for the three studies were not homogeneous. Namely, the points from the BASF cohort fell below the predicted curve. Because the heterogeneity was not judged to be

extreme by different statistical tests, however, the investigators used a common model in a combined analysis of the data from the three studies. The linear model provided an adequate fit of the data, and the slope associated with CSLC-ppt was 6.3×10^{-6} (95% CI = 8.8×10^{-7} to 1.3×10^{-5}). Based on goodness of fit analysis, the preferred estimate of ED_{01} was 45 pg/kg-day, which was six times higher than the estimate of 7.7 pg/kg-day derived by Steenland et al. (2001, 198589).

5.3.3.2. *EPA's Decision Not to Conduct a Meta-analysis*

From a statistical perspective, meta-analyses may not be very reliable when applied to a small number of studies. Crump et al. (2003, 197384) used only three studies. Had EPA undertaken a meta-analysis for the studies that met its criteria, most of the weight would come from the two large studies on the NIOSH and Hamburg cohorts. However, such an analysis relies on an assumption of a normally distributed between-study effect. This normality assumption cannot be assessed with only three observations, yet the meta-analysis estimate is highly sensitive to this distributional assumption (Higgins et al., 2009, 594339). Because of this limitation and the imprecision of the between-study variance estimate, statisticians often recommend forgoing meta-analysis in favor of discussing the individual studies when few studies are available (Cox, 2006, 594342; Higgins et al., 2009, 594339). Based on these considerations, EPA decided not to undertake a meta-analysis in this document.

As noted previously, Crump et al. (2003, 197384) has conducted a meta-analysis of the three cohorts considered here, i.e., the NIOSH, Hamburg, and BASF cohorts. However, Crump et al. modeled SMR data in which the cohorts were compared to the general population, rather than on internal exposure-response analyses as relied upon in this document. Their analysis included a total of 15 different SMRs from the three studies. A prior analysis of the dose-responses by Becher et al. (1998, 197173) was used (i.e., the categorical SMR analysis by Flesch-Janys et al. (1998, 197339)). Additionally, a prior analysis of the NIOSH cohort (Steenland et al., 1999, 197437) in which SMRs were calculated was used. Crump et al. (2003, 197384) found that a linear dose-response gave a good fit to the data, and used that for deriving an ED_{01}. However, they found that a supra-linear dose-response provided a better fit to the data, but rejected the supra-linear model (a power model) because of an infinite slope at zero dose. In the original publications by Becher et al. (1998, 197173) and Steenland et al. (2001, 198589),

both observed a supra-linear dose-response trend. Crump et al. (2003, 197384) concluded that the ED_{01} was 45 pg/kg-day, six times higher than the ED_{01} of 7.7 pg/kg-day calculated by Steenland et al. (2001, 198589) using the same dietary units (pg/kg-day).

Table 5-1. Cancer slope factors calculated from Becher et al. (1998, 197173), Steenland et al. (2001, 197433) and Ott and Zober (1996, 198408) from 2003 Reassessment Table 5-4

Study	ED_{01} (LED_{01}) (ng/kg)	Cancer slope factor per ng/kg-day above background[a] (UCL)
Hamburg cohort Power model Becher et al. (1998, 197173)	6 (N.A.)	5.1 (N.A.)
Hamburg cohort Additive model Becher et al. (1998, 197173)	18.2 (N.A.)	1.6 (N.A.)
Hamburg cohort Multiplicative model Becher et al. (1998, 197173)	32.2 (N.A.)	0.89 (N.A.)
NIOSH cohort Piecewise linear model Steenland et al. (2001, 198589)	18.6 (11.5)	1.5 (2.5)
BASF cohort, from Ott and Zober (1996, 198408), multiplicative	50.9 (25.0)	0.57 (1.2)

[a]Assumes 25% of body weight is lipid; in humans 80% of dioxin dose is absorbed from the normal diet; the TCDD half-life is 7.1 years in humans. Background all cancer mortality rate calculated through lifetable analysis to 75 years. Summary results are for male all cancer risk, because the male lifetime (to 75 years) all cancer risk is greater than for females, leading to correspondingly higher cancer slope factors. As detailed in Part III, Chapter 8, $RelRisk(ED_{01}) = 0.99 + 0.01/Risk_{(0\ dose)}$. Based on the manner in which the dose-response data were calculated using Cox regression rate ratio analyses, risks are given as cancer slope factors for 1 pg/kg-day above background, assumed 5 ppt TCDD in lipid.

UCL = upper confidence limit.

Source: U.S. EPA (U.S. EPA, 2003, 537122; Part III, Chapter 5, Table 5-4)

Table 5-2. Cox regression coefficients and incremental cancer-mortality risk for NIOSH cohort data

Model	Cox regression coefficient estimate $(ppt\text{-}year)^{-1}$	Incremental risk[a]
Steenland et al.(2001, 197433) (unlagged exposures)		
Piecewise linear	1.5×10^{-5}	7.0×10^{-4}
Cheng et al. (Cheng et al., 2006, 523122) (exposures lagged 15 years)		
Linear, lower 95% of observations	3.3×10^{-6} [b]	1.2×10^{-4}
Linear, full data	1.7×10^{-8} [c]	6.3×10^{-7}

[a]Compared to internal reference population (lowest exposure group),with a cancer mortality rate of 0.214; assumes background exposure of 5 ppt per year serum-lipid TCDD concentration.
[b]$p \leq 0.05$.
[c]$p \leq 0.05$.
[d]Not statistically significant ($p > 0.05$).

Source: Cheng et al. (2006, 523122; Table IV).

Table 5-3. Comparison of fat concentrations, risk specific dose estimates and associated oral slope factors based on upper 95th percentile estimate of regression coefficient[a] of all fatal cancers reported by Cheng et al. (2006, 523122) for selected risk levels

Risk level (RL)	AUC_{RL} (ppt-yr)	FAT_{RL} (ng/kg)	Risk specific dose[b] (D_{RL}) (ng/kg-day)	Equivalent oral slope factors (OSF_{RL}) per (mg/kg-day)
1×10^{-2}	1.262×10^{4}	1.803×10^{2}	8.79×10^{-2}	1.1×10^{5}
5×10^{-3}	6.432×10^{3}	9.189×10^{1}	3.14×10^{-2}	1.6×10^{5}
1×10^{-3}	1.307×10^{3}	1.867×10^{1}	2.88×10^{-3}	3.5×10^{5}
5×10^{-4}	6.546×10^{2}	9.352×10^{0}	9.56×10^{-4}	5.2×10^{5}
1×10^{-4}	1.311×10^{2}	1.873×10^{0}	1.29×10^{-4}	7.8×10^{5}
5×10^{-5}	6.558×10^{1}	9.368×10^{-1}	5.52×10^{-5}	9.1×10^{5}
1×10^{-5}	1.312×10^{1}	1.874×10^{-1}	8.94×10^{-6}	1.1×10^{6}
5×10^{-6}	6.559×10^{0}	9.370×10^{-2}	4.25×10^{-6}	1.2×10^{6}
1×10^{-6}	1.312×10^{0}	1.874×10^{-2}	8.08×10^{-7}	1.2×10^{6}
5×10^{-7}	6.559×10^{-1}	9.370×10^{-3}	4.00×10^{-7}	1.3×10^{6}
1×10^{-7}	1.312×10^{-1}	1.874×10^{-3}	7.92×10^{-8}	1.3×10^{6}

[a] Based on regression coefficient of Cheng et al. (2006, 523122, Table III), excluding observations in the upper 5% range of the exposures; where reported $\beta = 3.3 \times 10^{-6}$ ppt-years and standard error = 1.4×10^{-6}. Upper 95th percentile estimate of regression coefficient (β_{95}) calculated to be $6.04 \times 10^{-6} = (3.3 \times 10^{-6}) + 1.96 \times (1.4 \times 10^{-6})$; background cancer mortality risk is assumed to be 0.112 as reported by Cheng et al. (2006, 523122).

[b] To calculate the extra cancer risk (ER) and OSF for any TCDD daily oral intake (D):

5. For D in ng/kg-d, look up the corresponding fat concentration (ng/kg = ppt) from the conversion chart (nongestational lifetime dose metrics) in Appendix C.4.1.
6. Calculate the AUC in ppt-yrs by multiplying the fat concentration by 70 years.
7. Calculate Extra Risk (ER) using the following equation:
   ER = [exp(AUC × 6.04E-6) × 0.112 − 0.112] ÷ 0.888.
8. Calculate the OSF $(mg/kg\text{-}d)^{-1}$ = 1E6 × (ER ÷ D).

Example for risk at the RfD: D = 7×10^{-4} ng/kg-d; fat concentration = 6.93 ng/kg;
AUC = 70 years × 6.93 ppt = 485 ppt-year;
ER = exp(485 ppt-year × 6.04E-6 $(ppt\text{-}yr)^{-1}$) × 0.112 – 0.112) ÷ 0.888 = 3.7×10^{-4}
OSF = 1E6 ng/mg × ($3.7 \times 10^{-4} \div 7 \times 10^{-4}$ ng/kg-d) = 5.3×10^{5} $(mg/kg\text{-}d)^{-1}$.

Table 5-4. Comparison of fat concentrations, risk specific dose estimates and associated central tendency slope estimates based on best estimate of regression coefficient[a] of all fatal cancers reported by Cheng et al. (2006, 523122) for selected risk levels

Risk level (RL)	AUC_{RL}, (ppt-yr)	FAT_{RL} (ng/kg)	Risk specific dose (D_{RL}) (ng/kg-day)	Central tendency slope estimates $(mg/kg\text{-}day)^{-1}$
1×10^{-2}	2.312×10^{4}	3.303×10^{2}	2.21×10^{-1}	4.5×10^{4}
1×10^{-3}	2.393×10^{3}	3.419×10^{1}	6.97×10^{-3}	1.4×10^{5}
1×10^{-4}	2.402×10^{2}	3.431×10^{0}	2.74×10^{-4}	3.7×10^{5}
1×10^{-5}	2.403×10^{1}	3.432×10^{-1}	1.74×10^{-5}	5.7×10^{5}
1×10^{-6}	2.403×10^{0}	3.432×10^{-2}	1.50×10^{-6}	6.7×10^{5}
1×10^{-7}	2.403×10^{-1}	3.432×10^{-3}	1.46×10^{-7}	7.0×10^{5}

[a]Based on regression coefficient of Cheng et al (2006, 523122; Table III) excluding observations in the upper 5% range (≥252,950 ppt-year lipid adjusted serum TCDD) of the exposures; where reported $\beta = 3.3 \times 10^{-6}$ ppt-years; background cancer mortality risk is assumed to be 0.112 as reported by Cheng et al. (2006, 523122).

Table 5-5. Kociba et al. (1978, 001818) male rat tumor incidence data[a] and blood concentrations for dose-response modeling

Morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
	0	**1.56**	**7.16**	**38.72**
Stratified squamous cell carcinoma of hard palate or nasal turbinates	0/85	0/50	0/50	4/50[b]
Stratified squamous cell carcinoma of tongue	0/85	1/50	1/50	3/50[b]
Adenoma of adrenal cortex	0/85	0/50	2/50	5/50[b]

[a]Source: Kociba et al.(1978, 001818; Table 4).
[b]Statistically significant by Fischer Exact Test ($p < 0.05$).

Table 5-6. Kociba et al. (1978, 001818) female rat tumor incidence data[a] and blood concentrations for dose-response modeling

Morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
	0	**1.55**	**7.15**	**38.56**
Hepatocellular adenoma(s) or carcinoma(s)	2/86	1/50	9/50[a]	18/45[b]
Stratified squamous cell carcinoma of hard palate or nasal turbinates	0/86	0/50	1/50	4/49[b]
Keratinizing squamous cell carcinoma of lung	0/86	0/50	0/50	7/49[b]

[a]Source: Kociba et al. (1978, 001818; Table 5). Incidence for Hepatocellular adenomas or carcinomas is from Goodman and Sauer (Goodman and Sauer, 1992, 197667; Table 1); EPA calculated statistical significance as the study authors did not provide this.
[b]Statistically significant by Fischer Exact Test ($p < 0.05$).

Table 5-7. NTP (1982, 594255) female rat tumor incidence data[a] and blood concentrations for dose-response modeling

Morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
	0	**1.96**	**5.69**	**29.75**
Subcutaneous tissue: fibrosarcoma	0/75	2/50	3/50	4/49[b]
Liver: neoplastic nodule or hepatocellular carcinoma	5/75[c]	1/49	3/50	14/49[b]
Adrenal: cortical adenoma, or carcinoma or adenoma, NOS	11/73[c]	9/49	5/49	14/46[b]
Thyroid: follicular-cell adenoma	3/73[c]	2/45	1/49	6/47

[a]Source: NTP (1982, 594255; Table 10).
[b]Statistically significant by Fischer Exact Test ($p < 0.05$).
[c]Statistically significant trend by Chochran-Armitage test ($p < 0.05$).

Table 5-8. NTP (1982, 594255) male rat tumor incidence data[a] and blood concentrations for dose-response modeling

Morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
	0	**1.96**	**5.70**	**29.87**
Liver: neoplastic nodule or hepatocellular carcinoma	0/74[b]	0/50	0/50	3/50
Thyroid: follicular-cell adenoma or carcinoma	1/69[b]	5/48[c]	8/50[c]	11/50[c]
Adrenal cortex: adenoma	6/72	9/50	12/49[b]	9/49

[a]Source: NTP(1982, 594255; Table 9).
[b]Statistically significant trend by Chochran-Armitage test ($p < 0.05$).
[c]Statistically significant by Fischer Exact Test ($p < 0.05$).

Table 5-9. NTP (1982, 594255) female mouse tumor incidence data[a] and blood concentrations for dose-response modeling

Morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
	0	**1.95**	**5.84**	**32.06**
Subcutaneous tissue: fibrosarcoma	1/74[b]	1/50	1/48	5/47[c]
Hematopoietic system: lymphoma or leukemia	18/74[b]	12/50	13/48	20/47[c]
Liver: hepatooellular adenoma or carcinoma	3/73[b]	6/50	6/48	11/47[c]
Thyroid: follicular-cell adenoma	0/69[b]	3/50	1/47	5/46[c]

[a]Source: NTP (1982, 594255; Table 15).
[b]Statistically significant trend by Chochran-Armitage test ($p < 0.05$).
[c]Statistically significant by Fischer Exact Test ($p < 0.05$).

Table 5-10. NTP (1982, 594255) male mouse tumor incidence data[a] and blood concentrations for dose-response modeling

Morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
	0	**0.77**	**2.27**	**11.24**
Lung: alveolar/bronchiolar adenoma or carcinoma	10/71[b]	2/48	4/48	13/50
Liver: hepatocellular adenoma or carcinoma	15/73[b]	12/49	13/49	27/50[c]

[a]Source: NTP (1982, 594255; Table 14).
[b]Statistically significant trend by Chochran-Armitage test ($p < 0.05$).
[c]Statistically significant by Fischer Exact Test ($p < 0.05$).

Table 5-11. NTP (2006, 197605) female rat tumor incidence data[a] and blood concentrations for dose-response modeling[b]

System: morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	Low-med dose (ng/kg)	Median dose (ng/kg)	Med-high dose (ng/kg)	High dose (ng/kg)
	0	**2.56**	**5.69**	**9.79**	**16.57**	**29.70**
Liver: cholangiocarcinoma	0/49[c]	0/48	0/46	1/50	4/49	25/53[c]
Liver: hepatocellular adenoma	0/49[c]	0/48	0/46	0/50	1/49	13/53[c]
Oral mucosa: squamous cell carcinoma	1/49[c]	2/48	1/46	0/50	4/49	10/53[c]
Pancreas: adenoma or carcinoma	0/48[c]	0/48	0/46	0/50	0/48	3/51
Lung: cystic keratinizing epithelioma	0/49[c]	0/48	0/46	0/49	0/49	9/52[c]

[a]Source: NTP (2006, 197605; Table A3a).
[b]Incidence adjusted for animals <365 days on study.
[c]Statistically significant by Poly-3 Test ($p < 0.05$).

Table 5-12. Toth et al. (1979, 197109) male mouse tumor incidence data[a] and blood concentrations for dose-response modeling

Morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
	0	**0.57**	**14.21**	**91.21**
Liver tumors	7/38	13/44	21/44[b]	13/43

[a]Source: Toth et al. (1979, 197109; Table 1).
[b]Statistically significant by Chi^2 Test ($p < 0.01$).

Table 5-13. Della Porta et al. (1987, 197405) male mouse tumor incidence data[a] and blood concentrations for dose-response modeling

Morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	High dose (ng/kg)
	0	**38.00**	**67.77**
Hepatocellular carcinoma	5/43	15/51[b]	33/50[b]

[a]Source: Della Porta et al. (1987, 197405; Table 4).
[b]Statistically significant by Chi^2 Test ($p < 0.05$).

Table 5-14. Della Porta et al. (1987, 197405) female mouse tumor incidence data[a] and blood concentrations for dose-response modeling

Morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	High dose (ng/kg)
	0	**37.59**	**66.97**
Hepatocellular adenoma	2/49	4/42[b]	11/48[b]
Hepatocellular carcinoma	1/49	12/42[b]	9/48[b]

[a]Source: Della Porta et al. (1987, 197405; Table 4).
[b]Statistically significant by Chi^2 Test ($p < 0.05$).

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Table 5-15. Comparison of multi-stage modeling results across cancer bioassays using blood concentrations

Study	Species	Sex	Morphology: topography	Multi-stage modeling:[a] stage, GoF *p*-value, LL difference	BMD_{01} (ng/kg)	$BMDL_{01}$ (ng/kg)
Della Porta et al. (1987, 197405)	Mouse	Male	Hepatocellular carcinoma	2, $p = 0.52$	7.14	1.17
		Female	Hepatocellular adenoma	2, $p = 0.86$	14.49	2.34
			Hepatocellular carcinoma	1. $p = 0.019$	2.30	1.54
Kociba et al. (1978, 001818)	Rat	Male	Stratified squamous cell carcinoma of hard palate or nasal turbinates	1, $p = 0.81$	5.76	2.79
			Stratified squamous cell carcinoma of tongue	1, $p = 0.47$	6.09	2.60
			Adenoma of adrenal cortex	1, $p= 0.78$	3.25	1.85
			Combined tumors Bayesian analysis		1.57	0.96
		Female	Hepatocellular adenoma(s) or carcinoma(s)	1, $p = 0.24$	0.70	0.50
			Stratified squamous cell carcinoma of hard palate or nasal turbinates	1, $p = 0.97$	4.51	2.34
			Keratinizing squamous cell carcinoma of lung	1, $p = 0.63$	3.14	1.79
			Combined tumors Bayesian analysis		0.51	0.37
NTP (1982, 594255)	Rat	Female	Subcutaneous tissue: fibrosarcoma	1, $p = 0.18$	3.13	1.38
			Liver: neoplastic nodule or hepatocellular carcinoma	1, $p = 0.22$	1.17	0.74
			Adrenal: cortical adenoma, or carcinoma or adenoma, NOS	1, $p = 0.34$	1.61	0.81
			Thyroid: follicular-cell adenoma	1, $p = 0.57$	3.38	1.55
			Combined tumors Bayesian analysis		0.46	0.31
		Male	Liver: neoplastic nodule or hepatocellular carcinoma	1, $p = 0.85$	6.14	2.70
			Thyroid: follicular-cell adenoma or carcinoma	1, $p = 0.06$	1.21	0.70
			Adrenal cortex: adenoma	1, $p = 0.06$	3.98	1.22
			Combined tumors Bayesian analysis		0.74	0.44

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Table 5-15. Comparison of multi-stage modeling results across cancer bioassays using blood concentrations (continued)

Study	Species	Sex	Morphology: topography	Multi-stage modeling:[a] stage, GoF *p*-value, LL difference	BMD_{01} (ng/kg)	$BMDL_{01}$ (ng/kg)
NTP (1982, 594255) cont.	Mouse	Female	Subcutaneous tissue: fibrosarcoma	1, $p = 0.93$	3.40	1.69
			Hematopoietic system: lymphoma or leukemia	1, $p = 0.98$	1.14	0.61
			Liver: hepatocellular adenoma or carcinoma	1, $p = 0.34$	1.49	0.83
			Thyroid: follicular-cell adenoma	1, $p = 0.09$, no improvement with higher orders	3.05	1.44
			Combined tumors Bayesian analysis		0.44	0.29
		Male	Lung: alveolar/bronchiolar adenoma or carcinoma	1, $p = 0.09$	2.53	0.41
			Liver: hepatocellular adenoma or carcinoma	1, $p = 0.93$	0.21	0.14
			Combined tumors Bayesian analysis		0.16	0.11
NTP (2006, 197605)	Rat	Female	Liver: cholangiocarcinoma	3, $p = 0.99$, dLL = 2.93	7.57	4.13
			Liver: hepatocellular adenoma	3, $p = 0.93$, dLL = 2.10	10.22	6.53
			Oral mucosa: squamous cell carcinoma	1, $p = 0.27$	2.20	1.39
			Pancreas: adenoma or carcinoma	1, $p= 0.64$	10.52	4.63
			Lung: cystic keratinizing epithelioma	2, $p = 0.51$, dLL = 3.55	8.30	5.24
			Combined tumors Bayesian analysis		1.18	0.78
Toth et al. (1979, 197109)	Mouse	Male	Liver: tumors	1, $p = 0.29$	0.37	0.21

*Analysis uses a chi-square goodness of fit statistic for differences in the log likelihoods ($p > 0.05$).

Table 5-16. Individual tumor points of departure and slope factors using blood concentrations

Study	Tumor Site (Sex/Species)	$BMDL_{HED}$ (ng/kg-day)	OSF (per mg/kg-day)
NTP (1982, 594255)	Liver: adenoma or carcinoma (male mice)	1.7E−03	5.8E+6
Toth et al. (1979, 197109)	Liver tumors (male mice)	1.9E−03	5.2E+6
NTP, (1982, 594255)[a]	Lung: adenoma or carcinoma (male mice)	8.7E−03	1.1E+6
Kociba et al. (1978, 001818)	Liver: adenoma or carcinoma (female rats)	1.2E−02	8.6E+5
NTP (1982, 594255)	Hematopoietic: lymphoma or leukemia (female mice)	1.6E−02	6.4E+5
NTP (1982, 594255)[a]	Thyroid: follicular cell adenoma (male rats)	1.9E−02	5.2E+5
NTP (1982, 594255)	Liver: neoplastic nodule or hepatocellular carcinoma (female rats)	2.1E−02	4.8E+5
NTP (1982, 594255)	Adrenal: cortical adenoma or carcinoma or adenoma, NOS (female rats)	2.4E−02	4.1E+5
NTP (1982, 594255)	Liver: adenoma or carcinoma (female mice)	2.5E−02	4.0E+5
Della Porta et al. (1987, 197405)	Hepatocellular carcinoma (male mice)	3.1E−02	3.2E+5
NTP (1982, 594255)[a]	Adrenal cortex: adenoma (male rats)	4.5E−02	2.2E+5
Della Porta et al. (1987, 197405)[a]	Hepatocellular carcinoma (female mice)	4.9E−02	2.0E+5
NTP (1982, 594255)	Subcutaneous fibrosarcoma (female rats)	5.4E−02	1.8E+5
NTP (2006, 197605)	Oral mucosa: squamous cell carcinoma (female rats)	5.5E−02	1.8E+5
NTP (1982, 594255)[a]	Thyroid: adenoma (female mice)	5.7E−02	1.7E+5
NTP (1982, 594255)	Thyroid: follicular cell adenoma (female rats)	6.5E−02	1.5E+5
NTP (1982, 594255)	Subcutaneous fibrosarcoma (female mice)	7.4E−02	1.4E+5
Kociba et al. (1978, 001818)	Lung: carcinoma (female rats)	8.0E−02	1.2E+5
Kociba et al. (1978, 001818)	Adenoma of adrenal cortex (male rats)	8.5E−02	1.2E+5
Della Porta et al. (1987, 197405)	Hepatocellular adenoma (female mice)	9.4E−02	1.1E+5
Kociba et al. (1978, 001818)	Nasal/Palate: carcinoma (female rats)	1.2E−01	8.2E+4
Kociba et al. (1978, 001818)	Tongue: carcinoma (male rats)	1.4E−01	7.0E+4
NTP (1982, 594255)	Liver: neoplastic nodule or hepatocellular carcinoma (male rats)	1.5E−01	6.6E+4
Kociba et al. (1978, 001818)	Nasal/Palate: carcinoma (male rats)	1.6E−01	6.3E+4
NTP (2006, 197605)	Liver: cholangiocarcinoma (female rats)	2.9E−01	3.5E+4
NTP (2006, 197605)	Pancreas: adenoma or carcinoma (female rats)	3.4E−01	2.9E+4
NTP (2006, 197605)	Lung: cystic keratinzing epithelioma (female rats)	4.1E−01	2.4E+4
NTP (2006, 197605)	Liver: hepatocellular adenoma (female rats)	5.6E−01	1.8E+4

Table 5-17. Multiple tumor points of departure and slope factors using blood concentrations

Study	Sex/species: tumor sites	$BMDL_{HED}$ (ng/kg-day)	OSF (per mg/kg-day)
NTP (1982, 594255)	Male mice: liver adenoma and carcinoma, lung	1.1E−03	9.4E+6
NTP (1982, 594255)	Female mice: liver adenoma and carcinoma, thyroid adenoma, subcutaneous fibrosarcoma, all lymphomas	5.3E−03	1.9E+6
NTP (1982, 594255)	Female rats: liver neoplasitc nodules, liver adenoma and carcinoma, thyroid follicular cell adenoma ,adrenal cortex adenoma or carcinoma	5.7E−03	1.8E+6
Kociba et al. (1978, 001818)	Female rats: liver adenoma carcinoma, oral cavity, lung	7.3E−03	1.4E+6
NTP (1982, 594255)	Male rats: thyroid follicular cell adenoma, adrenal cortex adenoma	9.6E−03	1.0E+6
NTP (2006, 197605)	Female rats: liver cholangiocarcinoma, hepatocellular adenoma, oral mucosa squamous cell carcinoma, lung cystic keratinizing epithelioma, pancreas adenoma, carcinoma	2.3E−02	4.4E+5
Kociba et al. (1978, 001818)	Male rats: adrenal cortex adenoma, tongue carcinoma, nasal/palate carcinoma	3.1E−02	3.2E+5

Table 5-18. Comparison of cancer BMDs, BMDLs, and slope factors for combined or selected individual tumors for 1, 5, and 10% extra risk

Study	Species	Sex	BMD_{01} (ng/kg)	$BMDL_{01}$ (ng/kg)	SF_{01} $(ng/kg)^{-1}$	BMD_{05} (ng/kg)	$BMDL_{05}$ (ng/kg)	SF_{05} $(ng/kg)^{-1}$	BMD_{10} (ng/kg)	$BMDL_{10}$ (ng/kg)	SF_{10} $(ng/kg)^{-1}$
Kociba (1978, 001818)[a]	Rat	Female	4.9E−01	3.8E−01	**2.7E−02**	2.5E+00	1.9E+00	**2.7E−02**	4.9E+00	3.8E+00	**2.7E−02**
		Male	1.5E+00	9.6E−01	**1.0E−02**	7.2E+00	4.8E+00	**1.0E−02**	1.5E+01	9.6E+00	**1.0E−02**
NTP (1982, 594255)[a]	Rat	Female	4.4E−01	3.2E−01	**3.2E−02**	2.2E+00	1.6E+00	**3.2E−02**	4.4E+00	3.2E+00	**3.2E−02**
		Male	6.9E−01	4.5E−01	**2.2E−02**	3.5E+00	2.2E+00	**2.2E−02**	6.9E+00	4.5E+00	**2.2E−02**
	Mouse	Female	4.3E−01	3.0E−01	**3.4E−02**	2.1E+00	1.5E+00	**3.4E−02**	4.3E+00	3.0E+00	**3.4E−02**
		Male	1.5E−01	1.1E−01	**9.4E−02**	7.7E−01	5.4E−01	**9.4E−02**	1.5E+00	1.1E+00	**9.4E−02**
NTP (2006, 197605)[a]	Rat	Female	1.1E+00	7.8E−01	**1.3E−02**	4.8E+00	3.6E+00	**1.4E−02**	8.2E+00	6.6E+00	**1.5E−02**
Della Porta et al. (1987, 197405)[b]	Mouse	Male	7.1E+00	1.2E+00	**8.5E−03**	1.4E+01	5.0E+00	**1.0E−02**	2.0E+01	9.7E+00	**1.0E−02**
		Female	2.3E+00	1.5E+00	**6.5E−03**	1.0E+01	6.8E+00	**7.3E−03**	2.1E+01	1.4E+01	**7.1E−03**
Toth et al., (1979 197109)[c]	Mouse	Male	3.7E−01	2.1E−01	**4.8E−02**	1.9E+00	1.1E+00	**4.7E−02**	3.9E+00	2.2E+00	**4.6E−02**

[a]Combined tumors, Bayesian analysis.
[b]Hepatocellular carcinomas for both males and females.
[c]Hepatocellular carcinomas.
TCDD blood concentrations from Emond rodent PBPK models.
SF = BMR ÷ $BMDL_{BMR}$, where BMR = 0.01, 0.05, or 0.10.

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Table 5-19. TCDD human-equivalent dose (HED) BMDs, BMDLs, and oral slope factors (OSF) for 1, 5, and 10% extra risk

Study	Species	Sex	BMD_{01} (ng/kg-d)	$BMDL_{01}$ (ng/kg-d)	OSF_{01} $(ng/kg\text{-}d)^{-1}$	BMD_{05} (ng/kg-d)	$BMDL_{05}$ (ng/kg-d)	OSF_{05} $(ng/kg\text{-}d)^{-1}$	BMD_{10} (ng/kg-d)	$BMDL_{10}$ (ng/kg-d)	OSF_{10} $(ng/kg\text{-}d)^{-1}$
Kociba (1978, 001818)[a]	Rat	Female	1.1E−02	7.4E−03	**1.4E+00**	1.3E−01	8.6E−02	**5.8E−01**	3.8E−01	2.59E−01	**4.0E−01**
		Male	5.9E−02	3.1E−02	**3.3E−01**	6.6E−01	3.6E−01	**1.4E−01**	1.8E+00	9.7E−01	**1.0E−01**
NTP (1982, 594255)[a]	Rat	Female	9.7E−03	5.8E−03	**1.7E+00**	1.1E−01	6.6E−02	**7.6E−01**	3.2E−01	1.9E−01	**5.2E−01**
		Male	1.9E−02	9.7E−03	**1.0E+00**	2.2E−01	1.1E−01	**4.5E−01**	6.2E−01	3.3E−01	**3.1E−01**
	Mouse	Female	9.1E−03	5.4E−03	**1.9E+00**	1.1E−01	6.0E−02	**8.3E−01**	3.0E−01	1.8E−01	**5.7E−01**
		Male	1.9E−03	1.2E−03	**8.3E+00**	2.2E−02	1.3E−02	**3.8E+00**	6.4E−02	3.8E−02	**2.7E+00**
NTP (2006, 197605)[a]	Rat	Female	4.1E−02	2.3E−02	**4.4E−01**	3.6E−01	2.4E−01	**2.1E−01**	7.9E−01	5.7E−01	**1.8E−01**
Della Porta et al. (1987, 197405)[b]	Mouse	Male	5.2E−01	3.1E−02	**3.2E−01**	1.7E+00	3.8E−01	**1.3E−01**	2.8E+00	1.0E+00	**1.0E−01**
		Female	9.2E−02	4.9E−02	**2.0E−01**	1.1E+00	6.0E−01	**8.3E−02**	2.9E+00	1.7E+00	**5.9E−02**
Toth et al. (1979, 197109)[c]	Mouse	Male	5.1E−03	1.9E−03	**5.3 E+00**	6.7E−02	2.7E−02	**1.9E+00**	2.0E−01	8.5E−02	**1.2 E+00**

[a]Combined tumors, Bayesian analysis.
[b]Hepatocellular carcinomas for both males and females.
[c]Hepatocellular carcinomas.
HEDs from Emond human PBPK model corresponding to blood concentration BMDs and BMDLs in Table F3-1.
OSF = BMR ÷ $BMDL_{BMR}$, where BMR = 0.01, 0.05, or 0.10.

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Table 5-20. Illustrative RfDs based on tumorigenesis in experimental animals

Study	Species, strain (sex)	Protocol	Endpoint	$BMDL_{HED}$[a] (ng/kg-day)	RfD[b] (mg/kg-day)
NTP (1982, 594255)	Mouse, B6C3F1, male	2-year gavage; *n* = 50	Liver adenoma and carcinoma, lung	1.1E-3	3.6E-11
Toth et al. (1979, 197109)	Mouse, Swiss/ H/Riop, male	1-year gavage (1-year average); *n* = 38−44	Liver tumors	1.9E-3	6.3E-11
NTP (1982, 594255)	Mouse, B6C3F1, female	2-year gavage; *n* = 50	Liver adenoma and carcinoma, thyroid adenoma, subcutaneous fibrosarcoma, all lymphomas	5.3E-3	1.7E-10
NTP (1982, 594255)	Rat, Osborne-Mendel, female	2-year gavage; *n* = 50	Liver neoplasitc nodules, thyroid follicular cell adenoma, liver adenoma and carcinoma, adrenal cortex adenoma or carcinoma	5.7E-3	1.9E-10
Kociba et al. (1978, 001818)	Rat, S-D, female	2-year dietary; *n* = 50	Liver adenoma carcinoma, oral cavity, lung	7.3E-3	2.4E-10
NTP (1982, 594255)	Rat, Osborne-Mendel, male	2-year gavage; *n* = 50	Thyroid follicular cell adenoma, adrenal cortex adenoma	9.6E-3	3.2E-10
Della Porta et al. (1987, 197405)	Mouse, B6C3F1, male	1-year gavage; *n* = 40-50	Hepatocellular carcinoma	3.1E−02	1.0E-9
NTP (2006, 197605)	Rat, S-D, female	2-year gavage; *n* = 53	Liver cholangiocarcinoma, hepatocellular adenoma, oral mucosa squamous cell carcinoma, lung cystic keratinizing epithelioma, pancreas adenoma, carcinoma	3.1E-2	1.0E-9
Kociba et al. (1978, 001818)	Rat, S-D, male	2-year dietary; *n* = 50	Adrenal cortex adenoma, tongue carcinoma, nasal/palate carcinoma	3.1E-2	1.0E-9

[a]BMR = 0.01.
[b]UF = 30; UF_A = 3, UF_H = 10.

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Table 5-21. Illustrative RfDs based on hypothesized key events in TCDD's MOAs for liver and lung tumors

Key event	Endpoint and exposure duration	NO(A)EL$_{HED}$ (ng/kg-day)	LO(A)EL$_{HED}$ (ng/kg-day)	BMDL$_{HED}$[a] (ng/kg-day)	RfD[b] (mg/kg-day)	Study
Liver tumors						
Changes in gene expression	CYP1A1 mRNA, 1 day	**1.8E−05**	3.4E−04	2.3E−03[c] (Appendix H)	6E−13[d,e]	Vanden Heuvel et al. (1994, 594318)
Changes in gene expression	Benzo(a)pyrene hydroxylase (BPH) activity (CYP1A1), 1 day	9.2E−04	6.0E−03	**4.6E−04**[c,d] (Appendix H)	2E−11[d,e]	Kitchin and Woods (1979, 198750)
	EROD (CYP1A1), 53 weeks	none	1.4E−01	**9.5E−03**[c] (Appendix H)	3E−10[e]	NTP (2006, 197605)
Oxidative stress	DNA single-strand breaks, 90 days	none	3.3E−02	**2.2E−02**[c] (Appendix H)	7E−10[e]	Hassoun et al. (2000, 197431)
	TBARS, 90 days	–	–	**4.4E−02** (Appendix H)	2E−09[e]	Hassoun et al. (2000, 197431)
	Cytochrome C reductase, 90 days	–	–	**8.8E−02** (Appendix H)	3E−09[e]	Hassoun et al. (2000, 197431)
Hepatotoxicity	Toxic hepatopathy, 2 years	none	**1.4E−01**	1.8E−01[c] (Appendix E)	5E−09[f]	NTP (2006, 197605)
	Hepatocyte hypertrophy, 31 weeks	9.3E−02	3.3E−01	**8.8E−03** (Appendix E)	3E−10[e]	NTP (2006, 197605)
Hepatocellular proliferation	Labeling index, 31 weeks	none	1.4E−01	**6.6E−02**[c] (Appendix H)	2E−09[e]	NTP (2006, 197605)
Lung tumors						
Metabolic enzyme induction	EROD (CYP1A1), 53 weeks	none	1.4E−01	**2.9E−04**[c] (Appendix H)	1E−11[e]	NTP (2006, 197605)
Retinoid homeostatsis	Hepatic retinol and retinyl palmitate, 90 days	none	1.1E+00	**1.7E−01**[c] (Appendix E)	6E−09[e]	Van Birgelen et al. (1995, 198052)

[a]BMR for continuous endpoints—1 standard deviation; for quantal endpoints—10%.
[b]Bolded NOAEL, LOAEL, or BMDL is selected POD; poorly-fitting BMDLs above the LOAEL not used.
[c]Poor BMD model fit or no good model fit.
[d]Could be higher depending on the effect of background exposureoor (see Section 5.3.2.1).
[e]UF = 30; UF_A = 3; UF_H = 10.
[f]UF = 300; UFA = 3; UFH = 10; UFL = 10.

Table 5-22. Comparison of principal epidemiological studies

Strengths	Weaknesses	Study
Cumulative TCDD levels in the serum were estimated on an individual-level basis for the 3,538 workers. Evaluated effect of lag periods (0 and 15 years). Measured and back-extrapolated TCDD concentrations to refine and quantify job exposure matrices, which were then used to estimate dioxin cumulative dose for each member of their entire cohort. Internal cohort comparisons (Cox regression model). Background exposure estimated.	• Exposure to other chlorinated hydrocarbons (dioxin like compounds). • Extrapolation of dose from a small subset (roughly 5%, *n* = 170) of the cohort. • Serum fat or body fat levels of TCDD were back-calculated using a simple first-order model. Half-life of TCDD is variable but simulated as a constant. Changes in the lipid fraction of body weight or presence/absence of genetic differences in humans that alter the distribution and metabolism of TCDD were not considered. • Serum lipid levels of TCDD in 1988 were measured only at one of the eight plants in the study. No follow-up measures. The estimates of dose are based on blood samples taken decades after exposure.	NIOSH cohort Steenland et al. (2001, 197433)
Cumulative TCDD levels in the serum were estimated on an individual-level basis for the 3,538 workers. TCCD dose estimates were simulated with a kinetic model that included considerations of exposure intensity and age-dependent body weight and fat levels. Evaluated effect of lag periods (0 and 15 years). Background exposure estimated. Stratified risk estimates for smoking and nonsmoking. Race and age adjustments. Internal cohort noted an inverse-dose response for high-exposure groups and thus excluded the data resulting in stronger associations.	• Extrapolation of dose from a small subset (roughly 5%, *n* = 170) of the cohort. • The authors reported the CADM model provided an improved fit over the one-compartmental model, but no evidence was reported regarding any formal test of statistical significance. • Serum lipid levels of TCDD in 1988 were measured only at one of the eight plants in the study. No follow-up measures. The estimates of dose are based on blood samples taken decades after exposure. • Exposure to other chlorinated hydrocarbons (dioxin like compounds). • No consideration for recent exposures to TCDD, changes in the lipid fraction of body weight or presence/absence of genetic differences in humans that alter the distribution and metabolism of TCDD could cause misclassification.	NIOSH cohort Cheng et al. (2006, 523122)

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Table 5-22 Comparison of principal epidemiological studies (continued)

Strengths	Weaknesses	Study
• Repeated TCDD measures in serum in 48 individuals. Used to estimate half-life for study cohort. Took into account the age and body fat percentage of the workers. Measured and back-extrapolated TCDD concentrations to quantify exposures for the remaining cohort members using 5 different working areas of the plant. • Evaluated effect of lag periods up to 20 years. • Multiple statistical models used to evaluate fatal cancer slope estimates. • Background exposure estimated.	• Exposure to other chlorinated hydrocarbons (dioxin like compounds), HCH, and lindane. • Extrapolation of dose from a small subset (roughly 4%, n = 1,189) of the cohort. • Serum fat or body fat levels of TCDD were back-calculated using a simple first-order model. Presence/absence of genetic differences in humans that alter the distribution and metabolism of TCDD were not considered. • Serum lipid levels of TCDD for only 275 workers.	Becher et al. (1998, 197173); Hamburg Cohort
• Both internal and external analyses. • Adjustment for age, BMI, and smoking. • Both cancer incidence and cancer mortality data available, although results somewhat discordant, with steeper dose-response seen for cancer mortality.	• Acute dose due to accident may not be comparable to chronic dose accumulated over a long time, as in most environmental exposures. • Relatively small number of cancer deaths compared to NIOSH and Hamburg cohorts (n = 31). • Serum TCDD levels measured 30 years after accident, requiring extrapolation back in time to estimate cumulative dose over time. • Serum TCDD levels measured only on a sample of the cohort (138 out of 243), requiring assumptions about similarities in exposure scenario for other workers to estimate their exposure	Ott and Zober (1996, 198408)

Table 5-22 Comparison of principal epidemiological studies (continued)

Strengths	Weaknesses	Study
• TCDD levels measured in all 891 members of this female cohort. • Most TCDD measurements based on observed levels in stored serum at the time of the accident in 1976, no extrapolation needed to estimate past levels. • Internal analyses. • Evaluates female cancer incidence, other studies evaluate male cancer mortality. • Presumed adjustment for age and potential breast cancer confounders (15 of 21 cancers were breast cancer).	• Acute dose due to accident may not be comparable to chronic dose accumulated over a long time, which is typical of most environmental exposures. • Did not evaluate different lag periods. • Not clear if any adjustment for confounders. • Small number of cancers (n = 21). • Doses known in 1976, require assumptions about excretion over time to estimate cumulative dose (9 year half life assumed), presumed metric of primary interest. No more recent TCDD concentration data used. • Reported $\log_{10}$ transformation of the exposure estimates in their regression analysis.	Warner et al. (2002, 197489)

Table 5-23. Added background TEQ exposures to blood TCDD/TEQ concentrations in rats[a]

Background TEQ added				
None	**Est. TCDD only[b]**	**Est. TEQ[c]**	**2× Est. TEQ[d]**	**10× Est. TCDD[e]**
0	0.064	0.19	0.38	0.64
2.56	2.62	2.75	2.94	3.20
5.69	5.75	5.88	6.07	6.33
9.79	9.85	9.98	10.1	10.5
16.6	16.7	16.8	17.0	17.2
29.7	29.8	29.9	30.1	30.3

[a]Background exposures estimated from NTP (2006, 543749); rat TCDD concentrations from NTP (2006, 197605)).
[b]Estimated from TCDD fat concentration measurements in NTP (2006, 543749).
[c]Estimated from combined TCDD, PeCDF, and PCB-126 fat concentration measurements in NTP (2006, 543749).
[d]Assumes that measured congeners comprise 50% of actual TEQ exposure.
[e]Assumes that TCDD comprises 10% of total background TEQ exposure.

Table 5-24. Effect of added background TEQ exposure on $BMDL_{01}$ for cholangiocarcinomas in rats (NTP, 2006, 197605)

Background TEQ[a]	Added exposure (ng/kg blood TEQ)	$BMDL_{01}$[b] (ng kg blood)
None[c]	0	4.14
Est. TCDD only	0.064	4.19
Est. TEQ	0.19	4.30
2× Est. TEQ	0.38	4.45
10× Est. TCDD	0.64	4.65

[a]Scenarios as in Table 5-20.
[b]Multistage model results from BMDS version 2.1.1 (see Appendix I for modeling details).
[c]Same result as for the single tumor modeling presented previously in this section.

Table 5-25. NIOSH cohort septile data with added TEQ background[a]

Septile	TCDD serum level (ppt-yr)	TCDD + background TEQ (ppt-yr)	Relative increase (%)
1	260	2,960	1,040
2	402	3,102	770
3	853	3,553	320
4	1,895	4,595	140
5	4,420	7,120	60
6	12,125	14,825	20
7	59,838	62,538	5

[a]Septile data from Steenland et al. (2001, 197433); cumulative background TEQ estimate from Crump et al. (2003, 197384); both based on estimates by WHO (1998).

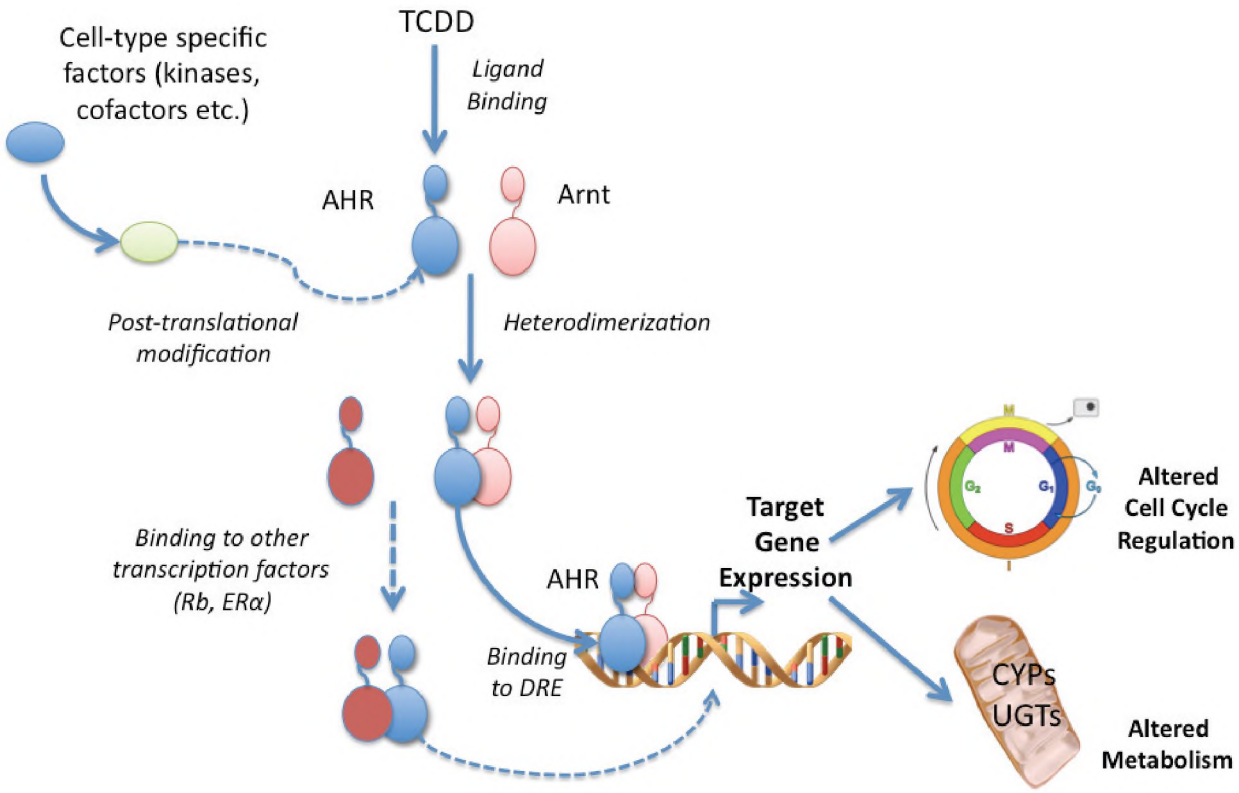


Figure 5-1. Mechanism of altered gene expression by AhR. The regulation of gene expression by TCDD in mammalian cells requires binding of the xenobiotic to the aryl hydrocarbon receptor (AhR). The AhR is part of a multi-protein complex that includes heat shock proteins and various kinases and other post-translational modifying factors. Upon ligand binding, the AhR heterodimerizes with the aryl hydrocarbon receptor nuclear translocator (Arnt) and binds to dioxin response elements (DREs) found in target genes. Alternatives to DRE-dependent gene expression exist whereby the AhR complex associates with other transcription factors and results in a cross-talk between these systems. The culmination of regulation of AhR targets genes (both increases ad decreases in transcription) results in an alteration in cellular phenotypes, including changes in intracellular metabolism and changes in cell cycle regulation.

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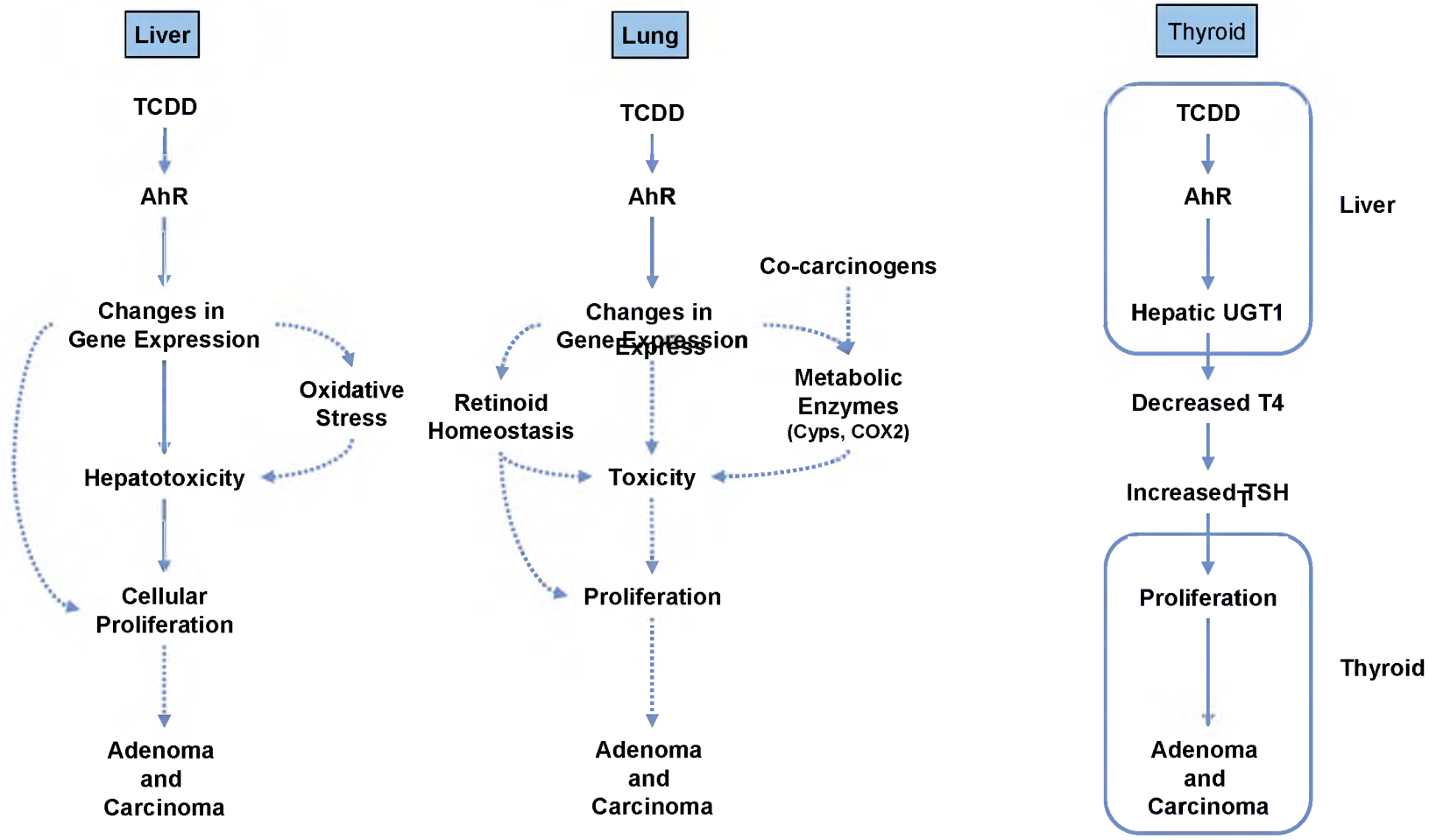


Figure 5-2. TCDD's hypothesized modes of action in site-specific carcinogenesis. See text for details. In each instance, the solid arrows depict pathways that are well-established and are associated with low uncertainty. The dashed arrows represent connections that are less established and are associated with higher uncertainty.

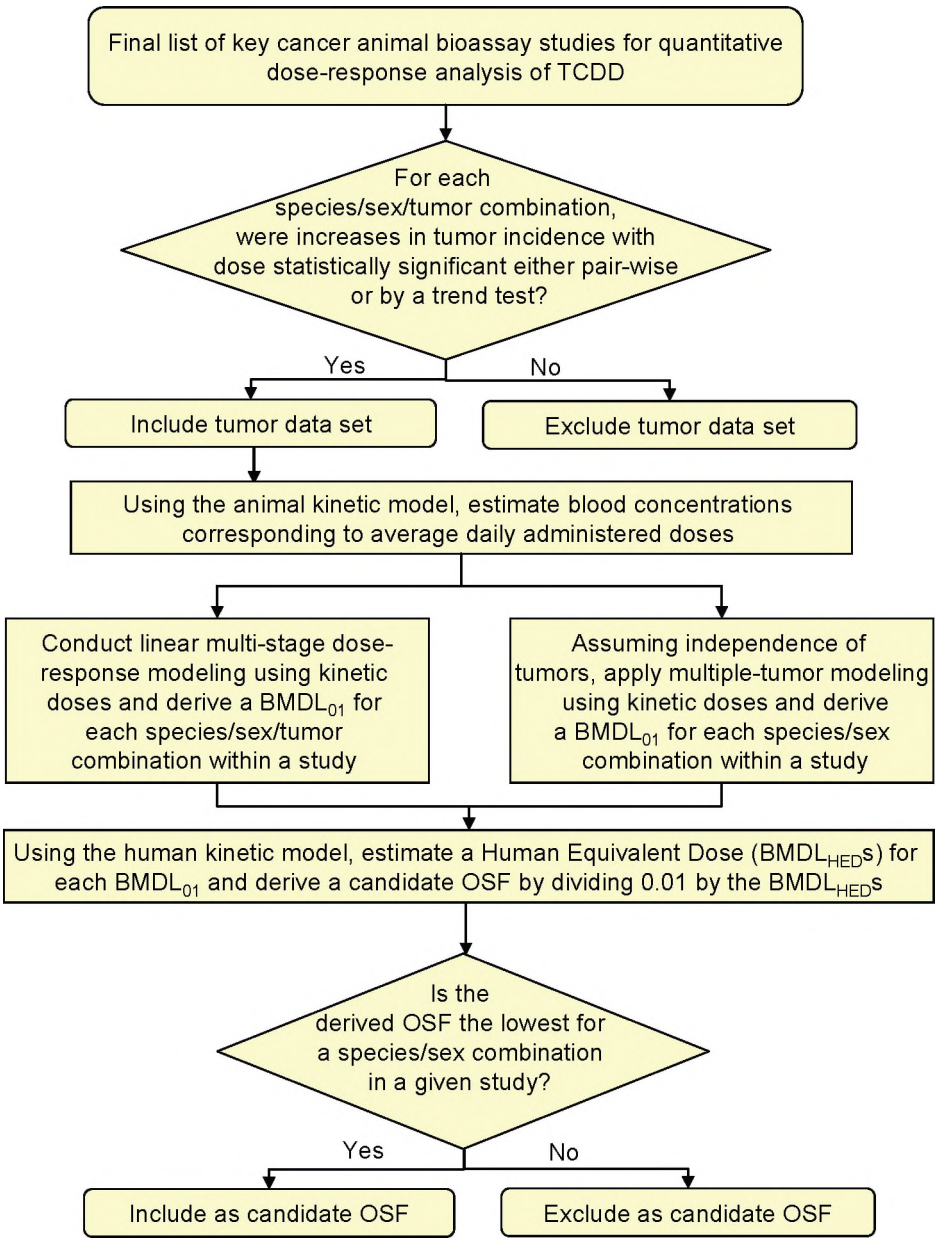


Figure 5-3. EPA's process to select and identify candidate OSFs from key animal bioassays for use in the cancer risk assessment of TCDD.

For each cancer study that qualified for TCDD dose-response assessment using the study inclusion criteria, EPA first selected the species/sex/tumor combinations with statistically significant increases in tumor incidence by either a pair-wise test between the treated group and the controls or by a trend test showing increases in tumors with increases in dose. Next, EPA used an animal kinetic model to estimate blood concentrations corresponding to the study average daily administered doses for use in dose response modeling. $BMDL_{01}$'s were then estimated for the blood concentrations by, (1) using the linearized multistage model for each species/sex/tumor combination within each study, and (2) using the linearized multistage model within a Bayesian Markov Chain Monte Carlo framework that assumes independence of tumors and modeling all tumors together for each species/sex combination within each study. Using the human kinetic model, human equivalent doses ($BMDL_{HED}$s) were then estimated for each of the $BMDL_{01}$s and oral slope factors were calculated by $OSF = 0.01/BMDL_{HED}$. The lowest OSF for a species/sex combination for either a single tumor type or all tumors combined was selected as a candidate OSF for TCDD risk assessment.

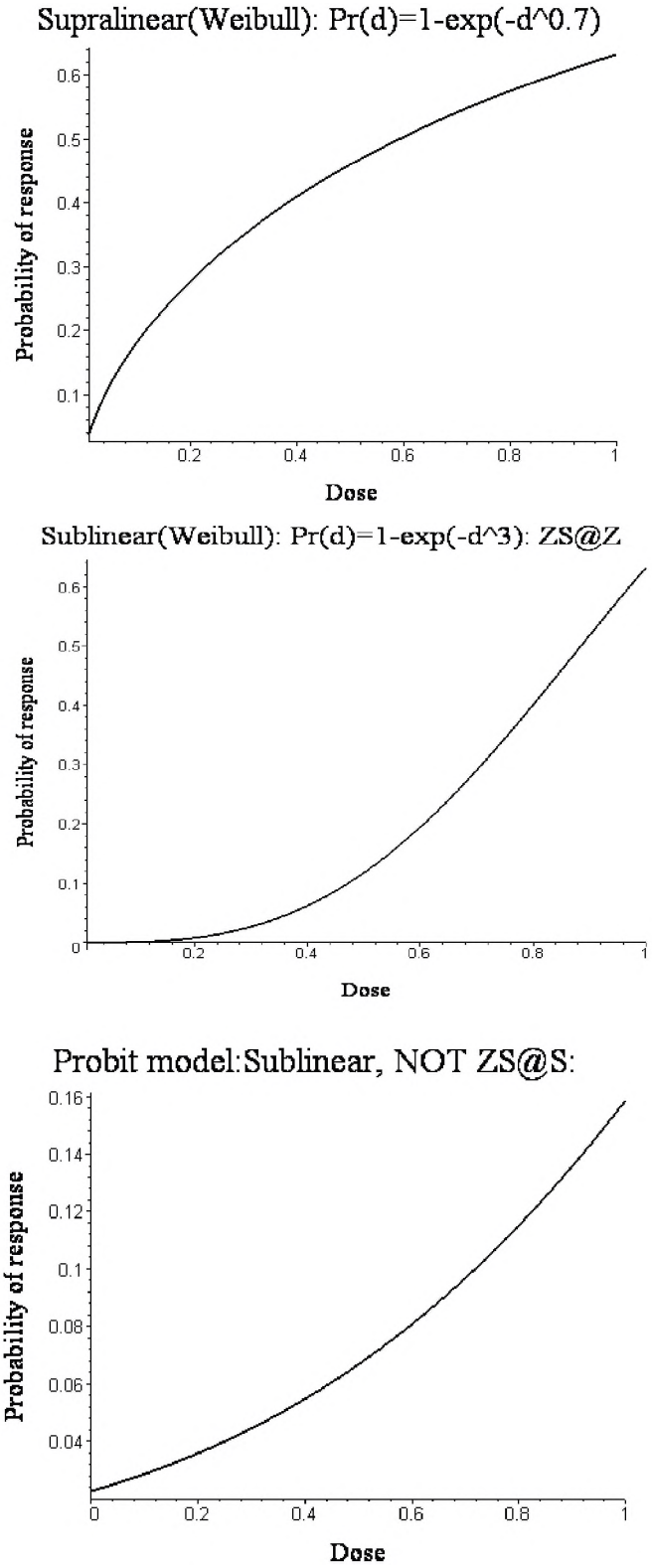


Figure 5-4. Dose-response model shape

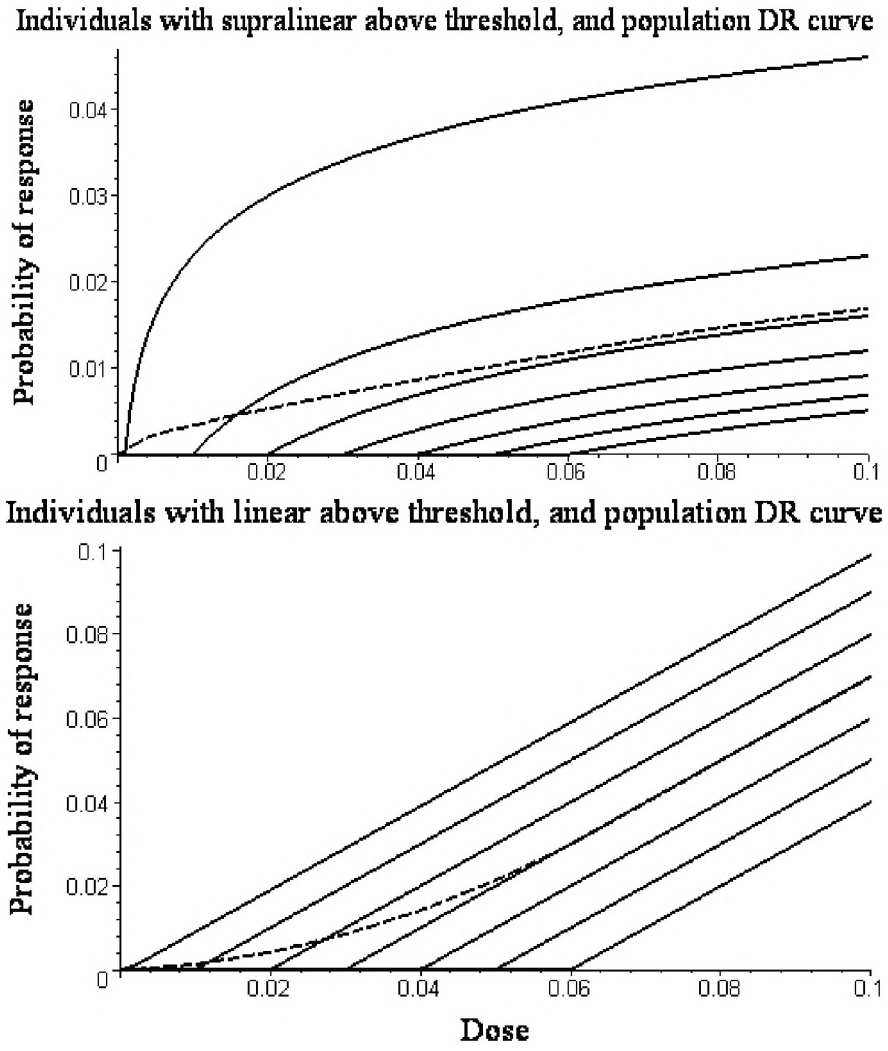
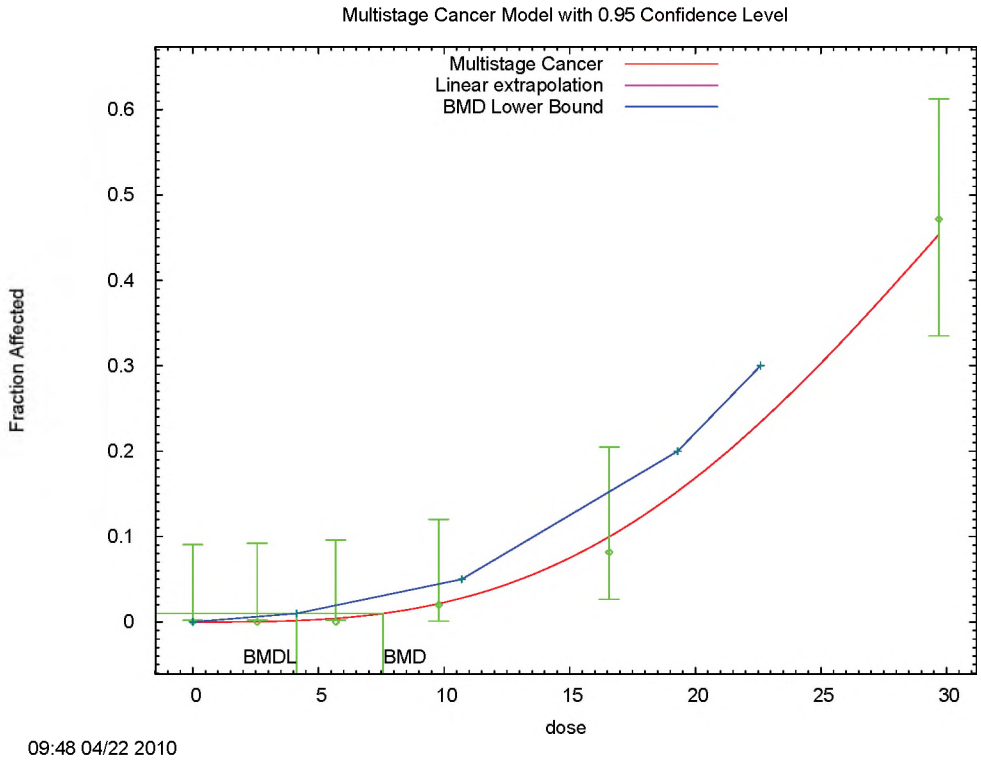


Figure 5-5. Comparison of individual and population dose-response curves; a simple illustration.

A. Full response range



B. Low-dose region

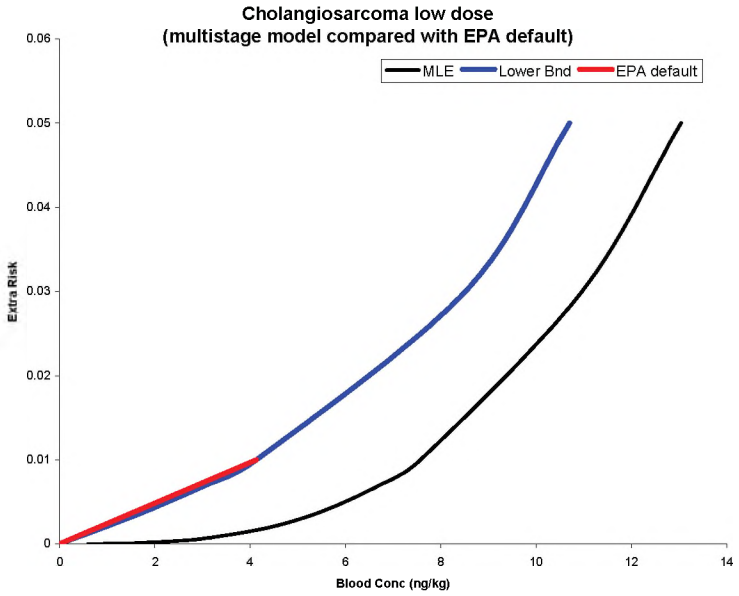
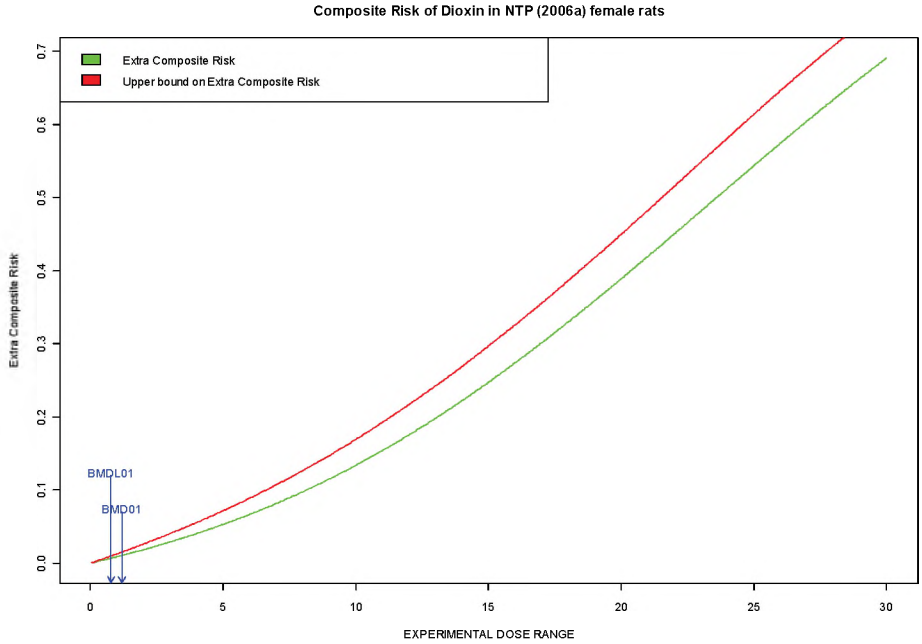


Figure 5-6. Multistage benchmark dose modeling of NTP (2006, 197605) cholangiosarcoma data.

A. Full response range



B. Low-dose region

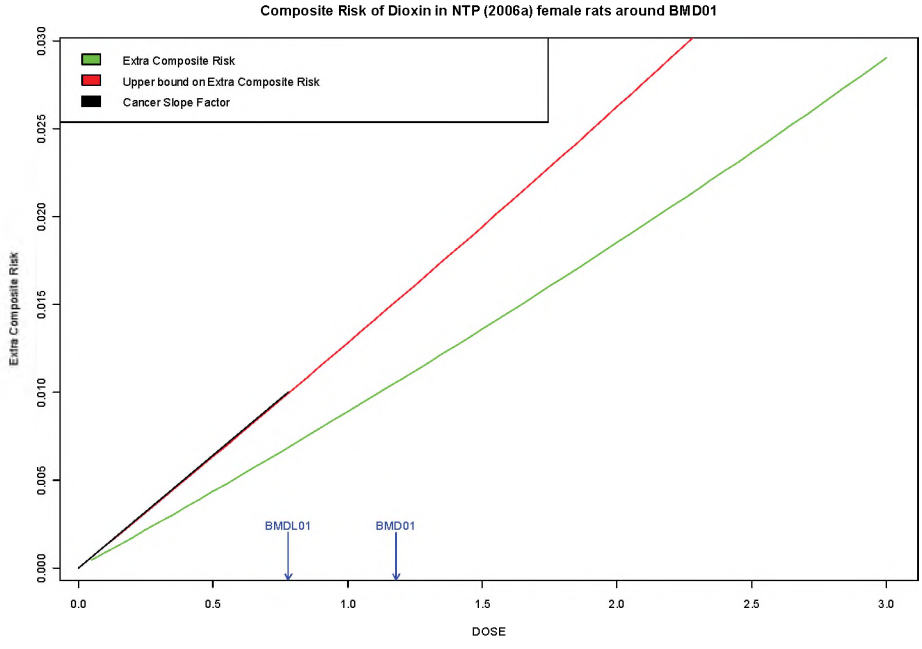
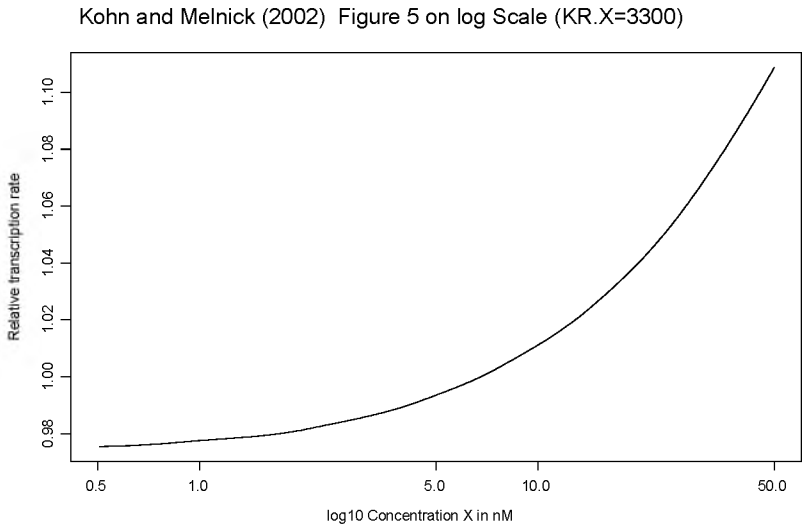


Figure 5-7. Multistage benchmark dose modeling of NTP (2006, 197605) combined tumor data.

A.



B.

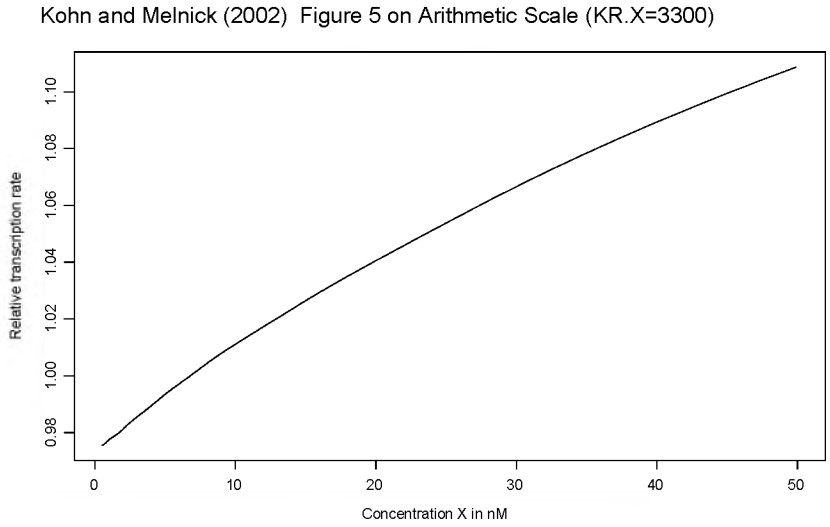


Figure 5-8. Estrogen receptor-mediated response-modeling plot from Kohn and Melnick (2002, 199104): low-dose region shown.

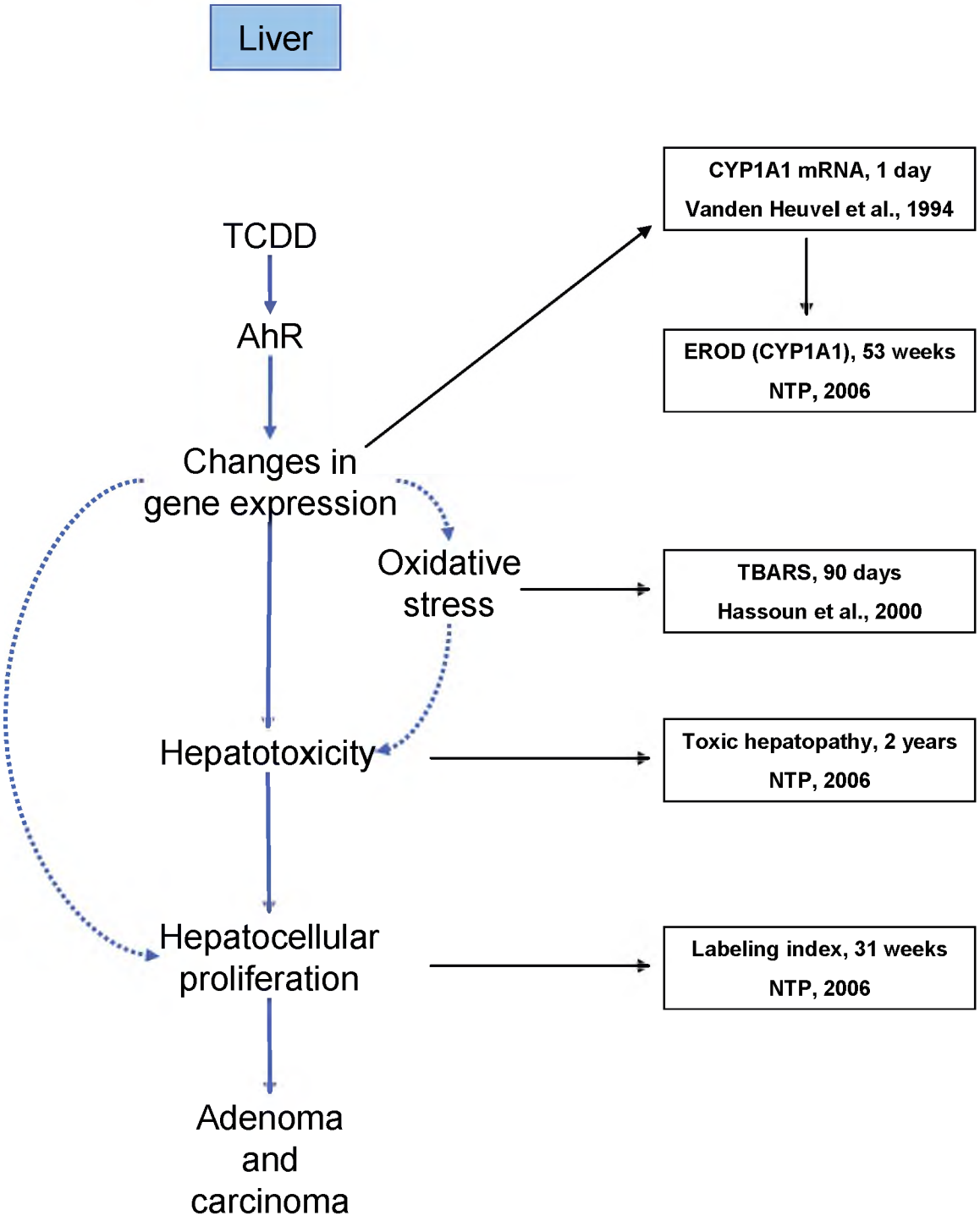


Figure 5-9. Representative endpoints for each of the hypothesized key events following AhR activation for TCDD-induced liver tumors.

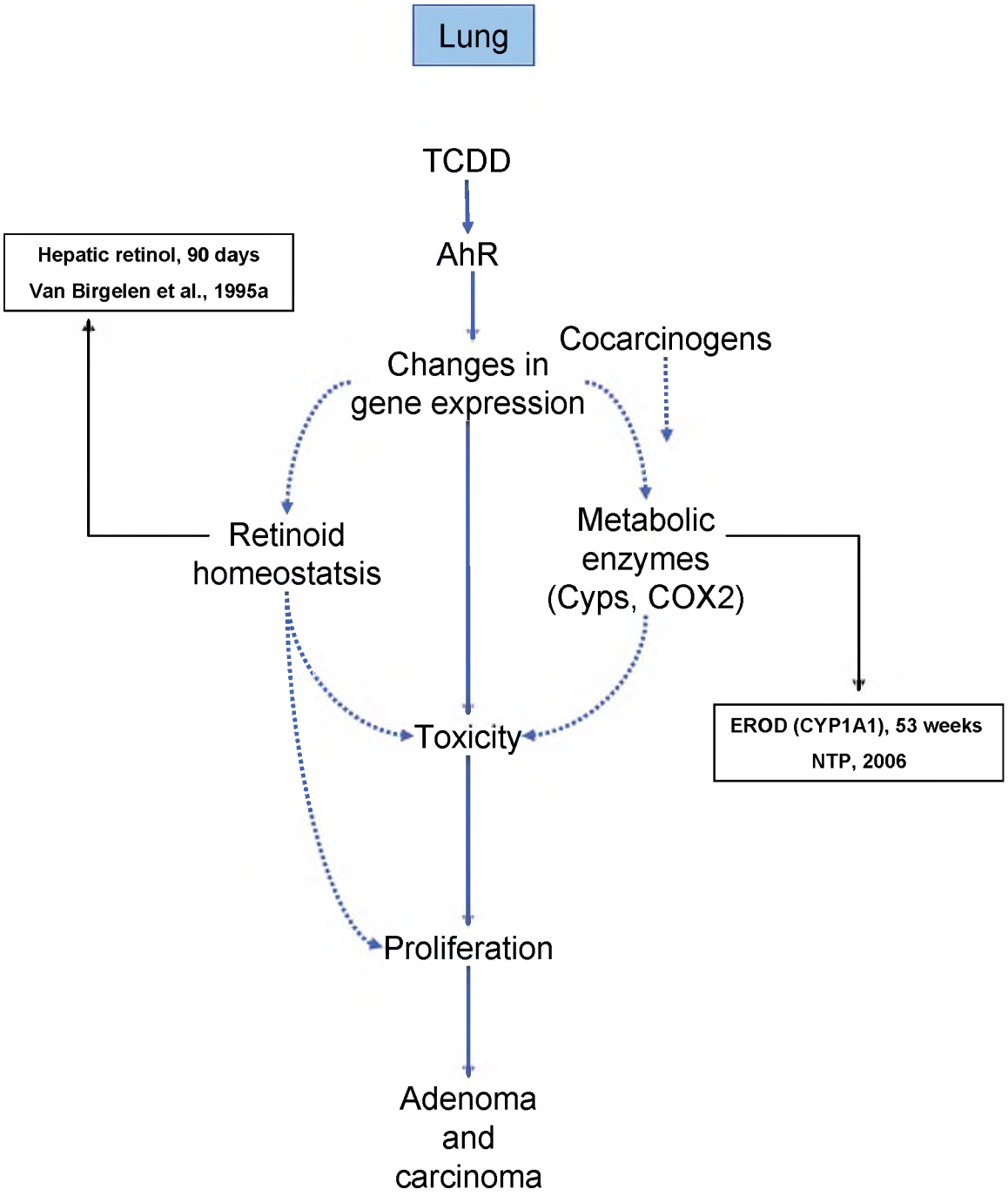


Figure 5-10. Representative endpoints for two hypothesized key events following AhR activation for TCDD-induced lung tumors.

This document is a draft for review purposes only and does not constitute Agency policy.

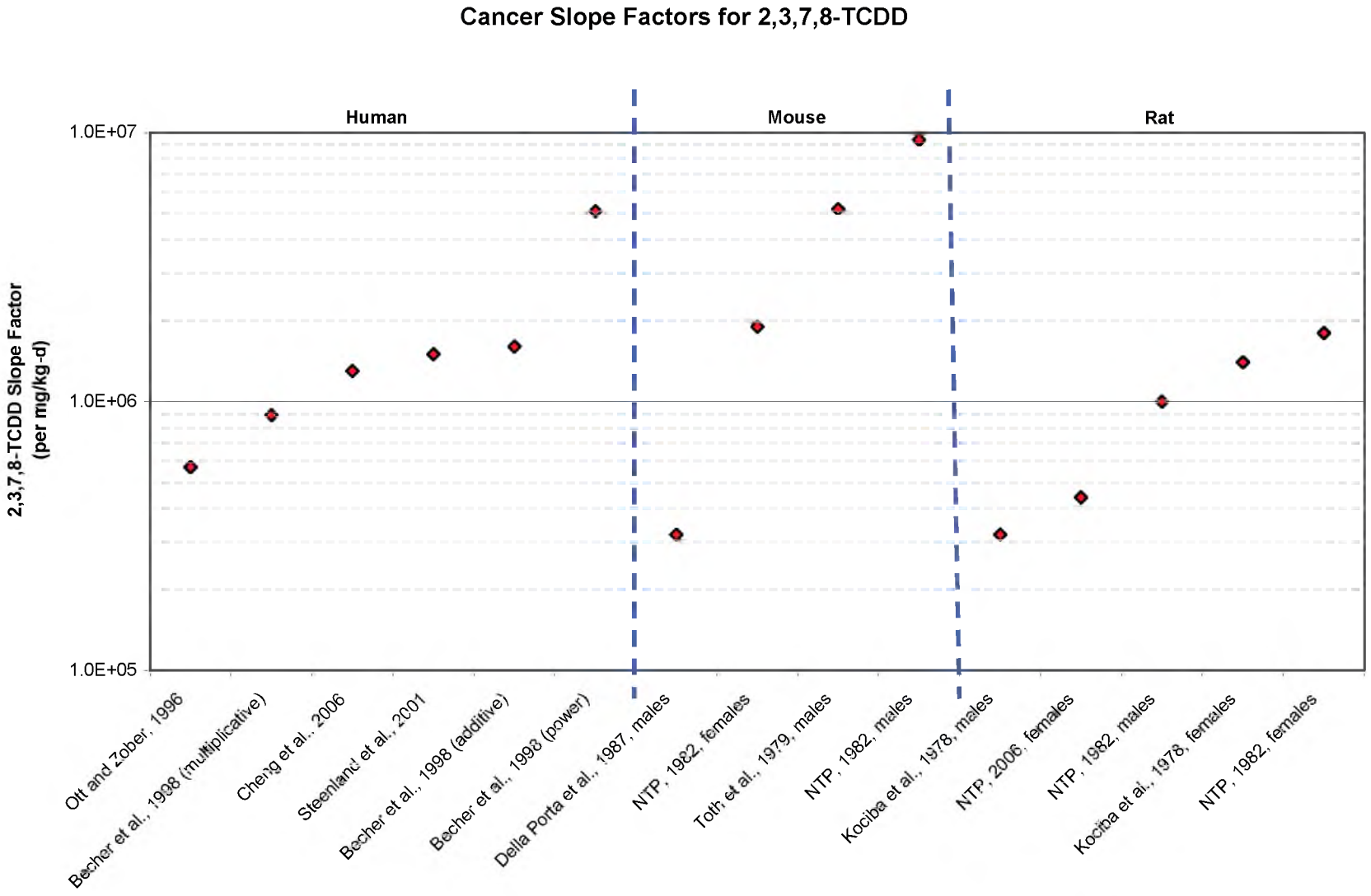


Figure 5-11. Candidate oral slope factor array.

6. FEASIBILITY OF QUANTITATIVE UNCERTAINTY ANALYSIS FROM NAS EVALUATION OF THE 2003 REASSESSMENT

6.1. INTRODUCTION

This section focuses on the third area for improvement in the 2003 Reassessment that was identified by the National Academy of Sciences (NAS) review committee (NAS, 2006, 198441), i.e., improving transparency, thoroughness, and clarity in *quantitative uncertainty analysis*. Although the NAS committee summarized the shortfalls in the 2003 Reassessment categorically, the elaborations within their report often contain the qualification "if possible" and do not take a position with regard to the feasibility of many of its suggestions. With appreciation for the extent of information available for dioxin, the goal of this section is to circumscribe the feasibility of a data-driven quantitative uncertainty analysis for TCDD dose-response assessment. Following brief highlights of the evolution of quantitative uncertainty analysis for such applications, this section lays out definitions of key terms, reviews EPA's position regarding cancer and noncancer endpoints, summarizes the NAS critique, and evaluates the feasibility of quantitative uncertainty analysis for TCDD within the framework of EPA's noncancer RfD and cancer slope factor dose-response methodologies.

6.1.1. Historical Context for Quantitative Uncertainty Analysis

The basic methods of probabilistic risk assessment (PRA) were developed in the aerospace program in the 1960s, and they found their first full-scale application in the U.S. Nuclear Regulatory Commission's (U.S. NRC's) *Reactor Safety Study of 1975*—including accident consequence analysis and uncertainty analysis (U.S. NRC, 1975, 543729). This study, commonly referred to as the Rasmussen Report after its lead author, is considered to be the first modern PRA. In the aftermath of the 1979 Three Mile Island accident, a new generation of PRAs appeared in which some of the methodological problems of the 1975 study were avoided. These advances were reflected in the Commission's *Fault Tree Handbook* (U.S. NRC, 1981, 543730) and PRA guide (U.S. NRC, 1983, 543732), which shored up and standardized much of the risk assessment methodology. An extensive chapter of the latter was devoted to uncertainty and sensitivity analysis. These documents formed the basis for standards and guidelines

established by other agencies, including the U.S. Department of Energy (U.S. DOE, 1992, 543733) and National Aeronautics and Space Administration (NASA, 2002, 543734).

In 1991, a set of U.S. NRC studies known as NUREG 1150 used structured expert judgment to quantify uncertainty and set new standards for uncertainty analysis, in particular with regard to expert elicitation (U.S. NRC, 1991, 543736). This was followed by a joint U.S.-European Union (EU) program for quantifying uncertainty in accident consequence models. Expert judgment methods were further elaborated in those evaluations, as well as screening, dependence modeling and sensitivity analysis (EC, 2009, 543738). Studies building off of this work have performed a large-scale uncertainty analysis of European consequence models and provided extensive guidance on identifying important variables; selecting, interviewing and combining experts; propagating uncertainty; inferring distributions on model parameters; and communicating results, as documented by Goossens et al. (1996, 548727; 1997, 543752; 1998, 548726; 2001, 548730; 2001, 548731; 2001, 548732; 2001, 548735; 2001, 548737; 2001, 548738; 2001, 548734) and others (Brown et al., 1997, 543739; Harper et al., 1995, 202317; 2002, 198124).

The National Research Council (NRC) has been a persistent voice in urging the government to enhance its risk assessment methodology beginning with its report on risk assessment in the federal government (NRC, 1983, 194806). The Council's 1989 report, *Improving Risk Communication*, inveighed against minimizing the existence of uncertainty and noted the importance of considering the distribution of exposure and sensitivities in a population (NRC, 1989, 000858). The issue of uncertainty was a clear concern in subsequent reports, including those assessing human exposure to airborne pollutants (NRC, 1991, 037823). Building on its evaluation of *Issues in Risk Assessment* (NRC, 1993, 078637), the landmark study *Science and Judgment in Risk Assessment* (NRC, 1994, 006424) gathered many of these themes in a plea for quantitative uncertainty analysis as "the only way to combat the false sense of certainty which is *caused* by a refusal to acknowledge and (attempt to) quantify the uncertainty in risk predictions." A subsequent report, *Estimating the Public Health Benefits of Proposed Air Pollution Regulations* (NRC, 2002, 035312), identified three barriers to the broad acceptance of recent EPA health benefit analyses: (1) the large amount of uncertainty inherent in these analyses, (2) the manner in which EPA deals with this uncertainty, and (3) "… projected health benefits are often reported as absolute numbers of avoided death or adverse health outcomes

without a context of population size or total numbers of outcomes." The Council encouraged EPA to "explore alternative options for incorporating expert judgment into its probabilistic uncertainty analyses."

In an early 2009 report, *Science and Decisions: Advancing Risk Assessment*, the NRC committee on improving risk analysis encouraged EPA to harmonize approaches for cancer and noncancer dose-response assessment (NRC, 2009, 194810), which involves uncertainty issues discussed in this section. Even more recently, EPA released a draft white paper, *Using Probabilistic Methods to Enhance the Role of Risk Analysis in Decision Making* (U.S. EPA, 2009, 522927). Although not focused specifically on quantitative uncertainty analysis, there is overlap with the issues treated here, and relevant insights are anticipated from ongoing efforts in this area.

6.1.2. Definition of Terms

For purposes of this study, the following definitions are adopted:[52]

Uncertainty Characterization. This consists of a *Structured Uncertainty Narrative* and, if the uncertainty is supported by quantitative models, *Quantitative Uncertainty Analysis*.

Structured Uncertainty Narrative. This identifies the assumptions conditional on which uncertainty is to be characterized and delineates the type of arguments with supporting evidence that buttress these assumptions.

Quantitative Uncertainty Analysis. This is a quantification of the uncertainty attending the use of quantitative models. It applies to a mathematical model of physical phenomena, some of whose parameter values are not known with certainty. A joint distribution is assigned to uncertain model parameters and propagated through the model to yield a joint distribution over the model output. Thus, a quantitative uncertainty analysis always has a joint distribution over model outputs as its result.

Joint Distribution/Marginal Distribution. For a set of uncertain quantities, a joint distribution is an assignment of probabilities (or probability densities) for each possible combination of values of these quantities. Each uncertain quantity has a marginal distribution, that is, an assignment of probabilities (or probability densities) to each possible value of that quantity. Assigning a marginal distribution to each quantity is not equivalent to assigning a joint distribution to the set of quantities, unless the quantities are independent; in this case the joint distribution is just the product of the margins.

[52]Many of these definitions are standard terms in probability and statistics, as described in Saltelli et al. (2000, 543756), Cox (2006, 594342), Kurowicka and Cooke (2006, 543758), and NRC (2007, 543748); some are reflected in current Agency practice (U.S. EPA, 2009, 522927).

Qualitative/Informal Uncertainty Analysis. This assembles the arguments and evidence and provides an assessment of their plausibility in terms of verbal modifiers. The meaning of verbal modifiers such as "likely/unlikely" or "plausible/implausible" in the natural language[53] is indeterminate and context dependent. The way in which these qualifiers combine in the natural language requires critical attention from a quantitative viewpoint. (For example, if A is likely and B is likely and C is likely, is A and B and C likely?) It is sometimes claimed that the probability formalism does not capture the way people reason with uncertainty, and many alternatives have been proposed.[54]

This is not the place to discuss foundational issues, except to remark that the practitioner wishing to depart from the standard probability formalism should carefully explore the whole range of alternatives and critically examine the operational meaning of the primitive notions.

Sensitivity Analysis. If a quantitative model uses "nominal values" (approximations of the real values) for various input parameters, a sensitivity analysis is performed by choosing different values for these parameters and re-running the model to assess the impact of changes in these parameters on model output. Applicable methods include one- and two-at-a-time methods, design of experiments and Morris's method (Saltelli et al., 2000, 543756). They aim at estimating first- and perhaps higher-order effects with a minimal number of model runs, by systematically varying the nominal values. In large uncertainty analyses, sensitivity analysis is used to screen variables for in-depth uncertainty quantification, and thus is part of a quantitative uncertainty analysis (Kurowicka and Cooke, 2006, 543758). As a note, the NAS committee report (NRC, 2006) does not distinguish between uncertainty and sensitivity analysis. In fields which have not developed a tradition in uncertainty quantification, the spread of values generated by a sensitivity analysis is sometimes presented as a representation of uncertainty (Murphy et al., 2004, 543741). The question of whether this is or is not the case is moot so long as the uncertainty on model input parameters is not quantified. Systematically varying input values is not the same as sampling input parameter values from their uncertainty distributions. In any event, a systematic approach to parameter variation is essential; simply choosing a few values of interest and generating different output is of limited scientific benefit and inevitably raises questions of selection bias. That said, if alternative values are commonly used and therefore recommend themselves, then running these through the models can help sensitize users to parameter variations and their impacts on model outputs.

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[53]*Natural language* denotes any discourse in which the meaning of the words is not formalized; rather, these words are just "as they come in off the street" with whatever meaning a participant may give them.

[54]Before the advent of personal computers, various shorthand techniques were developed for computing system risk. In control theory, schemes of 'interval probabilities' were proposed which could be propagated through a system to yield bounds on system reliability. Whereas these bounds originally reflected accuracy of shorthand approximations of complex formulae, their offspring have been proposed as quantifications of uncertainty. Alternative notions of uncertainty are also proposed with the goal of simplifying the assessment and computational burden or capturing putative features of uncertainty which are overlooked in probability theory. These include possibility theory, fuzzy numbers, qualitative algebra, imprecise probabilities, belief functions, certainty factors, and the like. Nonmonotonic reasoning systems attempt to capture reasoning about knowledge, or reasoning from partial knowledge; they include default logic, defeasible logic, abductive logic, and autoepistemic logic, to name a few.

Cognitive Uncertainty. This concerns uncertainty regarding what is the case. Not knowing "what is the case" may be conceived as uncertainty over the set of all possibilities, sometimes expressed as 'uncertainty over the set of possible worlds.' Uncertainty over possible worlds may be represented formally as probability; that is, the uncertainty of a given situation is represented as a number between zero and one, and the uncertainty of either of two mutually exclusive situations is the sum of the uncertainties of each situation.[55] Two interpretations or operationalizations of the probability formalism are current: the objective or frequentist interpretation and the subjective or Bayesian interpretation. These interpretations are not mutually exclusive, as subjective probabilities can and often do track relative frequencies.

Volitional Uncertainty. This concerns uncertainty regarding what to do. In the natural language, being unsure which course of action to choose is also called "uncertainty." Insofar as uncertainty on the best course of action can be translated into a claim about the state of the world, volitional uncertainty can be translated into cognitive uncertainty. For example, a regulatory body charged with setting a speed limit is obliged to make a decision. The decision may be cautious or reckless, well or poorly motivated, wise or foolish; but it cannot be true or false. Since the decision makes no claim about the state of the world, it cannot be uncertain in the cognitive sense. The uncertainty cannot be analyzed by sampling from some distribution. However, if the decision is based on the claim that the chosen speed limit minimizes accidents while maintaining a prescribed traffic volume, that claim may be uncertain and may be subjected to quantitative uncertainty analysis. A discretionary decision of a regulatory body may entrain cognitive uncertainty, but it becomes amenable for quantitative uncertainty analysis only when it is linked to a claim about the state of the world.

Aleatoric/Epistemic Uncertainty. This terminology has become standard in the technical uncertainty analysis literature, and it has been called *Variability/Uncertainty* in some areas, particularly dealing with human populations. A variable whose uncertainty is aleatoric for a given population takes different, uncertain, values for each member of the population. If its uncertainty is epistemic, it takes the same uncertain value for all members of the population. Issues involving uncertainty and variability or epistemic and aleatory uncertainty translate into issues of dependence, when conducting a quantitative uncertainty analysis (see Section 6.1.3.3). In its *Science and Judgment* report, NRC (1994, 006424) correctly remarks that "the amount of variability is generally itself an uncertain parameter." It is natural to ask whether a given uncertainty is aleatoric or epistemic, whereas it is awkward to ask whether this uncertainty is uncertain or variable—which explains the preference for the epistemic/aleatoric terminology.

[55]These are known collectively as Kolmogorov's probabililty axioms. The additivity of probability for exclusive alternatives states, e.g., that the probability of an unseen object being red or green is the sum of the probability that it is red and the probability that it is green. This of course assumes that "red" and "green" are clearly defined, such that nothing can be simultaneously red and green. Many alternative representations of uncertainty contest this additivity property.

6.1.3. Key Elements of a Quantitative Uncertainty Analysis

The uncertainty propagation can be performed by some rough estimation, as for example the delta method (Oehlert, 1992, 543742), or in rare cases it can be performed analytically, as in simple error propagation.[56] Most often, however, it will be performed using Monte Carlo simulation. A joint distribution is assigned to the parameters of a quantitative model and then propagated through the model by sampling repeatedly from this joint distribution, computing model output and generating a distribution of model output. Every uncertainty analysis is conditional on initial assumptions. A "complete" uncertainty analysis is an unattainable goal; the best that can be done in practice is to identify and motivate the assumptions that are used. This section is not a how-to guide, but a to-do list of key elements of any quantitative uncertainty analysis.[57]

6.1.3.1. *Quantitative Model*

The starting point of any quantitative uncertainty analysis is a mathematical model or procedure for calculating quantities of interest. A structured narrative explains the choice of quantitative models. If some values of input parameters for this calculation are not known with certainty, then the question arises: "What is the uncertainty attending the use of this model?" This is the question a quantitative uncertainty analysis seeks to answer.

6.1.3.2. *Marginal Distributions over Model Parameter*

If the model parameters are directly measurable with sampling error, then the sampling distribution may itself be used in the quantitative uncertainty analysis. If the model parameters are fit to data that are sampled from a known or hypothesized distribution, then by resampling this distribution and refitting the model, distributions over the model parameters may be constructed. Physically-based simulation models, such as pharmacokinetic models or environmental transport models, may be solved analytically if equilibrium reaction rates (the

[56]Simple measurement error is often represented by adding a normally distributed random variable with mean zero to a "true" value. If several measurements are performed in succession, and the errors on each measurement are assumed to be independent, then the error induced by adding the measurement results is also a normally distributed random variable whose mean is zero and whose variance is the sum of the variances on the individual measurements.

[57]These key elements of quantitative uncertainty analysis are discussed in many publications such as Saltelli et al. (2000, 543756), Cox (2006, 594342), Kurowicka and Cooke (2006, 543758), NRC (2007, 543748) and EPA (2009, 522927).

transfer coefficients) are constant. If these rates are not constant, as when concentrations are near saturation levels, then simulating the pharmacokinetics or transport is indicated. Structured expert judgment has been applied for uncertainty quantification within the engineering community since the time of the Rasmussen Report (U.S. NRC, 1975, 543729). More recently, this approach has been "test-driven" by EPA in assessing health effects of fine particulates (Walker et al., 1999, 198615), and its potential application has been identified in the Agency's *Guidelines for Carcinogen Risk Assessment*, commonly referred to as the Cancer Guidelines (U.S. EPA, 2005, 086237).[58]

6.1.3.3. *Dependence between Parameter Uncertainties: Aleatoric and Epistemic (Uncertainty and Variability)*

Two uncertain quantities are independent if knowledge about one of them does not alter our uncertainty regarding the other. The quantities are dependent if they are not independent. The role of dependence modeling in quantitative uncertainty analysis must be addressed. To illustrate, cigarette smoking and body fat are both thought to influence biomarkers for toxic response to dioxin exposure, such as ethoxyresorufin-*O*-deethylase (EROD) activity (Pereg et al., 2002, 199797). In an individual sampled at random from a target population, both percent body fat and whether (and how much) he or she smokes are uncertain.[59] However, these uncertainties are not independent, inasmuch as smokers tend to have less body fat (Vanni et al., 2009, 543754).

Issues involving uncertainty and variability, or epistemic and aleatory uncertainty, translate into issues of dependence when conducting a quantitative uncertainty analysis. For example, a constant used to estimate the biokinetic behavior of dioxin may be uncertain. If it is believed to be the same for every member of the population, the uncertainty is termed

[58]The EPA (2005, 086237) cancer guidelines state: "In many of these scientific and engineering disciplines, researchers have used rigorous expert elicitation methods to overcome the lack of peer-reviewed methods and data…." These cancer guidelines are flexible enough to accommodate the use of expert elicitation to characterize cancer risks, as a complement to the methods presented in the cancer guidelines. According to NRC (2002, 035312), the rigorous use of expert elicitation for the analyses of risks is considered to be quality science."

[59]Because dioxins generally distribute to body fat/lipid, the percent body fat is often used to estimate body burden; a default value of 25% is common (Connor and Aylward, 2006, 197632). However, body fat percentage varies widely between individuals, from a minimum essential level (e.g., 2% for men, 10% for women) to obesity (e.g., 38% or more for men, 42% for women). Considering that current estimates suggest 30% of the U.S. population are obese, an uncertainty analysis of dioxin risk in this population should sample individuals from their gender/body fat distribution and correlate this with other known or suspected covariates influencing toxic response (such as diet, smoking, natural and endogenous ligands, disease, and age).

"epistemic." In a quantitative uncertainty analysis, this factor would be sampled from its uncertainty distribution on each Monte Carlo run and used for *all* members of the population. Body fat, in contrast, is aleatoric. We do not sample one value from the body fat distribution and use this value for *all* members of the population on each Monte Carlo run. Instead we sample a body fat value for each individual on each run. Because body fat is correlated with other relevant variables (e.g., smoking, gender, age, and socioeconomic status), all of these variables should be sampled in a manner that reflects their dependences. Kinetic constants whose uncertainty is epistemic are completely correlated across individuals: if the value is 0.5 for one individual, it is 0.5 for everyone. Body fat values vary from individual to individual, and they are correlated through a host of other variables.

6.1.3.4. *Model Uncertainty*

All models, being idealizations, are false; on this there is no uncertainty to quantify. However, the choice of model may constrain the ability to represent uncertainty in observable phenomena. Thus, in a linear low-dose model, uncertainty over a cancer slope factor may be quantified, but uncertainty regarding changes in slope at distinct low-dose regimes cannot be captured. When the model choice imposes severe and potentially unwelcome constraints on uncertainty quantification, this must be addressed. Distributions over model parameters may be selected and evaluated based on their ability to reflect uncertainty distributions over observable phenomena predicted by the models.[60] In such cases, the uncertainty propagated through the quantitative model is not strongly model-dependent. In other cases, multiple model alternatives may be applied, whose "probability of being the true model" is known or assumed. Since different models can always be regarded as specializations of more general models, the distinction between parameter and model uncertainty is sometimes more apparent than real. For example, as illustrated in the EPA Benchmark Dose Software (BMDS) (U.S. EPA, 2000, 052150), the multistage and Weibull dose-response models both contain the model $Pr(x) = \gamma + (1 - \gamma)(1 - e^{-\beta 1x})$ as a submodel, to which they collapse if other parameters are zero (multistage) or one (Weibull). Recalling that the function $1/(1 + x)$ is first-order equivalent to $(1 - x)$ for

[60] Such techniques were first used on a large scale in the U.S. NRC-EU joint uncertainty analysis of consequence models for accidents at nuclear power plants, see Goossens et al. (1996, 548727; 2001, 548737; 2001, 548738; 2001, 548731; 2001, 548732; 2001, 548735) (Bock et al., 1998, 548752).

small x, the same may be said for logistic models as well. In this case, these models could easily be parameterized within one family, rendering the choice between them a choice of parameter values. Similarly, the choice between sub-, supra-, and linear models is sometimes reduced to parameter estimation within a more general class of model (Hoel and Portier, 1994, 198741).

In other cases, the reduction of model uncertainty to parameter uncertainty is less natural. For example, according to the "chemoprotection model" of Greenlee et al. (2001, 015400), dioxin's binding to the aryl hydrocarbon receptor (AhR) inhibits proliferation in tumor cells and thus suppresses mammary tumors. Dose-dependent protection and cancer induction can both be true, each applying to different tissues. Although mathematical models exhibiting these twin features have been suggested (e.g., Kohn and Melnick, 2002, 199104), these models are not yet readily estimable from data, and the choice between them is referred to the structured narrative.

6.1.3.5. *Sampling Method*

All sampling on a computer is "pseudo random." Significant issues arise in choosing a method for sampling high-dimensional distributions with dependence. If evaluating the quantitative model is very time consuming, various "quasi random" schemes may be applied, including Latin hypercube sampling, importance sampling, and Hammersley sampling. These methods involve trade-offs between economy and accuracy of the dependence modeling.

6.1.3.6. *Method for Extracting and Communicating Results*

When a large quantitative uncertainty analysis has been performed, the method for identifying important contributors and communicating this information to users is not straightforward. Analysts have proposed many ways to quantify the uncertainty contribution of one variable, or set of variables, on others,[61] and the analyst's decision at this juncture may strongly impact the "take-home" message from the study. An importance measure that averages

[61] A few examples may suffice. The standard Pearson correlation coefficient measures the linear dependence between two variables, positive or negative. The rank or Spearman correlation coefficient measures the monotone dependence. The correlation ratio measures the (unsigned) variance contribution of an explanatory variable on a target variable. The regression coefficient measures the expected change in standard (not natural!) units of a target variable, per standard unit change in an explanatory variable, and assumes this expected change is independent of the values of the explanatory variables. Multiple correlation measures the correlation between a given variable and its best linear predictor based on another set of variables. The partial correlation of two variables given a set of other variables is their correlation after discounting the influence of the other variables. The correlation ratio, multiple correlation, and the regression coefficient are not symmetric; the correlation ratio and multiple correlation are always non-negative (Kurowicka and Cooke, 2006, 543758; Saltelli et al., 2000, 543756).

over an entire sample space may obscure the features of real interest. For example, the drivers of cancer induction at low doses might be different from the drivers at high doses. If the drivers of low-dose cancer induction are of interest, then importance measures that average over all doses should not be considered.

6.2. EPA APPROACHES FOR ORAL CANCER AND NONCANCER ASSESSMENT

Different types of toxicity information have historically been used in EPA's oral cancer and noncancer dose-response assessments, although efforts to harmonize these approaches are ongoing. For oral exposures, noncancer endpoints are commonly assessed using the RfD methodology to derive "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime." In contrast, cancer endpoints are commonly assessed using a dose-response function with the probability of excess risk above background modeled as a linear function of dose, for doses down to zero. The RfD method relies on a POD. The cancer dose-response method uses a POD if the linear model is chosen. From the Cancer Guidelines, cancer endpoints can also be assessed using the RfD methodology if the proof burden is satisfactorily met (as described in Section 5.2.3.4.1.2).

Toxicity reference values have typically been derived for human noncancer endpoints based on a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) from animal bioassay studies. This terminology suggests a biological population threshold beneath which no harm is anticipated. Reference values such as the oral RfD or inhalation reference concentration are derived by applying uncertainty factors (UFs) to a POD. Depending on the nature of available data and modeling choice, a POD can be selected from values other than a NOAEL or LOAEL, such as an ED_x (effective dose eliciting x percent response), or a benchmark dose (BMD) or its lower confidence bound (BMDL). The BMD is the dose that induces a benchmark response (BMR), which is often chosen to represent a 5 or 10% increase in excess risk above background. The POD is divided by one or more uncertainty factors that represent knowledge gaps (see Section 6.4.1.2 for details on specific types of UFs).

An RfD is described as "likely to be without appreciable risk" but the probabilistic language has not as yet been operationalized. A quantitative definition of "appreciable" has not been articulated, and methods to compute risks above the RfD as a function of dose have not

been designated for use by the EPA; thus, it is not current practice to ascertain that the risk is indeed not appreciable. In addition, different participants in discussions over threshold/nonthreshold models for dioxin may have different perspectives regarding how to define "appreciable risk." Under the current POD/UF framework, dose-response functions are not provided for calculating the actual risk at or above the RfD. Instead, to provide a "risk indicator" for use in screening for health hazards, a hazard quotient (HQ) is computed as the ratio of a given oral exposure to the RfD, or a margin of exposure (MOE) is estimated as the ratio of the POD to the human exposure level.

For the cancer endpoint, an oral cancer slope factor may be derived for human health risk assessment, typically based on tumor incidence data from an animal bioassay or on cancer incidence or deaths from an epidemiologic study. In the EPA Cancer Guidelines, cancer is predominantly thought to have no population biological threshold and a linear extrapolation to zero is applied from the POD based on extra risk above background, i.e., the probability of the endpoint decreases linearly in dose from the POD to zero or to a population background level. In the absence of sufficient information on the cancer mode of action (MOA), the linear model is applied as a default. The linear model also can be applied when there is sufficient MOA evidence supporting this choice for low-dose cancer induction. Cancer endpoints could also be evaluated using a "nonlinear" model. In this case, the proof burden clearly rests on the nonlinear model; there must be sufficient evidence to override the health-protective default or scientifically-based choice of a linear model, as described in the Cancer Guidelines. These Guidelines state, "When adequate data on mode of action provide sufficient evidence to support a nonlinear mode of action *for the general population* (emphasis added) and/or any subpopulations of concern, a different approach—a reference dose/reference concentration that assumes that nonlinearity—is used." In current terminology, the RfD methodology applies to the cancer endpoint if there is sufficient evidence supporting a "zero slope at zero" model; otherwise, the linear nonthreshold model applies by default. (See Section 5.2.3 for a detailed discussion of linear vs. nonlinear extrapolations below the observed data, population vs. individual thresholds, and how the Cancer Guidelines are applied in choosing dose-response model forms for risk assessment.)

6.3. HIGHLIGHTS OF NAS REVIEW COMMENTS ON UNCERTAINTY QUANTIFICATION FOR THE 2003 REASSESSMENT

The NAS (2006, 198441; 2006, 543760) identified a number of uncertainty characterization issues for the 2003 Reassessment; key sources of uncertainty for which quantification is suggested are highlighted in Table 6-1. The discussion in this section focuses on comments related to dose response.

There are several nuances in the NAS position relative to the need for substantial improvement in transparency, thoroughness, and clarity in quantitative uncertainty analysis for the 2003 Reassessment. These nuances concern whether the nonlinear model (note that the NAS committee uses "sublinear" and "nonlinear" interchangeably) is scientifically better supported than the linear model, and if the sublinear model is better supported, whether this is based on data or on apodictic knowledge (knowledge without uncertainty) of the MOA. The NAS committee does not distinguish between individual and population dose-response models; however the criteria from the EPA Cancer Guidelines clearly apply to population models. Assuming that the AhR-mediated MOA implies a threshold for each individual, the step to a population "zero slope at zero" model requires the following, as identified and discussed in detail in Section 5.2.3.:

1. The distribution of the individual thresholds induced by the MOA, and
2. The dose-response function for values above the thresholds.

This information can either come from data or from known information of the MOA, but the burden of proof clearly rests on the nonlinear model. This section summarizes the NAS committee's overall positions. Responses to specific suggestions are given in Section 6.4 and summarized in Section 6.5. Several excerpts of specific comments from NAS (2006, 198441) illustrate key issues.

The NAS committee favors the nonlinear model with a threshold:

> …the committee concludes that, although it is not possible to scientifically prove the absence of linearity at low doses, the scientific evidence, based largely on mode of action, is adequate to favor the use of a nonlinear model that would include a threshold response over the use of the default linear assumption. *(p. 122)*

The committee does not state whether the threshold applies to the population, or whether each individual has his/her own threshold.

The NAS also comments on whether the nonlinear model should be used to compare with the linear default:

> Because the committee concludes that the data support the hypothesis that the dose-response relationship for dioxin and cancer is sublinear, it recommends that EPA include a nonlinear model for cancer risk estimates but also use the current linear models for comparative purposes. *(p. 16)*

The committee does not suggest what the (population/individual) threshold might be, nor how it might be supported on the basis of data. Rather, the apodictic knowledge that there *is* a (population/individual) threshold places the dioxin risk assessment within the RfD framework, using a HQ or MOE as the basis for indicating the potential risks from exposure. The committee further asks for a quantitative characterization of the range of uncertainty:

> The committee determined that the available data support the use of a nonlinear model, which is consistent with receptor-mediated responses and a potential threshold, with subsequent calculations and interpretation of MOEs. EPA's sole use of the default assumption of linearity and selection of ED_{01} as the only POD to quantify cancer risk does not provide an adequate quantitative characterization of the overall range of uncertainties associated with the final estimates of cancer risk. *(p. 24)*

Regarding the Cancer Guidelines' requirement of sufficient evidence to use a nonlinear approach for cancer risk assessment, the committee indicates that quantitative evidence will not decide the linearity/nonlinearity (nonthreshold/threshold) issue, but knowledge (without uncertainty) of the MOA should be used:

> Quantitative evidence of nonlinearity below the point of departure (POD), the ED_{01}[62] will never be available because the POD is chosen to be at the bottom end of the available dose-response data. ... EPA should give greater weight to knowledge about the mode of action and its impact on the shape of the dose-response relationship. *(p. 178)*

The comment continues, with the committee implicitly acknowledging that there is no evidence arguing against linearity, but that the lack of evidence should not justify using the linear model.

> The committee considers that the absence of evidence that argues against linearity is not sufficient justification for adopting linear extrapolation, even over a dose range of one to two orders of magnitude or to the assumption of linearity through zero, which would not normally be applied to receptor-mediated effects. *(p. 178)*

In addition, the committee recommended that EPA explore both linear and nonlinear approaches to TCDD cancer assessment:

> On the whole, the committee concluded that the empirical evidence supports a nonlinear dose response below the ED_{01}, while acknowledging that the possibility of a linear response cannot be completely ruled out. The Reassessment emphasizes the lack of such nonlinear models, hence its adoption of the approach of linear extrapolation below the POD level. Although this approach remains consistent with the cancer guidelines…., EPA should acknowledge the qualitative evidence of a nonlinear dose response in a more balanced way, continue to fill in the quantitative data gaps, and look for opportunities to incorporate mechanistic information as it becomes available. The committee recommends adopting both linear and nonlinear methods of risk characterization to account for the uncertainty of dose-response relationship shape below ED_{01} (*p. 72*).

In this document, EPA has applied its own guidance on cancer risk assessment and adopted linearity (and an assumption of no threshold) as a health-protective default approach in the absence of sufficient evidence of MOA involving a threshold for all tumors resulting from TCDD exposures (volitional uncertainty). (Note that the NAS report appears to view the absence of evidence as imposing a burden of proof on the linear model [cognitive uncertainty]; see Sections 5.2.3.4.1.2 and 6.2 regarding the burden of proof.) In addition, the NAS committee's request to apply nonlinear methods for the cancer assessment is addressed, in

[62] Eeffective dose (ED) is the dose corresponding to a X% increase (in this case a 1%) in an adverse effect such as a concer endpoint, relative to the control response.

Section 5.2.3.4.1.4 of this document. That evaluation describes the application of nonlinear methods to TCDD data and presents two illustrative examples of RfD development for carcinogenic effects: one based on tumorigenesis in experimental animals, and the other on hypothesized key events in TCDD's MOAs for liver and lung tumors.

The thrust of the NAS remarks regarding transparency, thoroughness and clarity in quantitative uncertainty analysis relevant to dose-response can be summarized as follows:

1. The uncertainty of cancer risks due to dioxin exposure should be quantified.
2. Dioxin cancer risk should be treated either as a threshold phenomenon, thus following the basic RfD methodology, or should be modeled using a sublinear dose-response function below the observed data, with the linear model used for comparison.
3. The POD should be subjected to quantitative uncertainty analysis.

A similar point of view has been indicated by others.[63] Detailed suggestions regarding specific improvements for quantitative uncertainty analysis in the 2003 Reassessment are outlined in the next section and summarized in Section 6.5.

6.4. FEASIBILITY OF CONDUCTING A QUANTITATIVE UNCERTAINTY ANALYSIS FOR TCDD

This section focuses on uncertainty analysis for TCDD dose response, which involves a range of issues as highlighted in Table 6-1.

6.4.1. Feasibility of Conducting a Quantitative Uncertainty Analysis under the RfD Methodology

This discussion applies to all noncancer endpoints of TCDD, and to cancer endpoints insofar as they fall under the RfD methodology. An RfD is obtained through the following steps:

1. Choose a POD, then
2. Apply uncertainty factors (UFs) to account for knowledge shortfalls.

[63]For example, from Popp et al. (2006, 197074). "Overall, the evidence indicates that (1) TCDD causes cancer via a receptor-mediated process; (2) this dose-response is non-linear; and (3) a threshold region exists for TCDD-induced cancer below which adverse effects are unlikely to occur."

The method of uncertainty factors harkens back to the engineering practice of safety factors (Lehman and Fitzhugh, 1954, 003195). To illustrate, if the reference load for an engineered structure is X, then engineers might design the structure to withstand load 3X, using a safety factor of 3 to create a margin of safety. If the structure functions in a corrosive environment, another factor could be multiplied to guarantee safety for that condition, and another factor could be applied for heat, another for vibrations, and so on. The choice of values is simply based on good engineering practice, i.e., reflecting what has worked in the past. Although safety factors are still common in engineering, they are giving way to probabilistic design in many applications. The reason is that compounding safety factors quickly leads to overdesigning. Compounding safety margins for spaceflight systems may render them too heavy to fly. As our understanding of a system increases, it becomes possible to guarantee the requisite safety by leveraging our scientific understanding of the materials and processes. That of course requires formulating clear probabilistic safety goals and developing the techniques to demonstrate compliance.

The engineering community has never sought to account for uncertainty by treating safety factors as random variables and assigning them (marginal) distributions; such an approach would not counteract the overdesigning inherent in safety factors. Many authors, including the recent national committee for *Science and Decisions* (NRC, 2009, 194810), have advocated just such a probabilistic approach to the apparent "overdesigning" of the RfD when multiple UFs are used in its derivation.

The NAS committee that evaluated the 2003 Reassessment does not discuss how to perform uncertainty analysis. But their call for substantial improvement in quantitative uncertainty analysis with TCDD falling under the RfD framework entails examining the *feasibility* of quantitative uncertainty analysis within this framework. (Note that the EPA Integrated Risk Information System (IRIS) database uses uncertainty factors without probabilistic interpretations; some context for this is offered in Section 6.4.1.2.)

6.4.1.1. *Feasibility of Conducting a Quantitative Uncertainty Analysis for the Point of Departure*

The POD plays a role in both the noncancer RfD methodology and the cancer dose-response methodology. The POD can be selected from various options, such as a NOAEL

or LOAEL, a BMDL, or an ED_x. The feasibility of quantitative uncertainty analysis for each of these three options is considered below.

By definition, the NOAEL is the highest of the tested doses in a toxicological experiment that is judged not to have caused an adverse effect (with dose expressed as a dose rate, in mg/kg-day). A quantitative uncertainty analysis for a NOAEL or LOAEL encounters the following problem. In an experiment involving a small, positive response, the probability of seeing no response can be calculated using a binomial model with the number of exposed animals and the observed number of responses. However, in an experiment with no response, the probability of having observed a response cannot be calculated without assuming a response probability. Such an assumption could not be based on observed data. The probability of a higher NOAEL or higher LOAEL can be computed, but not that of a lower NOAEL or LOAEL. In other words, the probability that an experiment with a positive result may have yielded a null response can be estimated, but not the probability that an experiment with a null response might have yielded a positive response.[64]

In addressing uncertainty quantification for a BMDL or ED_x, two questions must be distinguished regarding the response:

1. What is the distribution of possible doses that causes an x% increase over background?
2. What is the distribution for possible values of increase over background caused by a given dose?

The BMD is defined as the dose that realizes a BMR. It is an estimate from bioassay data that requires choosing a BMR and fitting a dose-response curve. The BMR, being a choice, is not amenable to quantitative uncertainty analysis, but the choice can be motivated in a structured narrative. The BMDL is the lower confidence limit on the dose that realizes a BMR (e.g., 95%) that can be found based on the uncertainty in the parameters of the dose-response relationship. Thus, the BMDL is addressed to the first question above, and represents in this case the 95% lower confidence band of the distribution of possible doses causing an x% increase over background. In the standard approach, the uncertainty captured by the BMDL is sampling

[64]The probability associated with a null response is often estimated by fitting a dose-response model to the data.

uncertainty *conditional* on the truth of the dose-response model. Different models might fit the data equally well yet lead to different BMDLs.

The BMDL is also influenced by the constraints imposed on the parameter fitting. Suppose that the slope is expected to be greater than one, and that the maximum likelihood estimate of the slope is slightly greater than one. Since the constraint is not binding, the constrained and unconstrained model would have the same Akaike Information Criterion and would be equivalent in this sense. However, computing the BMDL with the slope constraint can lead to very different values than without this constraint. In the latter case, slope values less than one contribute to the uncertainty in the dose causing the BMR (see Cooke, 2009, 543763).

The ED_x can also be taken as a POD. It is similar in spirit to the BMD; however, as used here, the term ED_x applies when the dose causing an x% extra risk over background has actually been observed, not estimated from a fitted dose-response model.[65] The observations are subject to sample fluctuations, and if the experiment on which the ED_x is based were repeated, different values might be found. It is helpful to consider a numerical example. Suppose a background response rate of 10% is established based on many observations of nonexposed individuals. In a given experiment, involving say 100 individuals given dose *d*, 14 individuals responded. The percent increase *x* over background (extra risk) is found by solving:

$$14/100 = 10/100 + x \times 90/100, \text{ or } x = 4.4\%.$$

We conclude that $d = ED_{4.4}$. We may assume that if the experiment were repeated with 100 new individuals sampled independently from the whole population, the response would be given by a binomial distribution with parameters (14, 100). The number of responses might be greater or smaller than four, there is a 16% chance of observing 10 or fewer responses. The response to dose *d* would not be distinguished from the background in that case, and a higher dose would be used for the POD.

The uncertainty analysis of ED_x as the POD involves addressing the second question above, without a quantitative dose-response model. A quantitative uncertainty analysis is hampered, however, by the possibility that dose *d* would produce a response less than or equal to

[65]This definition of ED_x is adopted to distinguish the modeled response (BMD) and the observed response (ED_x), and it is more restrictive than usages common in the literature.

the background, in which case the POD is indeterminate—another experiment with a different dose would be chosen as the POD. A true quantitative uncertainty analysis of ED_x as the POD would thus require a full bioassay experimental design, with binomial sampling of response rates at each dose level in the assay. Absent that, quantitative uncertainty analysis is not possible.

The interplay of choice and estimation ingredients in the POD depends on the type of POD. The main features of the above discussion are captured in Table 6-2. This table notes that the BMDL captures the uncertainty caused by sampling fluctuations *given* that the dose-response model is true. Other methods are available to compute the BMDL using (1) model-independent, observable uncertainty; (2) nonparametric Bayesian dose-response models; or (3) Bayesian model averaging (Cooke, 2009, 543763). Only the ED_x can be subject to a quantitative uncertainty analysis, and then only if a full bioassay data set is available.

6.4.1.2. *Feasibility of Conducting a Quantitative Uncertainty Analysis with Uncertainty Factors*

Uncertainty factors are chosen based on a structured narrative characterizing knowledge shortfalls involving the following issues:

1. Interspecies extrapolation (UF_A: from animal data to humans).
2. Intraspecies extrapolation (UF_H: to account for human interindividual variability, considering sensitive subgroups).
3. LOAEL to NOAEL extrapolation (UF_L: to estimate the dose corresponding to no adverse effect, from a reported LOAEL).
4. Subchronic to chronic extrapolation (UF_S: to estimate effects of chronic exposures, from a subchronic study).
5. Database deficiency (UF_D: to extrapolate from an incomplete data set, e.g., in terms of endpoints assessed or study design, i.e., from a poor to a sufficient or rich data context).

The standard chronic RfD can represent a sensitive human (H) response to a toxic substance under chronic (C) exposure conditions. Suppose a BMDL POD were based on animal (A) data from a subchronic (S) study. The database for that chemical could be rich (R), e.g., involving multiple (and at least sensitive) species/strains, both sexes, multiple life stages, with multiple endpoints observed under sound study designs. Or the data could be poor (P), with limited measurements from only a subchronic animal study (ASP) forming the basis for

estimating a general reference value for humans (including sensitive subgroups) under chronic exposure conditions. For that case, the UF method would be applied as follows:

$$RfD = \frac{ASP}{UF_A \times UF_S \times UF_D \times UF_H} \qquad \text{(Eq. 6-1)}$$

where UF_A, UF_S, UF_D, and UF_H are the uncertainty factors for extrapolating from animals to humans (UF_A), subchronic to chronic exposure conditions (UF_S), without adequate endpoint coverage (UF_D), and considering sensitive human subpopulations (UF_H). It is possible to assign distributions to the UFs in Eq. 6-1, and to perform a Monte Carlo analysis to produce a quantitative uncertainty distribution over the dose or value likely to be without appreciable risk to humans for chronic exposures. Many authors have proposed such an approach,[66] and the recent NRC (2009, 194810) report on science and decisions encourages EPA to move in this direction.

The idea of using a Monte Carlo analysis to develop quantitative uncertainty distributions for the RfD is simple, but the data on which the UFs are based and the assumptions that would need to be made should be further explored. For example, it is assumed that the extrapolation from subchronic to chronic exposure (UF_S) is the same whether applied to animals or humans, and whether applied to sufficient (rich) or deficient (poor) data contexts. Swartout et al. (1998, 093460) noted "Within the current RfD methodology, UF_S does not consider differences among species, endpoints, or severity of effects; the same factor is applied in all cases." In addition, due to the paucity of relevant human data, the same authors suggested the use of other endpoints as surrogates in estimating the extrapolation from animals to humans, UF_A. Further, few data exist

[66]There has been considerable work on giving a probabilistic interpretation of the UFs, including by Abdel-Rahman and Kadry (1995), Vermeire et al. (1999), Baird et al. (1996), Swartout et al. (1998, 093460), Slob and Pieters (1998, 087256), Evans and Baird (1998), Calabrese and Gilbert (1993), Calabrese and Baldwin (1995), Hattis et al. (2002, 548720), Kang et al. (2000, 548722), and Pekelis et al. (2003, 548723). These evaluations can be considered to frame what might be called a *random chemical* approach. Several authors adduce properties based on log normal distributions. Insightful studies by Kodell and Gaylor (1999;)(Gaylor and Kodell, 2000, 548724) suggest that uncertainty factors are independent log normal variables. Combining uncertainty factors involves multiplying the median values, and combining the "error factors" according to the formula $K_{S\times H} = exp[1.6449 \times \sqrt{(\sigma_S^2 + \sigma_H^2)}]$, where σ_S^2, σ_H^2 are the variances of $\ln(UF_S)$ and $\ln(UF_H)$. Thus $UF_S \times UF_H$ is a lognormal variable with Median($UF_S \times UF_H$) = Median(UF_S) × Median(UF_H), and 95th percentile given by Median($UF_S \times UF_H$) × $K_{S\times H}$. If U_S and U_H each have an error factor or 10, then the error factor of $UF_S \times UF_H$ is not 100 but 25.95. Several authors suggest that multiplying uncertainty factors might over-protect. Recent proposals from the National Research Council reflect the random chemical concept, and they inherit its problems (NRC, 2009, 194810).

in humans to accurately portray the interindividual variability represented by UF_H. Much of the data gathered to date on distributions of UFs have aggregated across other extrapolations; that is, data from subchronic to chronic ratios are aggregated over different species and different data contexts. Finally, it may be noted that an important issue is the data on which empirical distributions of response ratios are based. Brand et al. (1999, 007629; 2001, 543765) studied the sampling behavior of response ratios and raised concerns with regard to their informativeness.

Detailed analyses of the data underlying a Monte Carlo uncertainty analysis of Eq. 6-1 would afford the possibility of verifying at least some of the assumptions and numerical estimations such an analysis must make. Even if the assumption that the same UF_S is applicable for all species, endpoints, and effect severities is thought to be biological plausible, the question of whether a given set of chemicals reflects this assumption, and hence they are suitable for a Monte Carlo analysis of Eq. 6-1, can only be decided by data evaluation. Data are the ultimate arbiter of whether quantitative uncertainty analysis with uncertainty factors, as currently envisioned, has sufficient evidentiary support.

6.4.1.3. *Uncertainty Reduction Using Quantitative Data for Species Extrapolation*

Expressing dose in units of exposure that are more closely related to target tissue than to contact with administered feed (or an environmental medium) can reduce uncertainty in extrapolations of dose, route or species. This concept underlies EPA's establishment of the Inhalation Reference Concentration Methodology (U.S. EPA, 1994, 006488). Under this method, species differences in tissue exposure for inhalation toxicants serve as the basis for interspecies adjustments of dose. Likewise, the International Programme on Chemical Safety (IPCS) has established guidance for chemical-specific adjustment factors (IPCS, 2005), which also uses a measure of internal exposure (dose) to normalize (e.g., make equivalent) the dose between species. Certain more recent IRIS values also reflect such an approach, with data-derived extrapolation factors replacing default adjustments. Under such approaches, the relationship between external exposure and target tissue exposure is determined in each species, and the applied doses are normalized on the basis of the same level of the internal tissue exposure. One distinction between the two approaches is that the IPCS (2005) approach is based on the attainment of the same levels of the toxicant in the blood (the central compartment) rather than in the actual target tissue (a consideration based in part on the fact that typically the

only data available to evaluate a human toxicokinetic model will be venous blood concentrations, rather than concentrations in a responding tissue or organ). Further, it has been shown that species differences in internal dosimetry are more a function of species differences in blood solubility than differences in tissue solubility—that is, once distributed to blood, species differences in tissue exposure are less likely to be based on species differences in tissue solubility.

The approach to development of interspecies extrapolation factors for inter- and intraspecies extrapolation of effective dose for the oral RfD for dioxin, which is described in Sections 3 and 4 of this document, is in agreement with both of these approaches. All tissues in the body are exposed to dioxin via the bloodstream. Even in instances where the specific target tissues for observed effects may be other than the tissue where the effect is observed (e.g., effects mediated through the endocrine system), this biologically-based approach remains valid and reduces uncertainty in dose extrapolation. The approach to extrapolation of dosimetry—on the basis of circulating levels of dioxin in blood—makes optimal use of human exposure-response data, human biomonitoring data, and toxicokinetic modeling to estimate equivalent exposures for humans and test species without requiring that the target tissue be conclusively identified. The decision to base animal-to-human extrapolation on circulating levels of dioxin in blood, as predicted by a well-evaluated PBPK model, reduces some potential sources of uncertainty.

6.4.1.4. *Conclusion on Feasibility of Quantitative Uncertainty Analysis with the RfD Approach*

A quantitative uncertainty analysis of the POD is not feasible for PODs based on NOAELs or LOAELs. For the BMDL, such an analysis is not appropriate because the BMDL is already a quantile from an uncertainty distribution of the BMD. However, this uncertainty distribution can be obtained in different ways that capture different aspects of uncertainty. Quantitative uncertainty analysis is feasible if the POD is based on the ED_x (as defined above) and is supported by a full set of bioassay data. A quantitative uncertainty analysis based on a probabilistic interpretation of uncertainty factors in their present form invokes strong assumptions. The data on which the distributions of uncertainty factors are based could be used to check at least some of these assumptions.

6.4.2. Feasibility of Conducting a Quantitative Uncertainty Analysis for TCDD under the Dose-Response Methodology

Quantitative uncertainty analysis starts with a mathematical model and seeks to quantify the uncertainty attending the use of this model. Dose-response relations are mathematical models expressing the probability of response as a mathematical function of dose. For several decades, the uncertainty attending the use of dose-response models has been an abiding concern in many sectors, including the chemical and nuclear industries as well as the public health sector. Given a set of animal bioassay data, quantifying dose-response uncertainty may be approached in different ways. The differences reflect different types of uncertainty that are captured. A recent evaluation enumerates the following possible methodologies (Bussard et al., 2009, 543770):

Benchmark Dose Modeling (BMD): Choose the 'best' model, and assess uncertainty assuming this model is true. Supplemental results can compare estimates obtained with different models, and sensitivity analyses can investigate other modeling issues.

Probabilistic Inversion with Isotonic Regression (PI-IR): Define model-independent 'observational' uncertainty, and look for a model that captures this uncertainty by assuming the selected model is true and providing for a distribution over its parameters.

Non-Parametric Bayes (NPB): Choose a prior mean response (potency) curve (potentially a "non-informative prior") and a precision parameter to express prior uncertainty over all increasing dose-response relations, and update this prior distribution with the bioassay data.

Bayesian Model Averaging (BMA) (as considered here): Choose an initial set of models, and then estimate the parameters of each model with maximum likelihood. Use classical methods to estimate parameter uncertainty, given the truth of the model. Determine a probability weight for each model using the Bayes Information Criterion, and use these weights to average the model results.

The first of the above methods involves standard classical statistical methods and captures sampling uncertainty conditional on the truth of the model used. The other methods are "exotic" in the sense that they attempt to capture uncertainty that is not conditional on the truth of a given model. All have been subjected to peer review and published, but they do not enjoy the wide usage of the standard classical methods. The Bayesian models involve subjective choices of prior distributions. Insofar as the final result is largely independent of the choice of

prior, these methods conform to the current starting point of focusing on data-driven methods and not appealing to structured expert judgment. (Structured expert judgment can also be considered an exotic method; an explanation of this approach falls outside the scope of this report.)

A quantitative uncertainty analysis of TCDD capturing uncertainty in extrapolating data from animal bioassays to human reference values together with consideration of epidemiological data from studies of workers (routine exposures) or the general public (including dietary exposures and those reflecting discrete poisonings or accidental releases) would raise many issues. The major issues are summarized below.

6.4.2.1. *Feasibility of Quantitatively Characterizing the Uncertainties Encountered when Determining Appropriate Types of Studies (Epidemiological, Animal, Both, and Other)*

The risk assessor must choose the data set(s) that will serve as a starting point for dose-response modeling. With respect to TCDD, a wealth of animal bioassay data exist in the scientific literature, across species ranging from rats, mice, guinea pigs, and hamsters to mink, dogs and monkeys, and a variety of tissues, organs, and systems. In addition, a considerable amount of human data is available from clinical/case reports, accidental releases, and occupational exposures, including epidemiological data for several cohorts. As detailed in Sections 2, 4 and 5, some of the main sources of uncertainty in the TCDD epidemiological data include the healthy worker effect, confounding and exposure misclassification. Epidemiological data are usually attended with large uncertainties regarding the doses actually received by individuals. The difficulty in characterizing individual-level exposures largely stems from having limited internal measures of TCDD exposure, as biomonitoring data may only be available for one point in time or on a subset of the exposed population. Although there is little direct evidence of strong confounding in the cohorts of TCDD and dioxin-like compounds, some of the confounders that have been evaluated in a few of the epidemiological studies include gender, body mass index, age, cigarette and alcohol consumption, and hair and eye color (Baccarelli et al., 2005, 197053; 2006, 197036; Eskenazi et al., 2002, 197168; 2002, 197164; Pereg et al., 2002, 199797). As discussed in Section 5 on TCDD carcinogenicity, an additional limitation of the epidemiological evidence includes the lack of organ specificity, as many of the

studies have shown associations between TCDD exposure and all-cause mortality. With disagreement in the literature over the nature, scope, and quality of the epidemiological data for TCDD, given the lack of precedent for a multisite carcinogen without particular sites predominating, some have urged caution in the interpretation of the epidemiological data based on small relative risks Popp et al. (2006, 197074).

Despite these uncertainties, the EPA Cancer Guidelines express a clear preference for epidemiological studies over animal data. The question here is whether quantitative uncertainty analyses based on either a collection of bioassay data or on several epidemiological studies can be combined in some overall uncertainty assessment. Diverse human studies are sometimes combined into a meta-analysis, and the issues arising in this regard are instructive. A primary challenge of meta-analytical approaches is combining heterogeneous effects that may result from studies of different populations, study designs or analytical techniques. The question of whether uncertainty arising from combining such different studies can be taken into account in quantitative uncertainty analysis is similar to that of accounting for uncertainty due to missing covariates in Cox regression (see Section 6.4.2.2).

Existing standard statistical tools are insufficient to address this issue, as they quantify uncertainty in model parameters estimated from data. However, exotic methods, such as Bayesian methods, probabilistic inversion, or structured expert judgment may be applicable. These methods can be applied when a quantitative model *predicts* other phenomena, even though these phenomena could not be used to estimate the model. The question of whether such methods could remain sufficiently tethered to data, or whether structured expert judgment is unavoidable, is a subject for future research.

6.4.2.2. *Uncertainty in TCDD Exposure/Dose in Epidemiological Studies*

Uncertainties in epidemiological studies arise from a variety of study characteristics. There are many types of epidemiological study designs which determine the data structure, including intervention trials, case-control studies, cohort studies and cross-sectional studies. A variety of mathematical models some of these can be used to analyze epidemiological data; some of these includes Cox proportional hazard, Poisson regression, linear and logistic regression. The model outputs are based on different measures of association such as rate ratios, risk ratios, odds ratios, and standardized mortality ratios (SMRs, ratio of observed to expected deaths).

Exposure uncertainties often concern back-casted exposures based on current serum lipid concentrations, estimated/self reported dietary habits, fish consumption, placenta lipid concentrations, and other measures.

Uncertainty in exposure is often dealt with by coarsely grouping a cohort into exposed and unexposed groups. The output of such a study can be coarse grained in a similar way; instead of computing dose-dependent risk estimates, standard mortality ratios might be used to compare the exposed and unexposed groups. Packages computing the outputs routinely produce confidence intervals that reflect sampling fluctuations (e.g., can indicate the potential for chance to explain the association), assuming truth of the model. Additional uncertainty could be factored in with exotic methods. A significant issue in epidemiological studies is the effect of omitted covariates. Omitted covariates in Cox regression will bias the estimates of effects of included covariates. If the omitted covariates are independent of the included covariates, the bias is toward zero in absolute value (Bretagnolle and Huber-Carol, 1988, 543772); if the omitted covariates are not independent, little can be inferred.

With regard to individual studies, it might be possible to identify specific opportunities for uncertainty quantification. This is illustrated here using the study of Steenland et al. (2001, 198589) of more than 3,500 male workers exposed to TCDD-contaminated products at eight U.S. chemical plants. Each worker was assigned an exposure score based on an estimated level of contact with TCDD, the degree of TCDD contamination of product at each plant over time, and the fraction of a workday in contact with the product. For 170 workers, the serum TCDD levels were also measured. The serum levels were back-extrapolated to the last time of exposure using a constant biological half life, and regressed on the exposure scores. This regression model was used to predict the dose in all workers, and predicted dose was correlated with cancer mortality. Figure 6-1 shows a scatter plot of back-casted versus predicted TCDD serum levels for the 170 workers on which the regression was based.

Given a predicted TCDD level, the uncertainty on the back-casted TCDD value could be inferred from such data by various techniques. A key question is whether the actual cancer mortalities among 170 back-casted workers are randomly placed in the conditional distribution given predicted TCDD. Imagine, in other words, that the mortalities among the 170 back-casts are colored red in Figure 6-1. At any given level of TCDD prediction, are the red points evenly distributed, or are they shifted to the right? In principle, the correlation between mortality and

back-casted TCDD level, given the predicted level, could be estimated. This amounts to estimating heteroscedasticity in the regression model.[67] Then, for each of the 3,538 workers, given his predicted TCDD level, we could sample a back-casted TCDD level, appropriately correlating with mortality, and recompute the dose response analysis. Repeating this many times we could build up a distribution for excess lifetime cancer mortality risk.

It is instructive to step through similar issues with regard to biological half life, background and body fat. The Steenland et al. (2001, 197433) analysis assumed a constant TCDD biological half life (8.7 years). A distribution over this half life could plausibly be developed from published sources. Assuming this half life is constant for all workers, but uncertain (epistemic uncertainty), this distribution could easily supplement the previous distribution: first sample a half life (to be applied to all workers), then estimate the regression model for this half life, and sample back-casted TCDD levels given each worker's exposure score, taking account of correlation with mortality. This works if the half life uncertainty is epistemic. However, since the half life is estimated from data, it is more reasonable to suppose that the half life varies from worker to worker (aleatoric uncertainty). Here again the correlation with mortality must be taken into account, indeed it seems reasonable to suppose that the 256 cancer deaths involved workers with longer half lives. However, there is no way ex post of determining the biological half life in the deceased workers.

The potential impact of uncertainty regarding background exposure and body fat is likely similar to the uncertainty of estimating the half life of TCDD. Steenland et al. (2001, 197433) held the background level constant at the median level (6.1 ppt, range 2.0 to 19.7) for 79 nonexposed workers from whom blood was also drawn (see also Section 6.4.2.4). The full distribution of TCDD levels for these nonexposed workers could be used as well. Is it reasonable to suppose that responsive workers (i.e., those exhibiting the response) have background levels that are sampled randomly from this distribution, or might they not plausibly come from the high end of the distribution? The analysis also assumed a constant percentage of body fat (30%), whereas body fat percentage varies in the general population, e.g., for men this has been reported to range from 2 to 38% or more (see Footnote in Section 6.1.3.3). The body

[67] Heteroscedasticity occurs when the variance of the dependent variable in a regression analysis varies across the data, violating the assumption of equal variance commonly used in many regression models.

fat distribution in the worker population could have been ascertained, but again the question arises, are the responsive workers sampled randomly from this distribution?

These three factors, variable half life, variable background, and variable body fat percentage, might combine to make the effective dose level among the responsive workers significantly higher than would appear in a study that assumes these factors to be constant. However, such concerns cannot be addressed in a quantitative uncertainty analysis, unless cancer mortality can be correlated with these variables. In an optimal study design, this information could be retrieved from the data. However, in most observational epidemiological studies such data are not available, and it might be possible to estimate these correlations in some other defensible manner, in which case the effect of exposure uncertainty could be quantified and propagated. Such an analysis would involve substantial effort and should not be undertaken under assumptions that are themselves implausible. Protocols for epidemiological studies do not currently require such uncertainty quantification. In any event, Steenland et al. (2001, 197433) should be recognized for conscientiously identifying these key issues.

6.4.2.3. *Uncertainty in Toxicity Equivalence (TEQ) Exposures in Epidemiological Studies*

Toxicity equivalence factors (TEFs) are used to infer the health effects of dioxin-like compounds based on their relative potencies compared to TCDD. These factors are not known with certainty, and the question arises whether uncertainty in TEFs can be incorporated into a quantitative uncertainty analysis. The process of deriving TEFs applied by the World Health Organization (WHO, 2005, 198739) is described in Van den Berg et al. (2006, 543769). Distributions of relative potencies (REPs) were developed from the scientific literature, with preference for in vivo studies, as supplemented by in vitro studies. An expert panel used a consensus process to select a TEF value for each congener, in half log steps "Thus, the TEF is a central value with a degree of uncertainty assumed to be at least ± half a log, which is one order of magnitude. However, it should be realized that TEF assignments are usually within the 50th to 75th percentile of the REP distribution, with a general inclination toward the 75th percentile in order to be health protective" (Van den Berg et al., 2006, 543769) (see Figure 6-2 of this document).

The WHO considers the uncertainty in TEFs to span one order of magnitude (presumably log uniformly distributed). It would be tempting to use the distributions in Figure 6-2 to quantify

uncertainty in the TEFs in a quantitative uncertainty analysis. However, the issue of dependence in this case is daunting. For example, should values of 1,2,3,7,8,-pentachlorodibenzofuran and 2,3,4,7,8-pentachlorodibenzofuran be sampled independently? The choice of dependence structure will have a large effect. As described by (Van den Berg et al., 2006, 543769), the differences in REPs reflect differences in dosing regimens, species, endpoints, mechanisms, and calculation methods. In a quantitative uncertainty analysis one must insure that these are not double counted.

> Reasons for significant differences in REPs for the same congener can be caused by the use of different dosing regimens (acute vs. subchronic), different endpoints, species, and mechanisms (e.g., tumor promotion caused by at least two different mechanisms as for mono-*ortho*-substituted PCBs), as well as different methods used for calculating REPs. Thus, different methodological approaches used in different studies clearly provide uncertainties when deriving and comparing REPs. If future study designs to derive REPs were more standardized and similar, the variation in REPs when using the same congener, endpoint, and species might be expected to be smaller (Van den Berg et al., 2006, 543769).

Although the TEFs themselves and the distributions underlying them are based on expert judgment, it is possible to incorporate these into a quantitative uncertainty analysis; however, it is not simply a matter of taking the distributions in Figure 6-2 to predict the results, with uncertainty, of exposure to dioxin-like compounds. The issues of dependence and double counting must first be addressed. Inasmuch as the distributions are the result of expert judgment, this would reasonably involve structured expert judgment as well. (Procedures for this type of assessment have been developed and applied, and it would entail a significant level of effort.)

6.4.2.4. *Uncertainty in Background Feed Exposures in Bioassays*

TCDD is not produced intentionally but rather is formed as a byproduct of volcano eruptions, forest fires, manufacturing of steel and certain chemicals (including some pesticides and paints), pulp and paper bleaching, exhaust emissions, and incineration. It enters the food supply primarily via aerial transport and deposition of emissions, and it bioaccumulates in animal fat. In general, food of animal origin contributes to about 80% of the overall human exposure. For example, Schecter et al. (1997, 198396) measured dioxins in pooled food samples collected in 1995 from supermarkets across the United States. Reported as parts per trillion (ppt) toxicity

equivalences (TEQs), fresh water fish had the highest level (1.43); followed by butter (1.07); hotdog/bologna (0.54); ocean fish (0.47); cheese (0.40); beef (0.38); eggs (0.34); ice cream (0.33); chicken (0.32); pork (0.32); milk (0.12); and vegetables, fruits, grains, and legumes (0.07). More recent exposure studies indicate dietary levels have decreased over time. Values reported for the early 2000s by Lorber et al. (2009, 543766), in ppt TEQ, are: fish (0.33); beef (0.12); dairy, other than milk (0.079); eggs (0.06); pork (0.036); poultry (0.018); other meat (0.058); and milk (0.012).

These results illustrate that a person's dietary intake of dioxins depends on the relative intake of foods with high or low levels of contamination, and human background levels will vary accordingly. The same applies to experimental animals in bioassays, although in those cases the background intake can in principle be controlled. Some of the effects of TCDD and other AhR agonists in enhancing the early initiation stages of cancers are considered to occur as a result of prenatal exposures that are not included in the standard National Toxicology Program (NTP) bioassay protocol (Brown et al., 1998, 051311; Muto et al., 2001, 548713). Further, to enhance reproducibility and keep statistical fluctuations to a minimum, the standard NTP assays are deliberately run on groups of animals that are relatively uniform genetically, fed uniform diets, and have the minimum possible exposures to toxicants other than the agent(s) being tested. This tends to reduce the potential for observing the consequences of potential interactive effects that might occur in the diverse human population with its variety of dietary and other exposures to a wide range of potentially interacting substances and conditions.

A critical question is the extent to which the background exposure influences the dose-response curve, and how this background should be taken into account. One idea, articulated in the recent NRC (2009, 194810) report on science and decisions, involves an "interacting background."[68] This can be implemented by computing a virtual dose B which, according to the selected dose-response model, would explain a chosen fraction of the background response. If the chosen model for dose δ is $f(\delta)$, the model can be adapted to

[68]"Effects of exposures that add to background processes and background endogenous and exogenous exposures can lack a threshold if a baseline level of dysfunction occurs without the toxicant and the toxicant adds to or augments the background process. Thus, even small doses may have a relevant biologic effect. That may be difficult to measure because of background noise in the system but may be addressed through dose-response modeling procedures" (NRC, 2009).

account for an interacting background by writing $f^*(\delta) = f(\delta + B) - f(B)$. This can alter the model's behavior at zero dose.

For example, if $f(\delta) = \delta^n/(\delta^n + EC_{50}^n)$, the derivative $d(f)/d(\delta)$ is $n\delta^{n-1}EC_{50}^n/(\delta^n + EC_{50}^n)^2$, which goes to zero as $\delta \rightarrow 0$, if $n > 1$. However, replacing δ with $(\delta + B)$ evidently changes the derivative at zero to $nB^{n-1}EC_{50}^n/(B^n + EC_{50}^n)$. This model is not yet estimable from data, as we have no way of choosing from the available animal data the fraction of background response to be explained by the model when applied to humans (although judgments could be made if we had better information about the details of the processes that are involved in causing various human health effects). However, as a conceptual model, it serves to remind us that the manner of accounting for background exposures can influence a model's behavior in the low-dose region. (Note that sensitivity analyses can be done showing the consequences of assuming different amounts of interacting background within the context of a specific nonlinear model.)

6.4.2.5. *Feasibility of Quantifying the Uncertainties Encountered When Choosing Specific Studies and Subsets of Data (e.g., Species and Gender)*

Species, strain, gender, life stage, and other characteristics of experimental animals are selected for a given study based on previous knowledge (e.g., of the species sensitivity, availability of strains having little genetic variation for the endpoints in question, relevance of the MOA, and degree to which the endpoints are similar for humans). Many other decisions are made in designing a bioassay study; will the animals be sacrificed at the termination of the study (if not a lifetime study), or will they be allowed to live out their natural lives? What dosing regimen should be applied? How will the animals be fed and handled? Although such questions may engender uncertainty in the minds of the experimenters, and reviewers; such uncertainty is not amenable for quantitative uncertainty analysis unless and until there are quantitative models, with parameters estimable from data, that can predict the effect of these choices on the response function.

6.4.2.6. *Feasibility of Quantifying the Uncertainties Encountered when Choosing Specific Endpoints for Dose-Response Modeling*

Standard experimental protocols guide the selection of exposure/dosing conditions for a given bioassay, including the amount, delivery vehicle, route, timing, dosing frequency and

duration, and dose spacing. The goal is to find the dose range where the experimental animals begin to respond adversely, to help anchor the lower end of the dose-response relationship, and to avoid multiple experiments in which all or none of the animals respond. A common recommendation is that the dose levels be chosen such that the increments in probability of response are roughly equal. Hence, the choice of endpoint, dose spacing, and number of animals should be made with these factors in mind. Of particular importance is the number of animals at each dose level in relation to the choice of endpoint and probability of response. Using more animals at the lower dose levels increases the probability of seeing some animals respond; on the other hand, it will give higher weight to the low-dose responses in model fitting and uncertainty quantification. Including many low-dose groups in a study with no expected response can produce a bias in the event of model mis-specification (see Text Box 6-1). The conclusion with regard to the feasibility of this quantitative uncertainty analysis echoes that of the previous paragraph: such uncertainty is not amenable for quantitative analysis unless and until there are quantitative models, with parameters estimable from data, that predict the effect of these choices on the response function.

6.4.2.7. *Feasibility of Quantifying the Uncertainties Encountered when Choosing a Specific Dose Metric (Trade-Off between Confidence in Estimated Dose and Relevance of MOA)*

The concept of dose is not straightforward. To review, the Cancer Guidelines provide the following taxonomy:

- *Exposure* is contact of an agent with the outer boundary of an organism.
- *Exposure concentration* is the concentration of a chemical in its transport or carrier medium at the point of contact.
- *Dose* is the amount of a substance available for interaction with metabolic processes or biologically significant receptors after crossing the outer boundary of an organism.
- *Potential dose* is the amount ingested, inhaled, or applied to the skin.
- *Applied dose* is the amount of a substance presented to an absorption barrier and available for absorption (although not necessarily having yet crossed the outer boundary of the organism).

Text Box 6-1. Model Mis-Specification and Maximum Likelihood Estimation.

The maximum likelihood estimate (MLE) is widely used in statistics because of its attractive properties: *If* the true model generating the data is from the class whose parameters are being estimated, *then* under regularity conditions, the expected MLE converges to the true value, and its variance converges to zero. The caveat against what is called "mis-specification" is very important and easily overlooked. An illustration can be extracted from the NTP (2006a) data for female rat tumor incidence of cholangiocarcinoma, representative of the data which persuaded the NAS committee that the cancer dose response for dioxin was "sublinear."

NTP (2006a) Female Rat Tumor Incidence Data for Cholangiocarcinoma					
Blood concentration (ng/kg)	**2.56**	**5.69**	**9.79**	**16.57**	**29.70**
Number exposed	48	46	50	49	53
Number responding	0	0	1	4	25
Relative frequency	0	0	0.02	0.08	0.47

The Hill model with MLE in this case has zero slope at zero. The default Linear Low Dose (LLD) model fits a Hill model to doses with positive responses, but it extrapolates linearly from the lowest observed nonzero response frequency. Both models have the same two parameters, but the parameter values of the Hill model used in the LLD model are different from those in Hill model fit to all doses, including doses with zero response. Although the null responses are expected on the LLD model, the Hill model has greater log likelihood since it gives higher probability to the null responses (see below).

NTP (2006a) Female Rat Tumor Incidence Data for Cholangiocarcinoma: Low-Dose Linear and Hill Models					
Blood concentration (ng/kg)	**2.56**	**5.69**	**9.79**	**16.57**	**29.70**
Number exposed	48	46	50	49	53
Response probability: Linear Low Dose (LLD)	0.005	0.012	0.014	0.09	0.47
Response probability: Hill model	0.00009	0.0017	0.013	0.09	0.47
Probability of cohort null response: LLD	0.77	0.58			
Probability of cohort null response: Hill	0.99	0.92			
Log Likelihood	**LLD**		2.46		
	Hill		2.16		

Suppose, for the sake of illustration, that the data were generated with the response probabilities from the LLD model. The Hill model would be mis-specified in this case, as the model generating the data is not a Hill model. Because of the small cohort size, the probability of null responses is such that the Hill model has greater likelihood than the LLD model with probability (based on bootstrapping) about 0.43, even though the latter, by construction, is the true model. Averaging over many simulated responses from the LLD model, the Hill model underestimates the response probabilities for doses 2.56 and 5.69 by factors of 7.5 and 2.1 respectively. In the event of such mis-specification, the bias in the Hill model would be aggravated by including more 50-rat experiments with doses lower than 2.56.

- *Absorbed dose* is the amount crossing a specific absorption barrier (e.g., the exchange boundaries of skin, lung, and digestive tract) through uptake processes.
- *Internal dose* is a more general term, used without respect to specific absorption barriers or exchange boundaries. *Delivered dose* is the amount of the chemical available for interaction by any particular organ or cell

Due to their greater causal proximity to the affected organs, using the absorbed dose or internal dose would yield statistically more powerful results and enable more precise predictions than potential dose. If it is not possible to measure these or they were not measured during the conduct of the study (as is commonly the case), then other available dose metrics, such as potential dose or exposure, are used. Due to toxicokinetic variability, different individuals receiving the same exposure may not have the same absorbed dose. Hence, use of either exposure or exposure concentration adds variability to the predicted results. The dose metric should be selected that (1) has the most proximate possible causal relation to the production of an adverse health endpoint, and (2) can be readily related to the units of (external) exposure that will be the basis for assessing human exposures.

6.4.2.8. *Feasibility of Quantifying the Uncertainties Encountered When Choosing Model Type and Form*

The EPA (2009, 522927)draft white paper on probabilistic methods notes: "There is no consensus on any one well-accepted general methodology for dealing with model uncertainty, although there are various examples of efforts to do so." Model uncertainty was introduced in Section 6.1.3.4. Many statistical techniques are available to evaluate model adequacy or to choose a "best" model. Although it is tempting to qualify such deliberations as "uncertainty that a model is true," one must remember that all models, being idealizations, are false. Ultimately, one is interested in uncertainty with regard to observable phenomena, not with regard to models. Models are merely tools for describing the phenomena. Nonetheless, the choice of a model constrains the ways in which uncertainty can be represented, so the question is how to deal with these constraints. A recent study of uncertainty modeling in dose response (Cooke, 2009, 543763) addresses precisely this issue and provides technical details to frame possible options.

Before exploring exotic approaches to model uncertainty (i.e., those not yet widely used in dose-response analyses), one feature in the standard statistical treatment of uncertainty must

be appreciated. Consider a model based on experimental data, typically bioassay data, in which a certain number of study subjects are exposed to varying doses of a test substance, and in which the numbers of subjects exhibiting a response are tallied. Values for the parameters in the model are chosen by the principle of maximal likelihood: those values are chosen which render the data as likely as possible. According to standard practice, a model is chosen that best fits the data according to one of the accepted criteria, such as reduced R^2, or the Akaike Information Criterion. There might be many incompatible models that are nearly as good.

One can ask the following: If the experiments on which the model is based were repeated, sampling the same number of experimental subjects from the distribution posited by the model, how much could our parameter estimates change? This is described by a joint distribution over the model's parameters, which captures sampling uncertainty under the assumption that the model is true. Now, all models are false, and as our sample sizes grow the lack of fit in the model becomes increasingly apparent. At the same time, the sample fluctuations in parameter estimates—*assuming the model is true*—become smaller and smaller. In very large epidemiological studies, standard statistical methods can produce razor-thin confidence bands in this way, which fail to capture experts' uncertainty regarding observable phenomena.[69]

The exotic methods sketched in the beginning of Section 6.4.2 may be viewed as attempts to deal with this feature. Probabilistic inversion methods were deployed on a large scale in the joint U.S. NRC-EU uncertainty analyses noted in Section 6.1. Distributions over model parameters are intended to capture an antecedently defined uncertainty over observable phenomena predicted by the model. This method was applied in dispersion and deposition modeling and further environmental transport models (including uptake) for radionuclides. In most cases, the observable uncertainty was based on structured expert judgment, but it has also been based on binomial uncertainty in bioassay studies. A potential drawback is that it may not prove possible to capture the observable uncertainty in this way with a classically best-fitting model, and new models may be required.

Nonparametric Bayesian methods arose in the biomedical and reliability fields. They start with a prior distribution over all nondecreasing dose-response functions, and update these

[69]See, for example, Tuomisto et al. (2008, 548715, Table 6) for a comparison of experts' uncertainty in health effects of fine particulates with uncertainties derived from sampling uncertainty from large epidemiological studies. Although the experts generally agree with the studies' central estimates, their uncertainty bands are often much wider than those surrounding the published estimates.

with observations from a bioassay. No further assumptions regarding parametric form are introduced, but the prior distribution remains important for doses outside the range of observations. Bayesian model averaging starts with a prior distribution over a set of candidate models, and updates this distribution with bioassay data. The method is flexible and intuitive, though attenuation of the effect of the prior on the posterior must be verified.

All these approaches represent attempts to capture "extramodel uncertainty," that is, uncertainty that is not conditional on the truth of the model. This is an active research area, and improvements in methods for capturing extramodel uncertainty in quantitative uncertainty analysis are anticipated. A major effort with regard to TCDD dose-response would be indicated when the strengths and weakness of the exotic methods are well understood.

6.4.2.9. *Threshold MOA for Cancer*

The NAS committee avers that knowledge of the AhR binding MOA implies that there is a response threshold for TCDD cancer induction. The differences between individual and population thresholds are not discussed, but the following two possibilities are distinguishable:

1. The threshold is the same for each individual; since human variability in AhR binding affinity is rather large (see Section 5.2.3.3), this entails that the threshold is not affected by the binding affinity.
2. The threshold varies across individuals and is related to the individual AhR binding affinity.

These two positions are different. As shown in Section 5.2.3 it is quite possible that each individual in a population has a threshold, whereas the population dose-response relation is linear. Because the NAS committee does not distinguish which of these positions it holds, the feasibility of quantitative uncertainty analysis is examined here for both.

i. Quantitative uncertainty analysis concerns a mathematical model. In case (1), this model would show how the existence of the AhR binding would induce a threshold, independently of the strength of the binding. Assessing the feasibility of quantitative uncertainty analysis must await the elaboration of such a model.

ii. In case (2), it must be shown that the distribution of thresholds, and the dose-response function above the threshold, are able to induce a population "zero slope at zero dose" (ZS@Z) model. Recall, the burden of proof is on this (ZS@Z) model. Scoping the

population variability with regard to AhR-mediated mechanisms in general, and dioxin sensitivity in particular, is an active area of research. It involves phenotyping human AhR-mediated responsiveness and relating this to polymorphisms in the human population. Harper et al. (2002, 198124) report that a 10-fold variation in binding affinity of AhR for TCDD in human placental tissue did not reveal any polymorphisms, suggesting that the relation between phenotypical and genotypical variation is tenuous. Tuomisto et al. (1999, 548717) demonstrate large variations in efficacy in two rat strains whose binding affinity is similar (Long-Evans, K_d = 3.4, Han/Wistar, K_d = 3.9 (as also discussed in Connor and Aylward, 2006, 197632)), and they also show that this variation is endpoint-specific. The responses in both strains are similar for cytochrome P450 (CYP)1A1 induction, but very dissimilar for thymus atrophy, serum bilirubin, and mortality. Toide et al., (2003, 548792) suggest that common biochemical measures of EROD activity might be mediated by CYP1B1 and CYP1A2. The differences in serum bilirubin at doses around 10 µg/kg are about a factor of 30. Han/Wistar rats seldom die at this dose, while mortality of Long Evans rats is about 50%. The mechanisms are not understood.

Although the mass action dose-response model does not have a threshold, it is possible that certain enzymes block the receptor binding, and until these are overwhelmed, no response occurs. The availability of such enzymes may vary from individual to individual, and may or may not covary with the dissociation constant, K_d. Pursuing these lines of research may result in a convincing demonstration of a population (ZS@Z) model. Such a model would express the individual threshold in terms of parameters that could be estimated with uncertainty from the data.

6.4.2.10. *Feasibility of Quantifying the Uncertainties Encountered when Selecting the BMR*

The NAS committee explicitly requested that the uncertainty attending the choice of a BMR be quantified. Although selecting relevant alternative values for the BMR may provide information of interest, it does not constitute a quantitative analysis of uncertainty. The alternative values must be sampled from some uncertainty distribution. Since this concerns volitional uncertainty, there is no underlying distribution from which to sample, unless the choice of BMR is related to some claim about the state of the world.

However, in response to the NAS concerns, this document provides some limited quantitative comparisons of BMR choices. BMDs, BMDLs and OSFs from the animal cancer bioassay benchmark dose modeling assuming 1, 5, and 10% extra risk are compared in units of blood concentrations and human equivalent doses in Tables 5-18 and 5-19, respectively. In

addition, MLE and upper bound slope factor estimates based on Cheng et al. (2006, 523122) are presented (see Tables 5-3 and 5-4). For the noncancer effects, key animal study PODs (ng/kg-day) are shown based on different dose metrics: administered dose, first-order body burden HED, and blood concentration (see Tables 4-3 and 4-4).

6.5. CONCLUSIONS REGARDING THE FEASIBILITY OF QUANTITATIVE UNCERTAINTY ANALYSIS

In this section the main conclusions regarding the feasibility of quantitative uncertainty analysis are summarized in relation to specific suggestions made by the NAS committee (see Section 6.5.1). Following this, a suggested research agenda for moving forward in this area is provided (see Section 6.5.2).

6.5.1. Summary of NAS Suggestions and Responses

On page 130 of their report (NAS, 2006, 198441), NAS makes specific suggestions regarding uncertainty quantification. These are reformatted and presented in italics below. Following each suggestion, a summary of the discussion in this section is given, with reference to the section in which it is addressed.

EPA should have addressed quantitatively the following sources of uncertainty:

- *Basis for risk quantification:*
    1. *bioassay data,*
    2. *occupational cohort data.*

Response: (1) Classical statistical methods yield distributions on model parameters which reflect sample fluctuations, assuming that the model is true. This type of uncertainty is taken into account in the BMDL. Exotic methods can account for uncertainty which is not conditional on the truth of a model, at least for bioassay data (see Section 6.4.2). (2) For epidemiological data, the dose reconstruction often involves assumptions which may support data driven uncertainty analysis, if sufficient data can be retrieved. Examples discussed above include back-casted TCDD level, biological half life, body fat and background (see Section 6.4.2.2). Uncertainty in the choice of bioassay data sets or choice of occupational cohort data sets is volitional, and is not quantified by sampling an input distribution. To be amenable for quantitative uncertainty analysis, the choice must be linked to a statement about the state of the world (see Section 6.1.1).

- *Epidemiology data to use:*
    1. *risk estimate developed with data aggregated from all suitable studies,*
    2. *risk estimate or estimates developed using each study individually.*
- *Factors affecting extrapolation from occupational to general population cohorts, including differences in baseline health status, age distribution, the healthy worker survivor effect, and background exposures.*

Response: (1) Quantitative uncertainty analysis based on meta-analysis data poses challenges owing to differences in study protocols. Exotic methods might take us further, the question is whether the restriction to data driven methods (as opposed to expert judgment or Bayesian methods) could be maintained (see Sections 6.4.2.2 and 6.4.2.3). (2) If the general population is characterized by distributions over known confounders whose coefficients are estimated from the epidemiological studies, then uncertainty over these coefficients can be extracted with the methods mentioned in Section 6.4.2.1. Uncertainty due to missing covariates is intractable for data driven uncertainty analysis (see Section 6.4.2.2).

- *Bioassay data to use:*
    1. *risk estimate developed with the single data set implying the greatest risk (that is, single study, tumor site, gender),*
    2. *risk estimate developed with multiple data sets satisfying an a priori set of selection criteria.*

Response: (1) Uncertainty in choice of data sets is volitional and is not quantified by sampling an input distribution. To be amenable for quantitative uncertainty analysis the choice must be linked to a statement about the state of the world (see Section 6.1.1). (2) The issue here is similar to the meta-analysis addressed in (2.a).

- *Dose-response model:*
    1. *linear dose response,*
    2. *nonlinear dose.*

Response: (1) When low dose extrapolation is done using a linear model by default, the uncertainty is volitional. To be amenable for quantitative uncertainty analysis, the choice must be linked to a statement about the state of the world (see Section 6.1.1). The ED_x as POD for the linear extrapolation can be subjected to quantitative uncertainty analysis, if based on sufficient bioassay data. (2) With respect to nonlinear dose response, it is possible that human thresholds exist, and that the distribution of thresholds can be characterized in the human population. In as much as the mechanisms for this are not yet understood, there is no quantitative model expressing threshold as a function of parameters which could be estimated, with uncertainty, from data. This currently limits the application of uncertainty quantification (see Section 6.4.2.9).

- *Dose metric:*
    1. *average daily intake,*
    2. *area under the blood concentration-time curve,*
    3. *lifetime average body burden,*
    4. *peak body burden,*
    5. *other.*

Response: (1-5) The dose metric is chosen to maximize causal proximity to the endpoint, while maintaining the link to measured exposure (see Section 6.4.2.7). There may be uncertainty with regard to which metric is optimal. If an inappropriate metric is chosen in a bioassay study, this would be expressed in noisier responses which would tend to suppress the dependence of endpoint on dose. A data driven quantitative uncertainty analysis of dose metric would require a mathematical model expressing endpoints as a function, inter alia, of dose metric, with parameters estimated from data.

- *Dose metric—biological measure:*
    1. *free dioxin,*
    2. *bound dioxin.*

Response: (1−2) The issue is whether all TCDD available for AhR binding, or only the bound TCDD, should be used as a dose metric. Binding affinity is determined by more factors than genetic polymorphisms and these other factors are poorly understood (see Section 6.4.2.9). A quantitative uncertainty analysis must await the formulation of a quantitative model expressing binding affinity in terms of parameters which can be estimated from data.

- *POD:*
    1. ED_{10},
    2. ED_{05},
    3. ED_{01}

Response: (1−3) Uncertainty in choosing a POD is volitional. Uncertainty in the value of an ED_x can be quantified in a data driven manner if sufficient bioassay data is at hand (see Section 6.4.1.1).

- *Value from ED distribution to use:*
    1. *ED,*
    2. *lower confidence bound value for the ED (LED),*
    3. *upper confidence bound for the ED (UED).*

Response: (1−3) Given that uncertainty on the POD is quantified, a distribution of the slopes of a linear low dose extrapolation is readily derived, and hence a distribution of a risk specific dose.

- *Where alternative assumptions or methodologies could not be ruled out as implausible or unreasonable, EPA could have estimated the corresponding risks and reported the impact of these alternatives on the risk assessment results. The potential impacts of four sources of uncertainty are discussed below.*

    1. *The full range of plausible parameter values for the dose-response functions used to characterize the dose-response relationship for the three occupational cohort studies selected by EPA (Becher et al., 1998, 197173; Ott and Zober, 1996, 198408; Steenland et al., 2001, 197433)).*
    2. *Use of other points of departure, not just the ED_{01} (or LED_{01}), to develop a CSF.*
    3. *Alternative dose-response functional forms as well as goodness of fit of all models, especially at low doses.*
    4. *Uncertainty introduced by estimation of occupational exposures.*

Response: (1) The study of Steenland et al. (2001, 197433) was selected to illustrate the possibilities and limitations of quantitative uncertainty analysis for this type of study (see Section 6.4.2.2). (2) The possibilities for uncertainty quantification with regard to the POD are discussed in Section 6.4.1.1 and in the POD bullet above. (3) Goodness of fit at any measured dose is evaluated in standard packages. There may be different models with comparable goodness of fit at observed doses which differ strongly at doses outside the measured range. Extra model uncertainty, that is, uncertainty which is not conditional on the truth of any given model, is addressed by the exotic methods (see Section 6.4.2). (4) The feasibility of quantifying uncertainty in occupational exposure is study specific. The example of Steenland et al. (2001, 197433) was discussed in some detail (see Section 6.4.2.2). In general, the problem is not so much quantifying the exposure uncertainty, but in quantifying the dependence between the endpoints and the exposure uncertainty.

6.5.2. How Forward? Beyond RfDs and Cancer Slope Factors to Development of Predictive Human Dose-Response Functions

Uncertainty quantification is an emerging area in science. There are many examples of highly vetted and peer-reviewed uncertainty analyses based on structured expert judgment. Under this process, experts in effect synthesize a wide diversity of information in generating their subjective probability distributions. Where considerable data exist for an environmental pollutant, such as for the well-studied TCDD, it is natural to ask whether these extensive data can be leveraged more directly in uncertainty quantification. This is an area where research could be focused. The requisite knowledge does not yet exist, but there are promising lines of attack. It is

therefore not a question of convening blue-ribbon panels to reveal the proper approach; instead multiple approaches should be encouraged, to try out new ideas and share experiences.

An important idea that has been pioneered in Europe is to organize bench-test exercises where different approaches are applied to a common problem. This focuses the discussion on real issues and builds a community of capable practitioners. Such initiatives have proven much more productive than simply supporting individual researchers to explore their ideas.

Areas for which bench-test exercises might be appropriate include:

- Testing "exotic" methods for capturing model uncertainty;
- Combining bioassay and epidemiological data for uncertainty quantification;
- Assessing applicability of structured expert judgment, e.g., for low-dose extrapolation; and
- Conducting dependence modeling, dependence inference, and dependence elicitation (such as with regard to TEFs).

Looking beyond compounds for which considerable data exist, there will always be a need to evaluate new substances. The target will be a simple method that:

1. Can yield predictions of toxicological indicators with uncertainty via a valid probabilistic mechanism;
2. Could evolve from approaches based on similarities (such as a random chemical model) under which the new substance could be seen as a random sample from a reference distribution of chemicals considered sufficiently similar, e.g., in terms of structure, physicochemical properties, and biological activity (potency); and
3. Is consistent with current risk assessment science and approaches, peer-reviewed and accepted as EPA policy.

This last feature is important because advancements in risk assessment approaches should extend logically from current methodology based on data analysis and scientific methods. For example, the discussion surrounding uncertainty factors suggests that a probabilistically valid inference system could substantially differ from the current system. Nonetheless, to meld with current practice, it must initialize on the current system and allow this system to evolve in a measured fashion. Ideally, methodological changes should be undertaken in a forum where such issues are being addressed and not within an assessment of a single chemical.

Additional research topics relevant to dioxin that could further inform health assessments include population variability of biokinetic constants, threshold mechanisms for the mass action model, and low-frequency polymorphisms (e.g., less than 1%). Further data and improved methodologies in these areas, combined with developments illustrated elsewhere in this report, will help reduce uncertainties and strengthen our understanding of potential health implications of environmental contaminants.

Table 6-1. Key sources of uncertainty

Selection of endpoint and of species/strain, gender, life stage, other subject characteristics - critical effect - sensitivity (e.g., species, life stage) - human relevance
Selection of key study(ies): human data and bioassays (strength, inclusion criteria) - epidemiological studies, clinical/case reports (exposure estimate) - adequacy of study design, statistical power (exposure term, histopathology) - human relevance of bioassays (TK, MOA, endpoint) - data uncertainty, confidence in data; database deficiencies
Use of TK, dosimetry; body burden; species differences, cross-species extrapolation - bioavailability, dose dependence - half life, life stage, body fat, other compartments, age, other factors - body burden (peak, steady state, lifetime average) - physiologically-based pharmacokinetic (PBPK) modeling - scaling (human equivalents), adjustments (default and nondefault; with TD)
Selection of dose metric - intake (averaging time) - background (what place on the dose-response curve) - free vs. receptor-bound TCDD - tissue-specific concentration - lipid-normalized level
Selection of POD - selection (e.g., NOAEL/LOAEL, BMDL, $ED_{01, 05, 10}$) - derivation method (e.g., BMD) - choice of model form (e.g., Hill, Weibull) - statistical uncertainty at/confidence in POD
Selection of dose-response model (e.g., biologically based, multistage) and of BMR - biological plausibility, MOA - model type and form, alternative functional forms - range of plausible parameter values - goodness of fit, especially at low doses
Selection of low-dose extrapolation approach - linear/nonlinear - threshold/nonthreshold
Human population variability - subpopulations (e.g., occupational, general public, sensitive groups) - polymorphisms - life stage, other features - individual vs. population threshold
Characterization of risk/effect - adversity of effect (vs. in normal range of variation and adaptation) - uncertainty factors (TK; TD; chemical-specific vs. default; justification) - consistency of methods for endpoints with common MOA - back-extrapolation from occupational data - MOE, RfD; beyond a point estimate for SF

PBPK = physiologically-based pharmacokinetic; SF = slope factor; TD = toxicodynamic; TK = toxicokinetic. (Other acronyms are as defined elsewhere within this section.)

Table 6-2. PODs and amenability for uncertainty quantification

POD	Data profile	Choice	Uncertainty quantification
LOAEL	Experimental dose level from set of exposure-response data	Choose set of exposure-response measurements	No
NOAEL	Experimental dose level from set of exposure-response data	Choose set of exposure-response measurements	No
BMDL	Estimate from bioassay data	Choose BMR, choose dose-response relation	No, the BMDL is a quantile of an uncertainty distribution assuming that the dose-response model is true
ED_x	Estimate from set of exposure-response data	Choose bioassay experiments to estimate ED_x	Yes, if full bioassay data are available

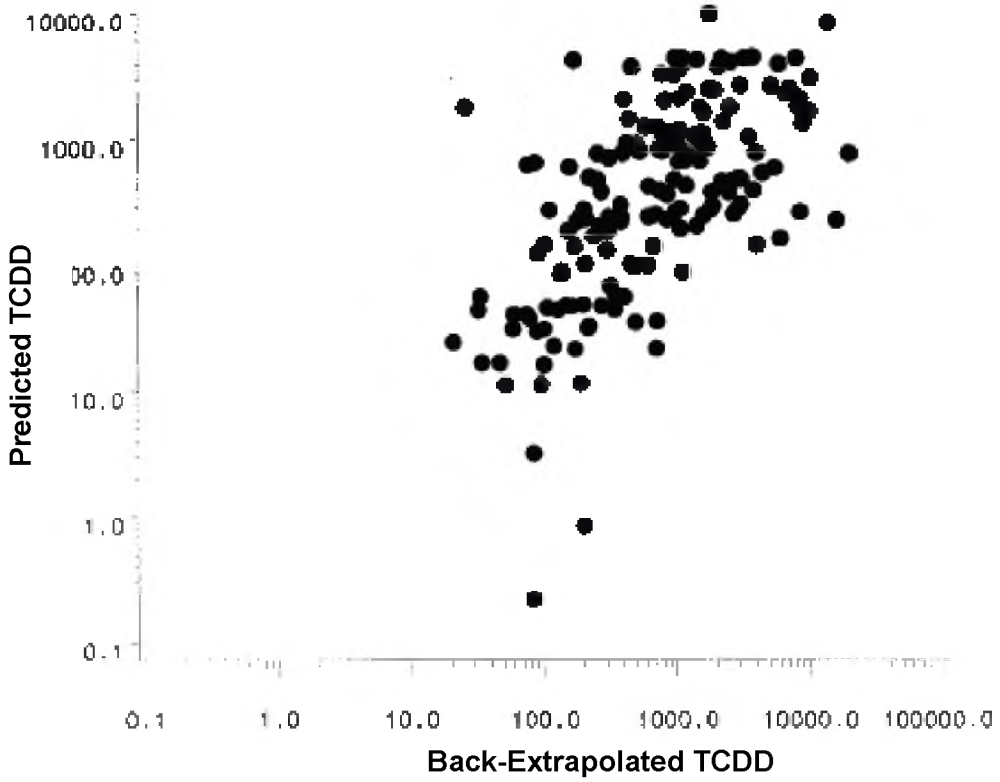


Figure 6-1. Back-casted vs. predicted TCDD serum levels for a worker subset.

Source: Steenland et al. (2001, 197433).

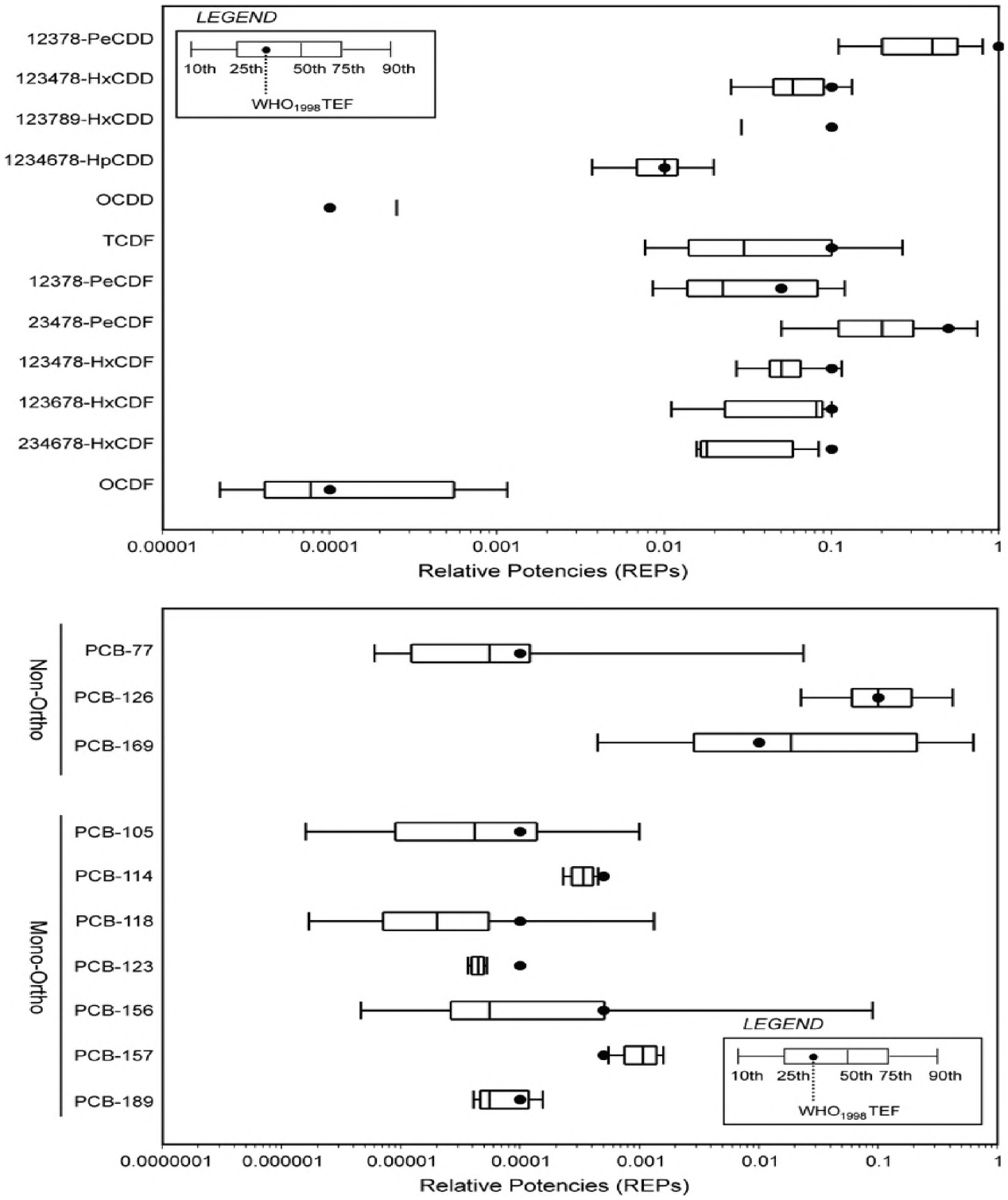


Figure 6-2. Distribution of in vivo unweighted REP values in the 2004 database.

Source: Van den Berg et al. (2006, 543769), reprinted with permission from Haws et al. (2006, 198416).

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APPENDIX A

Dioxin Workshop Report

NOTICE

THIS DOCUMENT IS AN EXTERNAL REVIEW DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

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Summary of U.S. EPA Dioxin Workshop

February 18–20, 2009

Cincinnati, Ohio

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

DISCLAIMER

This document summarizes the discussions presented at the Dioxin Workshop in February 2009, in Cincinnati, OH, as documented by the Session Co-Chairs. This document is not all inclusive or binding. Conclusions and recommendations to the U.S. EPA may not represent full consensus. The views expressed in this document are those of the Dioxin Workshop Panelists and do not necessarily reflect the views and policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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DIOXIN WORKSHOP TEAM

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INTRODUCTION

This document provides a summary of the Scientific Workshop to Inform EPA's Response to National Academy of Science Comments on the Health Effects of Dioxin in EPA's 2003 Dioxin Reassessment. The U.S. Environmental Protection Agency (U.S. EPA) and Argonne National Laboratories (ANL), through an inter-Agency agreement with the U.S. Department of Energy, convened this scientific workshop ("Dioxin Workshop") on February 18–20, 2009, in Cincinnati, Ohio. The goals of the Dioxin Workshop were to identify and address issues related to the dose-response assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). This report summarizes the discussions and conclusions from this workshop. Previously, at the request of the U.S. EPA, the National Academy of Sciences (NAS) prepared a report, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NAS, 2006), which made a number of recommendations to improve the U.S. EPA's risk assessment for TCDD (U.S. EPA, 2003). The 3-day Dioxin Workshop was convened specifically to ensure that the U.S. EPA's response to the NAS recommendations focuses on the key issues and reflects the most meaningful science.

The Dioxin Workshop included seven scientific sessions:

(1) Session 1: Quantitative Dose-Response Modeling Issues
(2) Session 2: Immunotoxicity
(3) Session 3A: Dose-Response for Neurotoxicity and Nonreproductive Endocrine Effects
(4) Session 3B: Dose-Response for Cardiovascular Toxicity and Hepatotoxicity
(5) Session 4A: Dose-Response for Cancer
(6) Session 4B: Dose-Response for Reproductive/Developmental Toxicity
(7) Session 5: Quantitative Uncertainty Analysis of Dose-Response

During each session, the U.S. EPA asked a panel of expert scientists to:

- identify and discuss the technical challenges involved in addressing the key NAS comments on the TCDD dose-response assessment in the U.S. EPA Reassessment (U.S. EPA, 2003);
- discuss approaches for addressing the key NAS comments; and
- identify important published, independently peer-reviewed literature, particularly studies describing epidemiologic and *in vivo* mammalian bioassays, which are expected to be most useful for informing the U.S. EPA's response.

The sessions were followed by open comment periods during which members of the audience were invited to address the Panels. At the conclusion of the open comment periods, the Panel Co-Chairs were asked to summarize and present the results of the panel discussions. The summaries could include minority opinions stated by panelists. The main points derived from the session summaries were used to prepare this document. Additionally, this document includes a list of the session panelists and their affiliations and three appendices. Appendix A presents the Dioxin Workshop Agenda. Appendix B identifies the charge questions presented to the Panel. Appendix C describes draft study selection criteria proposed by the Dioxin Workshop Team for consideration by the workshop panelists.

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NAS (National Academy of Sciences). 2006. Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment. National Academies Press, Washington, DC (July). Available at http://www.nap.edu/catalog.php?record_id=11688.

U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. NAS review draft, Volumes 1−3 (EPA/600/P-00/001Cb, Volume 1). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC (December). Available at http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/.

SCIENTIFIC WORKSHOP TO INFORM THE TECHNICAL WORK PLAN FOR U.S. EPA'S RESPONSE TO NAS COMMENTS ON THE HEALTH EFFECTS OF DIOXIN PRESENTED IN U.S. EPA'S DIOXIN REASSESSMENT

Dioxin Workshop Co-Chairs: Peter W. Preuss and Glenn Rice

The Dioxin Workshop session summaries were prepared by the session panel Co-Chairs with input from the panelists, as requested by the U.S. EPA prior to the workshop. The Co-Chairs subsequently presented these summaries to all of the workshop participants during designated periods at the workshop. In these summaries, the U.S. EPA asked that the Co-Chairs summarize the key issues from the panel discussions. Because the sessions were not designed to achieve consensus among the panelists, the summaries do not necessarily represent consensus opinions; rather, they reflect the essence of the panel discussions. Some of the specific points may represent the views of multiple panelists, while others only the views of a single panelist. Prior to the summarizations, there were opportunities for public comments on the discussion topics. Some Co-Chairs met with their sessions' panelists after their sessions ended to develop these summaries, while others developed reports based on their personal notes. Because Session 5 was the last session of the workshop—with little time provided to develop the summary—the Co-Chairs circulated a draft for comment by the Session 5 panelists after the workshop, prior to finalizing the session summary. The U.S. EPA collected the session summaries and then prepared this document. A draft of this document was distributed to all of the session Co-Chairs to provide them with a final opportunity to comment and make revisions. Finally, it should be noted that U.S. EPA was not prescriptive to the session Co-Chairs with respect to the format of the presentation materials and provided no specific instructions, resulting in unique formats among the session summaries.

SESSION 1: QUANTITATIVE DOSE-RESPONSE MODELING ISSUES

This session discussed the general dose-response modeling issues related to TCDD. Many of these issues were highlighted by NAS (2006). There was a general introductory presentation on TCDD kinetics, including information and uncertainties pertaining to the conversion of administered doses in animals to human body burden (BB) and additivity to background issues. This presentation was followed by a Panel discussion on the state of the science regarding dioxin dose-response modeling issues.

Session 1 Panelists (Session Co-Chairs are identified by asterisk)

- Bruce Allen, Bruce Allen Consulting
- Lesa Aylward, Summit Toxicology
- Roger Cooke, Resources for the Future
- Kenny Crump, Louisiana Tech University
- Mike DeVito, U.S. EPA
- Dale Hattis, Clark University
- Rick Hertzberg, Biomath Consulting
- Rob McDowell, U.S. Department of Agriculture
- Jim Olson, State University of New York, University at Buffalo

- *Lorenz Rhomberg, Gradient
- Woody Setzer, U.S. EPA
- *Jeff Swartout, U.S. EPA

Please note that the use of the term "concluded" or "recommended" in this summary does not mean that a consensus was reached. Session Summaries were written from the material prepared by the non-EPA/ANL Co-Chair and represent a synopsis of the panel discussions.

Key Study Selection Criteria

The Panel discussed the advantages and disadvantages of using key study criteria (Appendix C). They concluded that *a priori* criteria foster transparency and consistency, and could deflect *a posteriori* criticism. However, the Panel also acknowledged that having *a priori* criteria could introduce the potential for excluding useful data. Although the key study criteria provided by the U.S. EPA listed studies using TCDD only as a criterion, the Panel posed the possibility of using closely related dioxin-like compounds (DLCs) as surrogates for TCDD. The criterion for use of data from mammalian studies only was one criterion that received generalized support due to the lack of extrapolation protocols for nonmammalian species. The Panel also discussed the specific exposure-duration criterion and asked if there should be a preference for longer-term rather than acute studies. The Panel made three suggestions to modify U.S. EPA's key study selection criteria:

(1) Define more relevant exposure-level (i.e., dose) cut points using tissue concentrations.
(2) Reword statistical criteria to include do-it-yourself analysis.
(3) Reword the response criteria to clarify "outside of normal range."

Dose Metrics

The Panel discussed the relative merits of various measures of dose for modeling TCDD dose response. One general conclusion was that tissue concentration (TC) is the preferred metric, especially lipid-adjusted TC, because this measure more closely approximates exposures close to the target tissue when compared to administered doses. However, the Panel acknowledged that these data are often unavailable. They further noted that BB, which is defined as the concentration of TCDD in the body (ng/kg body weight) (U.S. EPA, 2003), might be useful as a surrogate for TC provided the two measures were proportional.

The Panel suggested that a linear approach to BB estimation, which was utilized by U.S. EPA (2003), is too simplistic because this approach does not take into account toxicokinetic issues related to TCDD—e.g., sequestration in the liver and fat, age-dependent elimination, and changing elimination rates over time. The Panel recommended the use of kinetic/mechanistic modeling to the extent possible to quantify tissue-based metrics.

The Panel raised the issue of whether the preferred dose metric would be different for different endpoints and exposure durations. This led to the Panel's comment that the peak exposure might be a more important metric than average BB for variable exposure scenarios. Given this discussion about different exposure durations being relevant to a specific endpoint, the Panel suggested that the U.S. EPA also consider peak measures in dose-response modeling.

The last point raised in this part of the discussion centered on the possibility of dose errors in experimental studies. The Panel highlighted the need for the U.S. EPA to consider dose error (i.e., uncertainty in the x-axis of the dose-response curve) when using dose surrogates.

Dose-Response Modeling of Mammalian Bioassays

The Panel considered several issues related to dose-response modeling of mammalian bioassay data for TCDD: supralinearity and incomplete response data ("anchoring"), defining the benchmark response (BMR) level with respect to establishing the point of departure (POD), and the use of threshold modeling—as further explained below.

The Panel discussed the specific issues of supralinearity and anchoring raised by the U.S. EPA with respect to modeling noncancer endpoints. The panel recognized that, for many of the most sensitive endpoints, the response at the lowest dose is high (e.g., quantal responses above 25% and continuous endpoints differ substantially from the mean, often implying 100% incidence in the treated animals). This lack of response anchoring at the low end of the dose-response curve (near the BMR) results in the higher responses determining the shape of the curve.

The Panel asked whether new tools might be needed or whether the current tools could be applied differently. In the context of developing new tools, the Panel emphasized the need for collaboration between biologists and mathematicians. When discussing application, the Panel suggested that the problem with supralinearity might be overcome by simply dropping the requirement for using the lower bound on the Benchmark Dose. In addition, the Panel posed several more approaches for further consideration in dose-response modeling by the U.S. EPA:

(1) Combine similar data sets to fill in data gaps.
(2) Use mechanistic approaches to model the data gaps.
(3) Dichotomize continuous data.

Finally, the Panel acknowledged that, in certain situations, there simply may not be enough information to provide meaningful answers.

The Panel discussed the BMR level for establishing a POD in the context of deriving a Reference Dose (RfD). The Panel generally agreed that, while the effective dose level (ED_{01}) used in the 2003 Reassessment may be useful for comparative analysis across endpoints, the ED_{01} estimates developed for all endpoints considered in the Reassessment were not appropriate for deriving an RfD because they were not based on the effect's adversity. The panel noted that ED_{01} also is much lower than typical EPA BMR levels. The Panel recommended that the U.S. EPA work to define endpoint-specific BMRs based on the consideration of adversity. Given that the same uncertainty factor framework is applied to all PODs, the Panel emphasized the need for consistency in BMRs; numerical consistency is needed for quantal BMRs and consistency in the choice of biological relevance should be applied for continuous BMRs.

The Panel generally discouraged threshold modeling by stating that thresholds are very difficult to pin down and suggested that the lower bound may always be zero.

Dose-Response Modeling of Epidemiological Studies

The Panel noted that many studies have been published with measured concentrations of TCDD that could be used for dose reconstruction. In this discussion, the Panel acknowledged that use of these data would entail dealing with toxicity equivalence (TEQ) issues and pharmacokinetic (PK) modeling. Pertaining to the use of these data for quantitative risk assessment by the U.S. EPA, the Panel posed the question, "At what point does indirect or confounded human data supersede controlled animal bioassay data?", or alternatively, "How much human data uncertainty can we tolerate?" The Panel suggested, at the least, that the epidemiologic data could be used to "ground-truth" the animal bioassay modeling results.

Supporting Information

The Panel acknowledged that Ah receptor (AhR) binding affinities are not necessarily tied to endpoint sensitivity, but they reiterated the need to consider mechanistic modeling to aid in developing appropriate dose metrics or filling in data gaps in the existing dose-response data.

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U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. NAS Review Draft (EPA/600/P-00/001Cb). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC. Available at http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/.

SESSION 2: IMMUNOTOXICITY

The U.S. EPA plans to consider development of a quantitative dose-response assessment for the immunologic effects associated with TCDD exposure. Such an assessment would be based on information in U.S. EPA (2003), NAS (2006) and key studies identified in this workshop. The purpose of this session was to identify and discuss key issues pertaining to dose-response assessment for dioxin-induced immunologic effects.

Session 2 Panelists (Session Co-Chairs are identified by asterisk)

- Roger Cooke, Resources for the Future
- Rob Goble, Clark University
- *Belinda Hawkins, U.S. EPA
- Nancy Kerkvliet, Oregon State University
- Manolis Kogevinas, Centre for Research in Environmental Epidemiology
- Robert Luebke, U.S. EPA
- Paolo Mocarelli, University of Milan
- *Allen Silverstone, State University of New York, Upstate Medical University

- Courtney Sulentic, Wright State University
- Nigel Walker, National Institute of Environmental Health Sciences

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Key Study Selection Criteria

The Panel first addressed the Key Study Selection Criteria proposed by the U.S. EPA (Appendix C). The Panel raised the issue that the key study criteria do not apply to most studies designed to investigate immunotoxicity, including those used to calculate ED_{01}s (U.S. EPA, 2003). The Panel observed that most dioxin immunotoxicity studies are relatively high dose (>200 ng/kg-d) acute studies and/or use parenteral rather than oral administration.

The Panel discussed several studies often considered important for assessing the immunotoxic effects of TCDD exposure. The Oughton et al. (1995) mouse bioassay was discussed and, although the study does meet the proposed criteria, it could not be considered a key study; specifically, the Panel contended that since there were no functional alterations observed or measured in this bioassay, the changes in cellular phenotypes are only "suggestive" of immune alterations and cannot be regarded as having immunopathologic significance.

The Panel discussed two additional studies for further consideration by the U.S. EPA:

- Baccarelli et al. (2002). The Panel discussed this as a potentially key human epidemiological study that should be reviewed and considered further by the U.S. EPA. It measured the level of IgG, demonstrating a significant decline relative to dioxin body burdens.
- Smialowicz et al. (2008). The Panel noted that this study identified the antibody response to sheep red blood cells (SRBCs) as the critical effect, labeling this protocol as a functional assay. The Panel stated that if modeled, the U.S. EPA could calculate the BMR for this endpoint as 1 standard deviation from the control mean.

References

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SESSION 3A: DOSE-RESPONSE FOR NEUROTOXICITY AND NONREPRODUCTIVE ENDOCRINE EFFECTS

The U.S. EPA plans to consider development of a quantitative dose-response assessment for neurological and/or nonreproductive endocrine effects associated with TCDD exposure. Such an assessment would be based on information in U.S. EPA (2003), NAS (2006) and key studies identified in this workshop. The purpose of this session was to identify and discuss key issues pertaining to dose-response assessment for dioxin-induced neurological and/or nonreproductive endocrine effects.

Session 3A Panelists (Session Co-Chairs are identified by asterisk)

- *Maryka Bhattacharyya, Argonne National Laboratory
- Mike DeVito, U.S. EPA
- Mary Gilbert, U.S. EPA
- Rob Goble, Clark University
- Nancy Kerkvliet, Oregon State University
- Fumio Matsumura, University of California-Davis
- Paolo Mocarelli, University of Milan
- Chris Portier, National Institute of Environmental Health Sciences
- Lorenz Rhomberg, Gradient
- Allen Silverstone, State University of New York, Upstate Medical University
- Marie Sweeney, National Institute of Occupational Safety and Health
- *Bernie Weiss, University of Rochester

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What Are the Key Questions Regarding These Endpoints?

The Panel used the following question to initiate discussion: "*Are there identifiable indices of neurotoxicity and nonreproductive endocrine effects in animal studies and human populations?*" Under this discussion topic, the Panel discussed three endpoints: neurotoxicity (with focus on developmental exposures), thyroid dysfunction (e.g., thyroid hormone deficits), and diabetes. The Panel also addressed the relevance of windows of vulnerability to each

endpoint. The Panel acknowledged that, in some cases, the window of exposure may precede the window of expression of toxicity.

Epidemiological Study Selection

Developmental Neurotoxicity

The Panel recognized that an unusual feature for this endpoint is that there are sufficient human data for dose-response modeling (e.g., Dutch children [Huisman et al., 1995; Patandin et al., 1999] and U.S. children [Jacobson and Jacobson, 1996]) and there is an internal dose metric (serum concentrations). Additionally, the Panel discussed recent studies that address this endpoint in humans (from Japan [reference not provided] and Holland [e.g., Koopman-Esseboom et al., 1996; Vreugdenhil et al., 2002]). For continued investigation into this endpoint, the Panel raised two issues to the U.S. EPA:

- Conduct an evaluation of whether a modeled effect can be attributed to TCDD and not some other persistent organic pollutant (POP), although the Panel recognized that it is unlikely U.S. EPA will be able to distinguish among these exposures because other POPs are intrinsic confounders in the Dutch study.
- Allow animal data to inform the dose-response modeling of epidemiological data.

Thyroid Dysfunction

The Panel identified the availability of human data for this endpoint (e.g., Calvert et al., 1999; Koopman-Esseboom et al., 1994). Much of the thyroid dysfunction literature has been published since the 2003 Reassessment (e.g., Wang et al., 2005; Baccarelli et al., 2008). The Panel also noted the availability of an internal dose metric (serum concentrations). Additionally, the Panel discussed the mechanistic studies in animals that link TCDD to thyroid dysfunction. For continued investigation into this endpoint, the Panel raised three issues for the U.S. EPA to consider:

- Consider the newly available human data since the Reassessment.
- Investigate and clarify of the role of TCDD-induced thyroid dysfunction in developmental neurotoxicity.
- Evaluate and determine whether an effect can be attributed to TCDD or other contaminants.

Diabetes

The Panel discussed that data suggest that diabetes incidence in those under 55 years old may be associated with exposure to PCBs. They acknowledged that whether this is a dioxin-like compound (DLC) mediated effect or whether other POPs are responsible is still undetermined. The Panel also acknowledged that no animal model exists for the investigation of xenobiotic-induced diabetes, and that separating the injury dose level from the current body burdens would depend on good pharmacokinetics in humans. For continued investigation into this endpoint, the Panel listed two issues for the U.S. EPA to consider:

- Results from the Anniston study and the Great Lakes Fishermen study (references not provided) should be examined for dose metrics (both studies examine human PCB exposures).

- Changes of adipose tissue status need to be considered, given that dieting can cause release of lipid-soluble contaminants.

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U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. NAS Review Draft (EPA/600/P-00/001Cb). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC. Available at http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/.

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Wang S.L., P.H. Su, S.B. Jong, Y.L. Guo, W.L. Chou and O. Päpke. 2005. *In utero* exposure to dioxins and polychlorinated biphenyls and its relations to thyroid function and growth hormone in newborns. Environ. Health Perspect. 113:1645–1650.

SESSION 3B: DOSE-RESPONSE FOR CARDIOVASCULAR TOXICITY AND HEPATOTOXICITY

The U.S. EPA plans to consider development of a quantitative dose-response assessment for cardiovascular and/or hepatic effects associated with TCDD exposure. Such an assessment would be based on information in U.S. EPA (2003), NAS (2006) and key studies identified in this workshop. The purpose of this session was to identify and discuss key issues pertaining to dose-response assessment for dioxin-induced cardiovascular and/or hepatic effects.

Session 3B Panelists (Session Co-Chairs are identified by asterisk)

- Bob Budinksy, Dow Chemical
- Manolis Kogevinas, Centre for Research in Environmental Epidemiology
- Rob McDowell, U.S. Department of Agriculture
- Jim Olson, State University of New York, University at Buffalo
- Marian Pavuk, Agency for Toxic Substances and Disease Registry
- *Jeff Swartout, U.S. EPA
- *Mary Walker, University of New Mexico
- Nigel Walker, National Institute of Environmental Health Sciences

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Key Study Selection Criteria

The Panel initially focused on the draft key study selection criteria offered by the U.S. EPA (Appendix C). The panel recommended that for cardiovascular effects, which are not usually observed in rodents, the use of knockout mouse models (ApoE KO and LDLR KO) be moved to the "primary" column because only these studies establish the cardiovascular toxicity model in mice.

The panel also was concerned that the gavage procedure can increase mouse blood pressure. Consequently, the panel recommended that gavage studies not be used for the blood pressure endpoint (i.e., only dietary dosing studies should be considered).

Human Health Endpoints

In relation to the hepatic endpoint, the Panel acknowledged the large body of dose response information on hepatic effects in rodents and that enzyme (mostly CYP1A1) induction was a sensitive effect. However, the Panel cited the lack of linkage of CYP1A1 to downstream events, which complicates the toxicological interpretation of this endpoint, and concluded that

the more important liver effects in rodents are probably on the "road to cancer." The Panel noted that hepatic effects were not seen in the epidemiological studies, but acknowledged that these studies were not designed to detect them.

In relation to the cardiovascular endpoint, the Panel identified hypertension and ischemic heart disease (IHD) as two key endpoints from the epidemiological studies. The Panel recommended that the U.S. EPA perform a meta-analysis of these data. The Panel also commented that recent animal studies support the observations linking TCDD exposure to IHD and hypertension. In particular, the National Toxicology Program (NTP) study shows inflammatory and structural effects on resistant vascular arterioles (NTP, 2006). Additional evidence from the study suggests that the vascular effects may be CYP1A1-dependent. The Panel suggested that the NTP study data might be used as a surrogate for dose-response modeling of hypertension and that such an approach would be supported by data on the role of AhR in vascular function and remodeling.

POD Issues

The Panel was not supportive of 1% of maximal response (ED_{01}), which was utilized in the 2003 Reassessment. The Panel concluded that the POD should depend on the specific endpoint and recommended the following to the U.S. EPA:

- For continuous measures, base the BMR on difference from control. Consider the adversity level—at what point does the endpoint become adverse?
- For incidence data, set the BMR to a fixed-risk level.

Supporting Information

The Panel posed several suggestions to the U.S. EPA for reducing uncertainty and improving the knowledge base for TCDD toxicity.

- Use in vitro data to define uncertainties, such as the relative sensitivity between rodents and humans and around the definition of a POD.
- Consider studies on dioxin-like compounds (DLCs).
- Use PK modeling to define the dose metric for hepatic effects.
- Use body burden or serum concentrations for cardiovascular endpoints.

Finally, the Panel recommended that U.S. EPA finish the reassessment quickly and establish a definitive plan to review and incorporate new data as they become available.

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SESSION 4A: DOSE-RESPONSE FOR CANCER

The U.S. EPA plans to consider development of a quantitative dose-response assessment for cancer associated with TCDD exposure. Such an assessment would be based on information in U.S. EPA (2003), NAS (2006) and key studies identified in this workshop. The purpose of this session was to identify and discuss key issues pertaining to dose-response assessment for dioxin-induced cancer.

Session 4A Panelists (Session Co-Chairs are identified by asterisk)

- Lesa Aylward, Summit Toxicology
- Kenny Crump, Louisiana Tech University
- Dale Hattis, Clark University
- *Janet Hess-Wilson, U.S. EPA
- Karen Hogan, U.S. EPA
- Manolis Kogevinas, Centre for Research in Environmental Epidemiology
- Marian Pavuk, Agency for Toxic Substances and Disease Registry
- Chris Portier, National Institute of Environmental Health Sciences
- Lorenz Rhomberg, Gradient
- Jay Silkworth, General Electric
- *Nigel Walker, National Institute of Environmental Health Sciences

Please note that the use of the term "concluded" or "recommended" in this summary does not mean that a consensus was reached. Session Summaries were written from the material prepared by the non-EPA/ANL Co-chair and represent a synopsis of the panel discussions.

Key Study Selection

The Panel discussed both human and rodent studies. In reviewing the epidemiological data, the Panel agreed the EPA should focus on four cohort studies (Dutch cohort, NIOSH cohort, BASF accident cohort, and Hamburg cohort) and pointed out that there are numerous updates and reevaluations of data now in the literature and others will be published soon. The Panel stated that it is appropriate for the U.S. EPA to consider the increase in total cancers for modeling human cancer data, however, Non-Hodgkin's lymphoma, and lung tumors are the main TCDD-related cancer types seen in humans exposed to TCDD. The Panel suggested the U.S. EPA focus the quantitative dose-response modeling on the human data.

In reviewing the rat data, the Panel identified four new NTP rodent cancer bioassays with liver and lungs as the main target organs. However, they suggested that dose-response modeling efforts should model "all cancers" from these NTP data sets as well and use tumor incidence—not individual rats as measures.

Key Study Selection Criteria

The Panel discussed whether data for TCDD only should be used or if PCB126 could be used to develop a dose-response curve. From this discussion, the Panel reached a general agreement that limiting the dose-response modeling and cancer assessment to TCDD only would be the best approach.

Regarding the oral dosing regimens, the Panel discussed the differences in results from different bioassays. They concluded that there were insufficient data to pick between oral feed (Kociba et al., 1978) and oral gavage (NTP, 2006) studies, but stated "If all aspects of studies were equal, an oral feed study is preferred." However, given that current data sets are not equal, they agreed that U.S. EPA should consider both feed and gavage studies.

The Panel put forth the recommendation that studies that include initiation-promotion model data and TgAC transgenic model data from oral exposure studies should be excluded from the primary category in the key study selection criteria (Appendix C lists the draft study selection criteria distributed prior to the meeting). Studies from both classifications should be moved to the second tier.

The Panel was also unsupportive of the "response magnitude outside the range of normal variability" criterion, as they did not believe it was applicable to a cancer endpoint.

Critical Endpoints to Consider

The Panel recognized that the MOA for TCDD includes cell growth/differentiation dysregulation, that different endpoints (tumor types) across species may be expected, and that there are differences in tumor sites across species. The Panel further acknowledged that there is insufficient information to determine if rodent tumor types observed are relevant to humans. Thus, the Panel suggests the following:

- U.S. EPA should consider all the observed cancer endpoints in its evaluation.

Nonlinear (aka threshold) Versus Linear Dose-Response Modeling

The Panel agreed that NTP bioassays appear to demonstrate nonlinear dose response, but they expressed concern about using animal data to infer slope and dose response for humans. The Panel pointed out that there are differences in slopes across different bioassays, and specifically, that some appear linear while others appear nonlinear. Given the observation of both nonlinear vs. linear, the Panel concluded that neither could be ruled out for extrapolation below the POD simply based on the available data. One panelist noted that U.S. EPA Cancer Guidelines (U.S. EPA, 2005) state that only if one can demonstrate that the MOA has a threshold dose-response shape, and can exclude all other potential linear MOAs, can one use a nonlinear model. Lastly, the Panel noted that there are data and rationales to support use of both linear and

nonlinear response below POD. From this discussion, the Panel raised one possibility to the U.S. EPA:

- Both linear and nonlinear model functions should be considered in the dose-response analysis.

Dose Metrics

In considering human data, the Panel expressed a preference for lipid-adjusted serum levels over body burden (BB), and they expressed concerns over the assumptions used in the back calculation of the BB in the epidemiologic cohorts. In considering the rat data, the Panel supported the use of BB—especially lipid-adjusted BB. The Panel, however, did express concern over the sequestering of TCDD in liver and then the use of liver levels in BB calculations.

Supporting Information—Biologically-Based Dose-Response (BBDR) Models and MOA

The Panel discussed BBDR. Though once considered an attractive proposition, BBDR models may mask uncertainty within the models, necessitating them to be used with greater caution. The Panel suggested two issues for the U.S. EPA to consider:

- If there is a published model, use it if it is valid—do not generate a new model.
- Focus on the actual experimental data to drive the analysis.

References

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SESSION 4B: DOSE-RESPONSE FOR REPRODUCTIVE/DEVELOPMENTAL TOXICITY

The U.S. EPA plans to consider development of a quantitative dose-response assessment for reproductive and developmental effects associated with TCDD exposure. Such an assessment would be based on information in U.S. EPA (2003), NAS (2006) and key studies identified in this workshop. The purpose of this session was to identify and discuss key issues pertaining to dose-response assessment for dioxin-induced reproductive and developmental effects.

Session 4B Panelists (Session Co-Chairs are identified by asterisk)

- Barbara Abbott, U.S. EPA
- Bruce Allen, Bruce Allen Consulting
- Roger Cooke, Resources for the Future
- George Daston, Procter & Gamble
- Mike DeVito, U.S. EPA
- Rob Goble, Clark University
- *Fumio Matsumura, University of California-Davis
- Paolo Mocarelli, University of Milan
- Brian Petroff, University of Kansas
- *Glenn Rice, U.S. EPA
- Marie Sweeney, National Institute of Occupational Safety and Health
- Mary Walker, University of New Mexico
- Bernie Weiss, University of Rochester

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A Major Question Posed During this Workshop Session was "Are Human Embryos and Infants Less Sensitive to Dioxin Exposures Than Some Experimental Animals?"

The Panel recognized that animal data show a wide range of species sensitivity to dioxin for a given developmental or reproductive endpoint. Presently, there are data for some endpoints that show that human sensitivity is comparable to experimental animals (e.g., semen quality), and for other endpoints the data demonstrate that humans are insensitive compared to other species (e.g., cleft palate). Lastly, the Panel recognized that there are some endpoints for which relative human sensitivity remains uncertain.

Key Study Selection

The Panel reviewed the charge questions (Appendix B), discussed them, and listed two issues for the U.S. EPA to consider:

- Concerning key study determination, use a stepwise approach that is dependent upon the information available and needed to address the question.

- Concerning the key studies informing the POD and the POD endpoint choice, use the POD to depart from what is certain and use a high-confidence study that has found effects at a low enough level at which other effects are protected.

The Panel also developed Table 1, based on the information presented in this session. Table 1 identifies specific reproductive and developmental effects of concern, listing whether an effect has been observed in test animals and epidemiologic cohorts. It also identifies the ED_{10} estimated by the U.S. EPA (2003) for health effects observed in rodent bioassays. If the U.S. EPA did not report an ED_{10} for an effect, the table identifies a study where the effect was reported and the lowest study dose where the effect was observed. Table 1 also identifies the epidemiologic cohort where the specific reproductive and developmental effects were observed.

Epidemiological Study Utility

The Panel reviewed the charge questions (Appendix B), discussed them, and made two suggestions to the U.S. EPA:

- Concerning the ability of epidemiological studies to inform critical effects, start with concordance across species (including humans) for the spectrum of effects.
- Concerning the ability of epidemiological studies to inform dose-response modeling, start with the epidemiology and then go to animal data if the dose response has not been well characterized for an endpoint of interest and compare to animal data as a reality check.

Animal Model Utility

The Panel reviewed and discussed the charge questions (Appendix B). Table 1, which identifies the effects that occur in animals and also have relevance to humans, summarizes much of this discussion. Regarding the influence of mode of action (MOA) on animal model choice, the Panel concluded that by evaluating concordance among health effects reported in epidemiologic and animal bioassay data, the U.S. EPA could identify a set of plausible reproductive and developmental effects to consider. Actual animal and human MOA information is helpful in that it creates comfort with the animal models and in defining the boundaries of possible effects.

TABLE 1

Reproductive/Developmental Effects of Concern for Human Health

Endpoint	Rodent (ED_{10} ng/kg-d)	Human	Notes
Sperm Count/Motility	Yes (6.2−28; 66−200)	Yes	ED_{10} bases Mabley et al. (1992a,b) caudal sperm count and daily sperm production range from 6.2−28; Gray et al. (1997) epididymal sperm count and total testis sperm counts range from 66−200.
Sex Ratio	No	Yes, Seveso	
Delayed Puberty Males	Yes (94)	Yu-cheng	ED_{10} basis rat male puberty delay Gray et al. (1997). Need to qualify epidemiology data because of cohort PCDD/PCDFs exposures.
Delayed Puberty in Females	Yes	No in Seveso	Gray and Ostby (2002) report delayed puberty in female offspring of pregnant rats receiving a single dose of 1 µg TCDD/kg on GD 15.
Cleft Palate	Yes (6300−6400)	No	ED_{10} basis Birnbaum et al. (1989).
Premature Senescence	Yes	No, Seveso	Franczak et al. (2006) report that rats prematurely entered reproductive senescence, after receiving cumulative TCDD doses as low as 1.7 µg TCDD/kg. They considered first occurrence of prolonged interestrous interval (>6 d) as evidence of onset of reproductive senescence.
Hormones E2	Yes	Yes, Males—Seveso	Li et al. (1995) report serum estradiol-17β (E2) concentrations induced by equine Chorionic Gonadotropin injection were significantly elevated in female rats orally administered 10 µg/kg TCDD on PND 22. While E2 decreased dramatically in control animals during the preovulatory LH surge, it did not in TCDD-treated rats.
Low Birth Weight	Yes (190)	Suggestive effect in Seveso in first 8 years after exposure	ED_{10} basis Gray et al. (1997).
Reproductive Cycling (prolongation)	Yes	Yes, Seveso Prepubertal exposure	Franczak et al. (2006) report loss of normal cyclicity in female rats at 8 months of age following a cumulative dose of 1.7 µg TCDD/kg.

Supporting Information

The Panel reviewed the charge questions (Appendix B), discussed them, and made two suggestions to the U.S. EPA:

- Concerning deviation from default approaches for noncancer endpoints, there needs to be a careful assessment of the POD and the application of uncertainty factors in light of PK/pharmacodynamics (PD), population characteristics and variability, and MOA information.
- Concerning the MOA's ability to clarify endpoint and the incorporation of a cascade of cellular event into dose-response for noncancer endpoint, any study that helps inform the dose response should be considered—including studies not specific to dioxins. Complicated mechanistic models need not be developed. Standard dose-response models can be applied. One can look at the cascade of events in a stepwise, simple way.

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SESSION 5: QUANTITATIVE UNCERTAINTY ANALYSIS OF DOSE-RESPONSE

This session addressed the uncertainty analysis to be considered for the dose-response assessments. The session opened with a presentation on current estimates of dioxin exposure levels. Then it focused on the factors to include in the scope of an uncertainty analysis including dioxin kinetics.

Session 5 Panelists (Session Co-Chairs are identified by asterisk)

- Bruce Allen, Bruce Allen Consulting
- Lesa Aylward, Summit Toxicology
- Roger Cooke, Resources for the Future
- Kenny Crump, Louisiana Tech University
- Mike DeVito, U.S. EPA
- Dale Hattis, Clark University
- *Rick Hertzberg, Biomath Consulting
- Nancy Kerkvliet, Oregon State University
- Leonid Kopylev, U.S. EPA
- Rob McDowell, U.S. Department of Agriculture
- Lorenz Rhomberg, Gradient
- Woody Setzer, U.S. EPA
- Marie Sweeney, National Institute of Occupational Safety and Health
- *Linda Teuschler, U.S. EPA

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The Panel summarized the NAS comments regarding uncertainty. Areas for improvement include:

- Ensure "transparency, thoroughness, and clarity in quantitative uncertainty analysis."
- Describe and define (quantitatively to the extent possible) the variability and uncertainty for key assumptions used for each key endpoint-specific risk assessment, including choices of data set, point of departure, dose-response model, and dose metric.
- Incorporate probabilistic models to represent the range of plausible values.

- Assess goodness-of-fit of dose-response models.
- Provide upper and lower bounds on central tendency estimates for all statistical estimates.
- When quantification is not possible, clearly state it, and explain what would be required to achieve quantification.

Identification of Important Uncertainties

The Panel reviewed the charge questions (Appendix B), discussed them, and listed eight issues for consideration by the U.S. EPA:

- Concerning species and strain differences in the U.S. EPA's Response to NAS, current U.S. EPA procedures do not take this into account when selecting one data set for risk assessment. Issues include "Where are humans in the distribution of potencies that can be generated? How likely is it that human response is similar to the selected data? Can we infer inter-individual variability from these differences?"
- Concerning the use of animal data for cross species extrapolation to humans (PK and PD uncertainties), issues to consider include differences in distribution and responses following bolus doses from those of subchronic and chronic protocols; uncertainty in liver doses due to sequestration; differences in receptor binding affinity among congeners; and age factors (e.g., assumption of a lifetime constant daily dose for a cancer extrapolation).
- Concerning the description of AhR response, biochemical changes occur at lower doses than toxicological changes. There should be an effort to identify the biochemical changes that would mark Ah receptor binding to inform the BMR, and, thus, prevent toxicity.
- Concerning model uncertainty, the mathematical model choice depends on endpoint. There should be an effort towards determining what is the most sensitive endpoint(s) for humans and conducting animal studies to model that endpoint(s).
- Concerning exposure and dose response in human studies, ensure enough similarity to current human exposure profiles (mixture composition) so that a dose-response assessment can be done. Incorporate new epidemiological studies. Evaluate concordance with animal data and consistency across studies. Panel-acknowledged uncertainties include exposure estimates from person to person, shape of human dose-response curve, healthy worker effect, and age dependence.
- Concerning POD determination, uncertainty factors are inherently mathematically inconsistent and that should be conveyed in the discussion of uncertainties when interpreting the POD.
- Concerning dose metric, tissue concentration is preferred. It should be evaluated against a background of variability in AhR-binding expression. There is uncertainty in what level of binding should be considered, in different cell types, tissues, life stage (development). The relationship between dose metric and causation of adverse effects should be examined.

Low-Dose Extrapolation

The Panel reviewed the charge questions and discussed them (Appendix B). The Panel concluded that curve-fitting uncertainty (for a given dataset, dose metric, and model) can be characterized and is useful, but, by itself, it is an incomplete characterization of uncertainty. The Panel acknowledged the difficulty of fully characterizing uncertainty, especially quantitatively. Some panelists argued that the problem is insurmountable and that no meaningful uncertainty analysis is likely to be performable. Other panelists contended that, the difficulties notwithstanding, "good-faith" efforts to do something practical and forthright to characterize uncertainty in low-dose extrapolation would be useful and important. The Panel clarified "good faith" as meaning a characterization that is useful and not misleading to decision makers and is inclusive of approaches that have meaningful support in the scientific community as a whole. Being in "good faith" is more important than being complete (i.e., addressing every uncertain element), especially since completeness is not a realistic goal. From this discussion, the Panel listed four issues for consideration by the U.S. EPA:

- Review alternative data sets, dose metrics, and models to see where consequential uncertainties and impacts on low-dose implications arise.
- Consider the impacts of choices among plausible alternative data sets, dose metrics, models, and other more qualitative choices—issues include how much difference the choices make and also how much relative credence should be put to each alternative as a way of gauging and describing the landscape of imperfect knowledge regarding possibilities for the true dose-response.
    - Hard to do quantitatively, since the factors are not readily expressed as statistical distributions, but can describe the rationale for believing/doubting each alternative in terms of available supporting evidence, contrary evidence, and needed assumptions.
    - Expert judgment methods may be helpful in characterizing the relative weights of scientific credibility among alternatives. The expert judgment process, when conducted systematically, can be thought of as adding data to the assessment of credibility of alternatives, rather than as just an opinion poll.
    - Information on plausibility of alternative low-dose extrapolation approaches can come from external considerations of mode of action, and not just from statistical success at fitting particular (high-dose) data sets.
- Characterizing uncertainty through a variety of approaches could be tried, and their relative merits and shortcomings discussed, as a way forward.
- Consider the sources of potential error, particularly in epidemiological data (e.g., TEF uncertainty and variation in congener mixtures) and if possible quantify their impact on the dose-response assessment.

Considerations for Conducting Uncertainty Analysis

Overall, the Panel was split on whether U.S. EPA should do quantitative uncertainty analyses. The Panel noted that if done on only some of the uncertainties, then results would be misleading and could be misused. Ultimately, the Panel listed seven issues for consideration by the U.S. EPA:

- The Panel recapped what some consider as being the first integrated risk assessment, with structured expert judgment and uncertainty analysis, i.e., the Rasmussen Report (WASH-1400; U.S. Nuclear Regulatory Commission, 1975). In their discussion of the report, the Panel noted that in addition to standard event tree/fault tree modeling, this report also tackled difficult model uncertainty issues involved in accident progression, dispersion of released pollutants in the atmosphere, environmental transport, exposure, health, and economic impacts. And though the Panel also recognized that this method was no longer state-of-the-art, the Panel contended that it represents a good example of a structured approach and methodology that could be built upon.

- The Panel also discussed TEQs used in epidemiological studies, based on intake, and recognized that the key uncertainty in what was measured was not just intake but also involved PK/PD issues. The Panel acknowledged that the TEQ system is regularly used on a concentration basis, but they expressed concern that the qualification becomes lost. TEQs ignore pharmacokinetics and the common practice of rounding to orders of magnitude introduces more error.
- Structure the risk assessment along MOA steps—identify key biochemical measures (~5−10) common across toxic endpoints and identify the degree of meaningful change in effect or effect variance. Make a table with all options for data set, model, etc.; make best estimates/choices and determine which of these choices matter the most to the answer.
- Use expert panels—expert judgment can be collected scientifically (procedures are published). But there are known biases; central tendency estimates work much better than extremes.
- Use supporting studies to fill in critical data gaps—Info filling methods do exist (e.g., PK modeling). Put short-term studies into the "supporting info" category (unless, of course, the risk assessment is for acute exposures, such as chemical spills).
- Be creative in the analysis of uncertainty. Intermediate steps between AhR binding and the end processes can be hypothesized based on data, experiences, and analogies related to other chemicals.
- The 2003 Reassessment presented potency estimates on wide variety of endpoints/models; needed to be more transparent in that discussion. Statistical graphics can be used to convey uncertainties.

Reference

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APPENDIX A: 2009 U.S. EPA DIOXIN WORKSHOP AGENDA

SCIENTIFIC WORKSHOP TO INFORM THE TECHNICAL WORK PLAN FOR U.S. EPA'S RESPONSE TO NAS COMMENTS ON THE HEALTH EFFECTS OF DIOXIN PRESENTED IN U.S. EPA'S DIOXIN REASSESSMENT

Cincinnati, OH

Date: February 18–20, 2009

BACKGROUND/WORKSHOP OBJECTIVE

At the request of the U.S. Environmental Protection Agency (U.S. EPA), the National Academy of Sciences (NAS) prepared a report, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NAS, 2006), that made a number of recommendations to improve the U.S. EPA's risk assessment for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In response, the U.S. EPA will prepare a technical report that addresses key comments on the dose-response assessment for TCDD. The U.S. EPA intends to develop its response through a transparent process that provides multiple opportunities for input.

To assist in this effort, a Workshop will be held to inform the U.S. EPA's evaluation of the NAS recommendations. The Workshop will be open to the public. At the Workshop, the U.S. EPA will solicit input from expert scientists and the public.

The goal of the Workshop is to ensure that the U.S. EPA's response to the NAS comments focuses on the key issues and reflects the most meaningful science. The three main objectives of the Workshop are to (1) identify and discuss the technical challenges involved in addressing the NAS key comments on the TCDD dose-response assessment in the U.S. EPA Reassessment (U.S. EPA, 2003), (2) discuss approaches for addressing these comments, and (3) identify key published, independently peer-reviewed literature, particularly studies describing epidemiologic and *in vivo* mammalian bioassays, which are expected to be most useful for informing the U.S. EPA response.

Workshop participants will be encouraged to think broadly about the body of scientific information that can be used to inform the U.S. EPA's response and to participate in open dialogue regarding ways in which the science can best be used to address the key dose-response issues. This Workshop is similar to scientific workshops being conducted under the new review process for the National Ambient Air Quality Standards (NAAQS)[1] that assess health-related information for criteria pollutants.

[1] Please see http://www.epa.gov/ttn/naaqs/ for more information on the new NAAQS review process.

The Workshop discussions are expected to build upon two prior publications:

1. *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* (U.S. EPA, 2003). This external review draft provides a comprehensive reassessment of dioxin exposure and human health effects. This "dioxin reassessment" was submitted in October 2004 to the National Academy of Sciences (NAS) for review.
2. *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NAS, 2006).

Workshop participants are encouraged to review both of these documents and other relevant materials (e.g., the National Toxicology Program report on TCDD [NTP, 2006]) before the meeting because they provide important insights into the key questions and challenges. There are a number of open comment periods that are intended to facilitate a broad discussion of the issues.

Scientists with significant expertise and experience relevant to the health effects of TCDD or dioxin-like compounds and associated topics will be asked to serve on "expert panels" for discussions throughout the Workshop. Workshop panelists will include a wide range of experts representing many scientific areas needed to assess TCDD dose-response (e.g., epidemiology, human and animal toxicology, nuclear receptor biology, dose-response modeling, risk assessment, and uncertainty analysis). The Workshop panelists will be asked to highlight significant and emerging research and to make recommendations to the U.S. EPA regarding the design and scope of the technical response to NAS comments on the dose-response analysis for TCDD—including, but not limited to, recommendations for evaluating associated uncertainty. Open comment periods will follow each panel discussion session. Public participation will be encouraged by way of these designated open comment periods and, also, by participation in the scientific poster session planned for the second evening (February 19).

U.S. EPA will use the input received during this Workshop as the foundation for its development of a technical work plan for responding to the NAS comments on the TCDD dose-response analysis. The work plan will outline the schedule, process, and approaches for evaluating the relevant scientific information and addressing the key issues. The work plan also will identify the key literature to be utilized in U.S. EPA's response.

As a follow-on activity to this Workshop, a panel is being established under the Federal Advisory Committee Act (FACA) to guide and review the U.S. EPA's response to NAS comments. The FACA panel will be asked to conduct a consultation with the Agency on the draft technical work plan. At the same time, the public will also have the opportunity to provide comments to the FACA panel on the work plan. The final technical work plan will guide the development of the technical report that will constitute the U.S. EPA's response to NAS comments. During the development of this response, the U.S. EPA will seek advice from the FACA panel and the public several times. Finally, the FACA panel will be asked to review the technical report in a public forum.

The preliminary Agenda presented on the following pages may be revised prior to the Workshop following review by the session Co-Chairs; the dates and general timing of the

sessions, however, will not change. A final Agenda and a set of charge questions, intended to provide general direction for the Workshop discussions, will be posted on the Workshop Internet site (http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=199923) prior to the meeting.

A poster session will be held on the evening of the second day (February 19). The purpose of this poster session is to provide a forum for scientists to present recent studies relevant to TCDD dose-response assessment and to encourage open discussion about these presentations.

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U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds, NAS review draft, Volumes 1-3 (EPA/600/P-00/001Cb, Volume 1). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC (December). Available at http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/.

WORKSHOP AGENDA

Day 1

8:00–9:00	**Registration**
9:00–9:30	**Welcome/Purpose of Meeting/Document Development Process**
9:30–9:45	**Panel Comments/Questions on Charge**

9:45–2:45 Session 1: Quantitative Dose-Response Modeling Issues (Hall of Mirrors)

9:45–10:10	**Background/Introductory Remarks**
10:10–10:35	**TCDD Kinetics: Converting Administered Doses in Animals to Human Body Burdens** Presenter: Michael Devito
10:35–11:30	**Panel Discussion**
11:30–1:00	**Lunch**
1:00–2:00	**Panel Discussion cont.**
2:00–2:45	**Open Comment Period**

2:45–3:05 Break

3:05–5:15 Session 2: Immunotoxicity (Hall of Mirrors)

3:05–3:15	**Background/Introductory Remarks**
3:15–4:45	**Panel Discussion**
4:45–5:15	**Open Comment Period**

Day 2

8:00–8:30 **Report-Outs for Sessions 1 and 2 (Hall of Mirrors)**

8:00–8:15 **Report-Out for 1: Quantitative Dose-Response Modeling Issues**

8:15–8:30 **Report-Out for 2: Immunotoxicity**

8:30–11:30 **Sessions 3A and 3B (concurrent sessions)**

8:30–11:30 **Session 3A: Dose-Response for Neurotoxicity and Nonreproductive Endocrine Effects (Hall of Mirrors)**

8:30–8:45 **Background/Introductory Remarks**

8:45–11:00 **Panel Discussion**

11:00–11:30 **Open Comment Period**

8:30–11:30 **Session 3B: Dose-Response for Cardiovascular Toxicity and Hepatotoxicity (Rookwood Room)**

8:30–8:45 **Background/Introductory Remarks**

8:45–11:00 **Panel Discussion**

11:00–11:30 **Open Comment Period**

11:30–1:00 **Lunch**

1:00–2:00 **Report-Outs for Sessions 3A and 3B (Hall of Mirrors)**

The structure of the session report-outs will include the following:

- Summary of session presentation including minority opinion
- Public comments
- Discussion

1:00–1:15 **Report-Out for 3A: Dose-Response for Neurotoxicity and Nonreproductive Endocrine Effects**

1:15–1:30 **Open Comment Period**

1:30–1:45 **Report-Out for 3B: Dose-Response for Cardiovascular Toxicity and Hepatotoxicity**

1:45–2:00 **Open Comment Period**

2:00–5:15 Sessions 4A and 4B (concurrent sessions)

2:00–5:15 Session 4A: Dose-Response for Cancer (Hall of Mirrors)

2:00–2:15 **Background/Introductory Remarks**

2:15–4:45 **Panel Discussion**

4:45–5:15 **Open Comment Period**

2:00–5:15 Session 4B: Dose-Response for Reproductive/Developmental Toxicity (Rookwood Room)

2:00–2:15 **Background/Introductory Remarks**

2:15–4:45 **Panel Discussion**

4:45–5:15 **Open Comment Period**

6:45–8:15 Poster Session (Rosewood Room)

Day 3

8:30–9:30 Report-Outs for Sessions 4A and 4B (Hall of Mirrors)

8:30–8:45 **Report-Out for 4A: Dose-Response for Cancer**

8:45–9:00 **Open Comment Period**

9:00–9:15 **Report-Out for 4B: Dose-Response for Reproductive/Developmental Toxicity**

9:15–9:30 **Open Comment Period**

9:30–3:30	**Session 5: Quantitative Uncertainty Analysis of Dose-Response (Hall of Mirrors)**
9:30–9:40	**Background/Introductory Remarks**
9:40–10:10	**Evidence of a Decline in Background Dioxin Exposures in Americans Between the 1990s and 2000s** Presenter: Matt Lorber
10:10–10:30	**Break**
10:30–11:30	**Panel Discussion**
11:30–1:00	**Lunch**
1:00–2:15	**Panel Discussion cont.**
2:15–2:30	Break
2:30–3:00	**Open Comment Period**
3:00–3:15	**Report-Out for 5: Quantitative Uncertainty Analysis of Dose-Response**
3:15–3:30	**Closing Remarks**
3:30	**Adjourn**

APPENDIX B: 2009 U.S. EPA DIOXIN WORKSHOP QUESTIONS TO GUIDE PANEL DISCUSSIONS

SESSION 1

Dose Metric

Considering all of the endpoints or target tissues, and species that U.S. Environmental Protection Agency (U.S. EPA)'s dose-response modeling might evaluate, what are the best measures of dose (e.g., ingested, tissue concentrations, body burden, receptor occupancy, other surrogate) and why?

Developing Dose-Response Models from Mammalian Bioassays

How best can the point of departure (POD) be determined when the response range is incompletely characterized (i.e., high response at the lowest dose or low response at the highest dose; observed in several key 2,3,7,8-Tetrachlorodibenzo-p-Dioxin [TCDD] studies)?

If considered to be biologically plausible, how can a threshold be incorporated into a dose-response function (e.g., for TCDD cancer data)?

How can nonmonotonic responses be incorporated into the dose-response function?

Developing Dose-Response Models from Epidemiological Studies

How can the epidemiological data be utilized best to inform the TCDD exposure-response modeling? Which epidemiological studies are most relevant?

Supporting Information

For those toxicological endpoints that are Ah receptor-mediated, how would the receptor kinetics influence the shape of the dose-response curve? How would downstream cellular events affect the shape of the dose-response curve? How can this cascade of cellular events be incorporated into a quantitative model of dose-response?

SESSIONS 2, 3A, 3B, 4A, AND 4B

Key Study Selection

For this endpoint, what refinements should be made to the draft criteria for selection of key studies?

What are the specific effects of concern for human health for this endpoint?

Based on the draft criteria for the selection of key studies, what are the key studies informing the shape of the dose-response curve above the POD and the choice of the POD for this endpoint?

Epidemiological Study Utility

How and to what extent do the epidemiological data inform the choice of critical effect?

How can the epidemiological data inform the quantitative dose-response modeling?

Animal Model Utility

Are there types of effects observed in animal models that are more relevant to humans than others? To what extent does information on mode of action (MOA) influence the choice of animal model (species, strain, sex)?

Supporting Information

Are there studies that establish a sufficient justification for departure from the default procedures that address the shape of the dose-response curve below the POD under the cancer guidelines?

Are there studies that establish a sufficient justification for departing from U.S. EPA's default approaches for noncancer endpoints?

To what extent can MOA information clarify the identification of endpoints of concern and dose-response metric for this endpoint? How can the cascade of cellular events for this endpoint be incorporated into a quantitative model of dose response?

SESSION 5

For cancer and noncancer TCDD dose-response assessments, U.S. EPA is interested in developing a quantitative uncertainty analysis addressing both parameter and model uncertainty, if feasible. Uncertainties will include, among others, choice of endpoint; underlying study uncertainties; choice of dose metric; interspecies extrapolations such as kinetic uncertainties; and choice of dose-response model, including threshold models. The U.S. EPA is currently examining techniques and tools for uncertainty analysis—including Bayesian and frequentist approaches.

Identification of Important Uncertainties

What are the major uncertainties pertaining to modeling the animal data?

Consider the dose metric (species or tissue specificity), vehicle of administration, exposure frequency, exposure duration, and POD determination (e.g., benchmark response selection or no-observed-adverse-effect level/lowest-observed-adverse-effect level identification).

What are the major uncertainties pertaining to dose-response modeling below the POD?

Consider how receptor kinetics and downstream cellular event information might be used to bound the uncertainties associated with dose-response modeling below the POD.

What are the major uncertainties in cross-species extrapolation (e.g., half-lives, tissue distribution, and toxicodynamics)?

Consider the primary species dosed with TCDD: mice, hamsters, rats, guinea pigs, and monkeys.

What are the major uncertainties pertaining to intrahuman variability?

Consider what data sets would be useful to represent sensitive subpopulations.

What are other significant sources of uncertainty for the cancer and noncancer assessments?

Considerations for Conducting Uncertainty Analysis

What data sets could be used to quantify uncertainties in cancer and noncancer TCDD dose-response assessments?

Consider dioxin-like compound dose-response data.

Consider MOA information.

What are the appropriate techniques for the TCDD dose-response uncertainty analysis, and what are their respective strengths and weaknesses of these approaches as applied to TCDD?

APPENDIX C: 2009 U.S. EPA DIOXIN WORKSHOP DRAFT SELECTION CRITERIA TO IDENTIFY KEY *IN VIVO* MAMMALIAN STUDIES THAT INFORM DOSE-RESPONSE MODELING FOR 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)[a]

Study Feature	Selection Rationale		
	Primary[b]	*Secondary*[c]	*Currently Excluded*
Chemical, purity, matrix/medium	TCDD-only doses included, purity specified, matrix in which TCDD is administered is identified	TCDD purity or matrix not clearly identified	Studies of dioxin-like compounds (DLCs) or mixtures
Peer review	Independently peer-reviewed, publicly available	Supplementary materials accompanying peer-reviewed publication	Not formally peer-reviewed; literature not publicly available
Study design, execution, and reporting	Clearly documented and consistent with standard toxicological principles, testing protocols, and practice (i.e., endpoint-appropriate, particularly for negative findings)	Testing protocol provides incomplete coverage of relevant endpoint-specific measures, particularly for negative findings	Studies not meeting standard principles and practices
Study subject: species, strain, and sensitivity for given endpoint; litter; life stage; gender	Mammalian species Strain and gender identified Animal age at beginning of treatment identified Litter confounders (within/between) accounted for	Mammalian species, *in vivo*, but only studying an artificially sensitive subject (e.g., knockout mouse)	Non-mammalian or not *in vivo*
Exposure route	Oral	Parenteral (e.g., intravenous, intramuscular, intraperitoneal, subcutaneous)	Inhalation, dermal, ocular
Dose level	Lowest dose ≤200 ng/kg-d for noncancer endpoints and ≤1 µg/kg-d for cancer	Lowest dose >200 ng/kg-d for noncancer endpoints, or >1.0 µg/kg-d for cancer	
Exposure frequency, duration, and timing	Dosing regimen characterized and explained		Characterization/explanation missing or cannot be determined
Controls	Appropriate and well characterized	Effect reported, but with no negative control	
Response	Effect relevant to human health Magnitude outside range of normal variability	Precursor effects, or adaptive responses potentially relevant to human health	Lethality
Statistical evaluation	Clearly described and appropriate to the endpoint and study design (e.g., per error variance, magnitude of effect)	Limited statistical context	

[a] NAS (2006) commented that the selection of data sets for quantitative dose-response modeling needed to be more transparent. These draft criteria are offered for consideration at the kickoff workshop. These criteria would be used to identify candidate studies of non-human mammals that would be used to define the point-of-departure (POD). These criteria are not designed for hazard identification or weight-of-evidence determinations. Studies addressing data other than direct TCDD dose-response in mammals (including toxicokinetic data on absorption, distribution, metabolism, or elimination; information on physiologically-based pharmacokinetic [PBPK] modeling, and mode of action data) will be evaluated separately.

[b] Presents preliminary draft criteria for evaluating a study being considered for estimating a POD in a TCDD dose-response model.

[c] Presents preliminary draft criteria that could qualify a study as primary with support from other lines of evidence (e.g., PBPK modeling), when no study for an endpoint meets the "primary" criteria.

This document is a draft for review purposes only and does not constitute Agency policy.

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APPENDIX B

Evaluation of Cancer and Noncancer Epidemiological Studies for Inclusion in TCDD Dose-Response Assessment

NOTICE

THIS DOCUMENT IS AN EXTERNAL REVIEW DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

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APPENDIX B. EVALUATION OF CANCER AND NONCANCER EPIDEMIOLOGICAL STUDIES FOR INCLUSION IN TCDD DOSE-RESPONSE ASSESSMENT

B.1. EVALUATION OF CANCER STUDIES

B.1.1. NIOSH Cohort Studies

Table B-1. Fingerhut et al., 1991—All cancer sites, site-specific analysis

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. The data sources to ascertain vital status and cause of death information were the Social Security death files, the National Death Index, and the Internal Revenue Service. Vital status could be determined for 98% of the cohort.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. While the authors provide compelling arguments that suggest risks are not unduly biased by lack of cigarette smoking data, they acknowledge potential biases that could exist for other occupational exposure (e.g., asbestos) for which data were lacking.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. There was not a statistically significant linear trend of increasing mortality with increased duration of exposure.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration not satisfied. This study used duration of exposure, at an individual level, as a surrogate measure of TCDD. Duration of exposure determined by number of years workers were involved in processes involving TCDD contamination. Exposure was determined by reviewing, at each plant, operating conditions, job duties, records of TCDD levels in industrial hygiene samples, intermediate reactants, products, and wastes. Exposure assessment was limited and the uncertainty related to exposure measures not fully addressed.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. This is the largest of the occupational cohorts that has been exposed to TCDD. The cohort consisted of 5,172 workers and a total of 265 cancer deaths. Site-specific mortality analyses, including soft tissue sarcoma (n = 4), was limited by small numbers.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.

Response	Criteria satisfied. New England Journal of Medicine, 1991; 324:212–218. Authors address the possibility of bias from lack of control for potential confounders such as smoking and other occupational exposures. They address limitations of using death certificates for identifying certain causes of deaths, and limitations of using duration of employment as an exposure metric.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. Since this study used duration of exposure as the exposure metric, dose-response relationships cannot be quantified.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose-is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. Models incorporated period of latency, and a surrogate measure of cumulative TCDD exposure was modeled. The follow-up interval was sufficiently long (1942–1987).
Conclusion	Overall, quantitative exposure data are lacking on an individual-level basis. Further dose-response analysis should consider updated data for this cohort that includes serum-based measures of TCDD, in addition to an extension of the follow-up period. Given these limitations, this study is not further evaluated for TCDD dose-response assessment.

Table B-2. Steenland et al., 1999—All cancer sites combined, site-specific analysis

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. The study evaluated mortality from all cancer sites (combined). As described in the paper, the sources of vital status and cause of death information were received from the Social Security death files, the National Death Index, and the Internal Revenue Service. Vital status was known for 99.4% of the cohort members, cause of death information is available for 98% of the decedents.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Occupational exposure to asbestos and 4-aminobiphenyl contributed to some excess cancer, but no evidence of confounding for the relationship between TCDD and all cancer mortality was detected following removal of workers who died of bladder cancer. No information is available for cigarette smoking, although dose-response patterns were stronger for nonsmoking related cancers. This finding suggests that smoking is not responsible for excess cancer risk that was observed in the cohort.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.

Response	Consideration satisfied. When a 15-year lag interval was incorporated into the exposure metric a statistically significant dose-response pattern was observed for all cancer sites combined with both a continuous measure of TCDD ($p = 0.05$) as well as one that was log-transformed ($p < 0.001$).
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. The study conducted detailed sensitivity analyses and evaluated different assumptions regarding latency, log-transformed TCDD exposures, and half-life values for TCDD.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. This is the largest of the occupational cohorts with exposures to TCDD. The cohort consisted of 5,132 male workers and a total of 377 cancer deaths. This permits characterization of risk for all cancer sites (combined).
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Journal of the National Cancer Institute, 1999; 91(9):779–786. The authors discussed the potential for bias from smoking, and other occupational exposures for which data for both were lacking at an individual basis.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Exposure scores assigned on an individual level using a job-exposure matrix. The job-exposure matrix was based on estimated factor of contact with TCDD in each job, level of TCCD contamination of materials at each plant over time, and proportion of day worker could be in contact with materials. These factors were multiplied together to derive a daily exposure score, which was accumulated over the working history of each worker to obtain a cumulative measure of TCDD.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. The follow-up of the cohort extended from 1942 until the end of 1993. Greater than 25 years of follow-up have accrued in cohort allowing for latency to be examined. Different assumptions on the half-life of TCDD were evaluated and produced similar results. Latency intervals were incorporated, with strongest associations noted with an interval of 15 years.
Conclusion	This study meets the criteria and considerations noted above but has been superseded and updated by Steenland et al. (2001). Therefore, this study was not considered for further dose-response analyses.

Table B-3. Steenland et al., 2001—All cancer sites combined

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. The study evaluated mortality from all cancer sites (combined). As described by Steenland et al., (1999) the sources of vital status and cause of death information were received from the Social Security death files, the National Death Index, and the Internal Revenue Service. Vital status was known for 99.4% of the cohort members, cause of death information is available for 98% of the decedents.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Occupational exposure to asbestos and 4-aminobiphenyl contributed to some excess cancer, but no evidence of confounding for the relationship between TCDD and all cancer mortality was detected following removal of workers who died of bladder cancer. No information is available for cigarette smoking, although dose-response patterns were similar between smoking and nonsmoking related cancers.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Increased risk estimates were observed in the higher cumulative exposure categories. The dose-response curve was not linear at higher doses.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Exposure metrics considered included cumulative TCDD, log10TCDD, average exposure, and a cubic spline model was also evaluated. Exposure response relationships were also evaluated using TEQs. Exposure scores were assigned on an individual level using a job-exposure matrix. The job-exposure matrix was based on estimated factor of contact with TCDD in each job, level of TCCD contamination of materials at each plant over time, and proportion of day worker could be in contact with materials. Serum levels were measured in 199 workers at one of 8 plants in 1998. Different estimate of the half-life of TCDD were used, and similar results were produced. The paper presented a range in risk estimates thereby conveying the range of uncertainties in risk estimates derived using different measures of exposure.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. This is the largest of the occupational cohorts with exposures to TCDD. The cohort consisted of 3,538 male workers and a total of 256 cancer deaths.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied Am J Epidem, 2001, 154(5):451–458. However, additional details to assess uncertainties associated with characterizing serum data in a subset of workers to remainder of cohort are lacking.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.

Response	Criteria satisfied. The metrics considered included cumulative TCDD, log10TCDD, average exposure, and a cubic spline model was also evaluated. Exposure response relationships were also evaluated using TEQs. Serum lipid TCDD measurements from 170 workers whose TCDD levels were greater than 10 ppt (the upper ranges of a background level) were used along with JEM information, work histories, and a pharmacokinetic elimination model to estimate dose rates per unit exposure score. In this regression model, the estimated TCDD level at the time of last exposure was modeled as a function of exposure scores. The coefficient relating serum levels and exposure scores was then used to estimate serum TCDD levels over time from occupational exposure (minus the background level) for all 3,538 workers. Time-specific serum levels were then integrated over time to derive a cumulative serum lipid concentration due to occupational exposure for each worker.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. Greater than 25 years of follow-up have accrued in cohort allowing for latency to be examined. Different assumptions on the half-life of TCDD were evaluated producing similar results.
Conclusion	Overall, criteria have been satisfied. This study was modeled in the 2003 Reassessment and is considered for further dose-response evaluations herein.

Table B-4. Cheng et al., 2006—All cancer sites combined

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. The study evaluated cancer mortality. The vital status and the information regarding the cause of death were extracted from the Social Security death files, the National Death Index, and the Internal Revenue Service (Steenland et al., 1999). Vital status was known for 99.4% of the cohort members, while cause of death information is available for 98% of the decedents.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. This is the same data set used in the Steenland et al., (2001) paper. Occupational exposure to asbestos and 4-aminobiphenyl contributed to some excess cancer, but no evidence of confounding for the relationship between TCDD and all cancer mortality was detected following removal of workers who died of bladder cancer. No information is available for cigarette smoking, although dose-response patterns were similar between smoking and nonsmoking related cancers.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Slope coefficients are available for all cancers combined under a varying set of assumptions. Little evidence of an association was found when lag interval was not taken into account. Associations strengthened with incorporation of a 10 to 15 year lag interval. Dose-response was nonlinear at higher exposures, suggesting a nonlinear relationship or increased exposure misclassification at higher levels.

4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Compared to the 1st order models, the concentration, and age dependent model (CADM) provided a better fit for the serum sampling data. CADM model exposure estimates are higher than those based on an age only, constant 8.7-year half-life model. As discussed by Aylward et al. (2005b), model exposure estimates are influenced not only by choice of elimination model, but also by choices in regression procedure (e.g., log transformation, use of intercept, and incorporation of background dose term). Other limitations or uncertainties in exposure assessment include the following • Job-exposure matrix based on limited sampling data, and subjective judgment on contact times and factors • Inability to take into account interindividual variability in TCDD elimination kinetics • Dose-rate regressions are based on a small sample of the cohort with serum measures; therefore, regression results may not be representative of remainder of the cohort.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. Largest cohort of TCDD exposed workers. The risk estimates are based on a total of 256 cancer deaths.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Risk Analysis, 2006; 4:1,059–1,071. Additional details to assess uncertainties associated with characterizing serum data can be found in Aylward et al. (2005b); Risk Anal. 25(4):945–956.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Cumulative serum lipid concentrations were estimated for each worker. No other dioxin-like compounds were assessed in this analysis.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. Concentration and age-dependence of TCDD elimination and two compartments (hepatic and adipose tissue) were taken into account when estimating TCDD exposures. Nearly 50 years of follow-up were available permitting an evaluation of latency.
Conclusion	This study met the main criteria and considerations. The study is considered for further dose-response analyses.

Table B-5. Collins et al., 2009—All cancer sites combined, site-specific analysis

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.

Response	Consideration satisfied. Vital status complete for all but two workers.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. No information collected on smoking status, but no excess in lung cancer or nonmalignant respiratory diseases noted. Analyses took into account potential for exposure to pentachlorophenol.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. No dose-response pattern was observed with all cancer sites combined, however, a dose-response pattern was observed with soft tissue sarcoma. The study found no association between TCDD and death from most types of cancer.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. The authors used these serum from 280 former TCP workers to estimate historical exposure levels of TCDD, furans, and polychlorinated biphenyls for all 1,615 workers. Exposure assessment included detailed work history, industrial hygiene monitoring, and the presence of chloracne cases among groups of workers. This data was integrated into a 1-compartment, first-order pharmacokinetic to determine the average TCDD dose associated with jobs in each group, after accounting for the presence of background exposures estimated from the residual serum TCDD concentration in the sampled individuals. The authors did not evaluate departures from linearity, or examine skewness at higher exposures. Exposure levels were not provided.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. Largest study of workers employed in one center, and a total of 177 deaths from cancer were observed. Limited precision in the relative risk estimate was noted for soft tissue sarcoma and TCDD exposures.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Published in Am J Epidemiol, 2009, 170(4):501–506. The authors discuss limitations of using death certificates for identifying deaths from soft tissue sarcoma for which a positive association was noted, assumptions in exposure characterization, and effects of cigarette smoking.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. This study has the largest number of serum samples obtained from a specific plant.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. Although specific analyses of latency were not reported, this cohort had a sufficient length of follow-up for cancer mortality outcomes.

Conclusion	The authors found a statistically significant dose-response trend for soft tissue sarcoma mortality and TCDD exposures. The all-tumor results are not amenable to dose-response analysis because they found no effect. Therefore, this study is considered for quantitative dose-response analysis for the soft tissue sarcoma mortality results, only.

B.1.2. BASF Cohort Studies

Table B-6. Zober et al., 1990—All cancer sites combined, site-specific analysis

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. A large component of the cohort (94 out of 247 workers) was assembled by actively seeking out workers who were alive in 1986 through the "Dioxin Investigation Programme." As a result, it is likely a number of deaths were missed due to the recruitment of survivors. This underascertainment is supported by much lower all cancer SMR one component of the cohort (SMR = 0.48, 95% CI: 0.13–1.23) relative to the general population.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. See above discussion of underascertainment in mortality for some of the cohort members. Although it is likely that other coexposures occurred (e.g., among firefighters), confounding could only occur if these coexposures were associated with both the endpoint and exposure (TCDD) being considered.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. Workers were not categorized on the basis of their exposure, but rather their mortality experience compared to control cohort and the general population. The design of the study does not allow for dose-response to be examined.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration not satisfied. Although years since first exposure was examined, exposure assessment was based on working in various occupational cohorts. Since there was no quantitative assignment of TCDD exposures, the associated uncertainties could not be evaluated.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration not satisfied. There were only 23 cancer deaths in the entire cohort. As such, this study lacked adequate statistical power to detect cancer mortality differences that were moderate in magnitude.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.

Response	Criteria satisfied. Int Arch Occup Envir Health, 1990, 62:139–157. The authors address issues related to the healthy worker effect, multiple comparisons, smoking, and small size of the cohort.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. Risks were derived by comparing mortality rates of the three cohort subsets relative to a control cohort and the general population by time since first exposure categories. Workers were not assigned exposures. There were no quantitative estimates of TCDD exposure.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria not satisfied. While the study was able to indirectly look at variations in risk estimates related to latency by using time since exposure, there were no quantitative estimates of TCDD exposure.
Conclusion	This study is not suitable for dose-response analysis, as it failed the inclusion criteria. Most notably, the lack of exposure data does not permit the use of these data for a dose-response analysis.

Table B-7. Ott and Zober, 1996—All cancer sites combined

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Mortality ascertainment appeared to be fairly complete. The ascertainment of cancer incidence is more difficult to judge as geographical area not covered by a cancer registry.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Information was collected on smoking status, body mass index, and other occupational exposures, however a large portion of the cohort was firefighters who may have been exposed to other occupational carcinogens. However, the recruitment of survivors may results in under-ascertainment of mortality.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Increased cancer incidence was observed in the highest TCDD cumulative exposure category. Risks were most pronounced when a period of 20 years since first exposure was incorporated into the model.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.

Response	Consideration satisfied. Cumulative measure of TCDD expressed was derived from serum measures. Exposure was also estimated by chloracne status of the cohort members. The authors have not addressed the potential implication of deriving TCDD exposure estimates for the whole cohort using sera data that were available for only about half of the cohort.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. For all cancer sites combined, there were 31 deaths. It is the smallest of the occupational cohorts, but the deaths can be grouped into quartiles to allow for evaluation of dose-response relationships.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Occupational and Environmental Medicine, 1996, 53:606–612. A large component of the cohort (94 out of 247 workers) was assembled by actively seeking out workers who were alive in 1986 through the "Dioxin Investigation Programme." As a result, it is likely a number of deaths were missed due to the recruitment of survivors. This underascertainment is supported by much lower all cancer SMR one component of the cohort (SMR = 0.48, 95% CI: 0.13–1.23) relative to the general population (Zober et al., 1990).
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum samples, taken in 1989, were available for 138 surviving workers out of 254 and allowed for cumulative TCDD levels to be estimated using regression techniques in the remainder of the cohort.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. Exposure assignment took into the affect that body mass index had on TCDD half-lives. TCDD levels estimates through back-extrapolation of serum levels based on half-life estimates obtained from previous studies. Latency was considered with stronger association observed in external comparisons incorporating a latency of 20 years. The follow-up of the cohort was lengthy (>50 years).
Conclusion	Given a part of the cohort was based solely on survivors in the in the mid-1980s, the SMR statistic derived from this study underestimates excess mortality relative to the general population. The cohort also includes some firefighters who are recognized to be exposed to other carcinogenic agents—these exposures may be confounding the associations that were reported. However, exposure to TCDD was quantified and the effective dose and oral exposure estimable. Overall, criteria have been satisfied. This study was modeled in the 2003 Reassessment and is considered for further dose-response evaluations herein.

B.1.3. The Hamburg Cohort

Table B-8. Manz et al., 1991—All cancer sites combined, site-specific analyses

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Deaths were identified through medical records of the cohort members. A review of death certificates of the identified cancer deaths found a high degree of concordance (51/54). One of the 136 noncancer death certificates examined indicated an "occult" neoplasm.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Smoking data were similar between exposed and nonexposed cohort based on independent samples. Occupational exposure for which individual data are lacking unlikely to explain dose-response with TCDD.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Dose-response patterns across three levels of exposure observed among those who started work before 1954, and among those who worked for 20 years or longer. Dose-response patterns not evident across whole cohort, among those with less than 20 years of employment, or among those who started after 1954.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Categorical exposures were based on TCDD concentrations in precursor materials, products, waste, and soil from the plant grounds, measured after the plant closed in 1984. Exposure uncertainty examined using a separate group of 48 workers who provided adipose tissue samples. Other surrogate measures of exposure were considered in this study, including duration of exposure and year of first employment.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. For all cancer sites combined, there were 65 cancer deaths for the comparison to the comparison cohort of gas workers. The study is underpowered to look at site-specific cancers.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Lance,t 1991, 338:959–964. The authors discussed potential for misclassification using death certificates, healthy worker effect and their related use of a comparison cohort of gas supply workers, other occupational exposures present at the plant, potential impact and the lack of smoking data.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. Exposure consisted of a large DLC component that was not quantified. Given crude TCDD exposure categorization data, no quantitative exposure metric was derived.

3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. Exposure metrics were constructed that took into account duration of exposure, and periods when exposure was highest. However, exposure estimates did not consider lagged exposure.
Conclusion	This study is not amenable to further TCDD dose-response analysis and is not considered further here because it consisted of a large DLC component that was quantified and no quantitative exposure metric was derived. The dose-response patterns of risks observed across the three exposure groups provide compelling support for an association between TCDD and cancer mortality, particularly, given the associations observed when analyses restricted to those who were hired when TCDD exposures were known to be much higher, and among those who worked for at least 20 years. Subsequent studies improved the exposure assessment through the use of serum measures.

Table B-9. Flesch-Janys et al., 1995; Flesch-Janys et al., 1996 erratum—All cancer sites combined

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Medical records used to identify deaths over the period 1952–1992.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Similarity in smoking rates between control cohort and the exposed workers was similar based on independent surveys. Occupational exposures to benzene, and dimethyl sulfate were unlikely to bias dose-response pattern observed as these exposures occurred in production departments with low-medium levels of exposure.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Dose-response relationship observed across 6 exposure categories, with the cohort of gas supply workers used as the referent.
4. Consideration	Consideration satisfied. Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	The exposure measure was an integrated TCDD concentration over time estimate that back calculated TCDD exposures to the end of the employment. Categorical and continuous TCDD exposures were examined in relation to the health outcome. These efforts improve the exposure assessment of earlier studies.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. For all cancer sites combined, there were 124 deaths in the exposed cohort, and 283 in the cohort of gas supply workers. No site-specific cancers were examined in this paper.

1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Am J Epidemiol, 1995, 1442:1165–1175. The authors discuss the potential role of other occupational exposures (i.e., dimethyl sulfate, solvents, and benzene), smoking, and suitability of the comparison cohort of gas supply workers.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum and adipose tissues were used to estimate TCDD exposure in 190 workers. A one-compartment first-order kinetic model was used to estimate exposure at end of exposure for these workers. Regression methods were then used to estimates TCDD exposures for all workers.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria not satisfied. Exposure was based on half-life estimates from individuals with repeated serum measures. Other dioxin-like compounds were considered with the TOTTEQ exposure metric. No consideration, however, was given to latency or lagged exposures.
Conclusion	The exposure data used within this study are well-suited to a dose-response analysis given the associations observed, the characterization of exposure using serum, and quality of ascertainment of cancer outcomes. However, subsequent methods have been applied to the cohort to derive different exposures to TCDD using area under the curve approaches, which updates the analysis herein. Therefore, subsequent studies (i.e., Becher et al., 1998) will supersede this evaluation.

Table B-10. Flesch-Janys et al., 1998—All cancer sites combined, site-specific analysis

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Mortality follow-up was extended until the end of 1992, an increase in 3 years from previous analyses of the cohort.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Exposure was well characterized using sera data. While serum samples provided only from a subsample of surviving workers, these levels were consistent with expected levels in different production departments. The authors examined other potential occupational coexposures (e.g., β-hexachlorocyclohexane) and indirectly examined the potential effect of smoking on the associations that were detected.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.

Response	Consideration satisfied. A dose-response relationship across quartiles of TCDD was observed with cancer mortality based on the SMR statistic (SMRs = 1.24, 1.34, 1.34, 1.73), and a linear test for trend was statistically significant ($p = 0.01$).
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. The exposure measure was an integrated TCDD concentration over time estimate that back-calculated TCDD exposures to the end of the employment. Categorical and continuous TCDD exposures were examined in relation to the health outcome. These efforts improve the exposure assessment of earlier studies.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. For all cancer sites combined, there were 124 cancer deaths.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Environ Health Perspect, 1998, 106(2):655–662. The authors address uncertainties in the estimation of exposure, describe the potential for confounding from β-2,4,5-T, hexachlorocyclohexane, and cigarette smoking. In fact, they showed that blood levels of TCDD were not associated with smoking in a subsample suggesting little bias from lack of smoking data.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum samples, taken from 190 workers were used to derive TCDD levels for the entire cohort. Methods used to estimate exposure took into account elimination of TCDD during employment periods when exposure took place, and the methods of the area under the curve was used as it takes into account variations in concentration over time, and reflects cumulative exposure.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. Exposure estimated based on half-lives observed in individuals with repeated samples. Area under the curve approach was used which is an improvement from past characterizations of exposure in this cohort.
Conclusion	The study provides data suitable for dose-response modeling. Derivation of exposure was done using current understanding of elimination of TCDD. Estimates of risks were derived from external comparisons to the general population that are unlikely to be biased by healthy worker effect, but risks generated using internal cohort comparisons would be preferable. Becher et al., (1998) assessed this same data taking cancer latency into account, therefore Flesch-Janys et al., (1998) will not be further considered for dose-response modeling.

Table B-11. Becher et al., 1998—All cancer sites combined

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Medical records used to identify deaths over the period 1952–1992. The follow-up interval was lengthy.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Risks adjusted for exposures to TEQ, β-hexachlorbenzene, and employment characteristics. Smoking was shown to be similar to the comparison cohort of gas workers.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. A variety of exposure measures for both TCDD and TEQs found positive associations with cancer mortality.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. The exposure measure was an integrated TCDD concentration over time estimate that back-calculated TCDD exposures to the end of the employment. Categorical and continuous TCDD exposures were examined in relation to the health outcome. Different models explored the shape of the dose-response curve. These efforts improve the exposure assessment of earlier studies.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. For all cancer sites combined, there were 124 cancer deaths.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Environ Health Perspect, 1998, 106(2):663–670. The authors discuss uncertainties associated with their use of exposure metrics, inability to evaluate effects for PCDD/Fs other than dioxin due to high correlations with β-HCH, and inability to characterize risks associated with exposures in children.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. The authors derived a measure of cumulative dose as a time-dependent variable ("area under curve") using serum measures available in a sample of 275 workers.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. TCDD levels estimates through back-extrapolation of serum levels based on half-life estimates obtained from previous studies. Latency was considered, and a variety of exposure metrics including nonlinear relationships were evaluated.

Conclusion	In this paper, a variety of exposure metrics were found to be positively associated with cancer mortality. The additional lifetime risk of cancer corresponded to a daily intake of 1pg ranged between .01 and 0.001. This study was modeled in the 2003 Reassessment and is considered for further dose-response evaluations herein.

B.1.4. The Seveso Cohort Studies

Table B-12. Bertazzi et al., 2001—All cancer sites combined, site-specific analyses

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Mortality appears to be well captured from the vital statistics registries in the region (99% complete). Vital status was ascertained using similar methods for both the exposed and reference populations. Both cancer and noncancer mortality outcomes were evaluated. Ideally, would have evaluated incident rather than decedent outcomes for cancer.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Individual-level data on potential confounders (i.e., age, calendar period, and gender) were adjusted for. Information from other independent surveys suggests similarity between smoking behaviors across the regions. Comparison of cancer mortality rates before the time of the accident between the regions also revealed no differences.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied (for all cancers combined). No statistically significant excesses noted in Zone A, or Zone B relative to reference area. Evidence of an exposure-response relationship was detected for lymphatic and hematopoietic tissues by number of years since first exposure.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration not satisfied. Subjects were assigned to one of the zones (A, B, R, or reference) based on official residence on the day of the accident or at entry into the area. Exposure misclassification is likely and lack of individual-level data precludes an examination of this source of error.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. In total, 27, and 222, cancer deaths were found among residents of Zones A, and B, respectively. This allowed examined of gender-specific effects.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.

Response	Criteria satisfied. Am J Epidemiol, 2001 Jun 1; 153(11):1031–1044. Authors discuss completeness of mortality ascertainment, diagnostic accuracy of death certificates particularly with respect to diabetes, limited available of blood dioxin measures that did not permit estimation of TCDD dose on an individual-level basis.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. Individual-level exposure data are unavailable. Exposure based on place of residence at time of the explosion. Soil sampling performed indicated considerable variability in TCDD levels within each region. In addition, place of residency at time of explosion does not ensure individuals were at their home around the time of the accident.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria not satisfied. An ecological measure of exposure (region of residency at time of accident) was used to categorize individuals according to their possible exposure. Latencies were considered. While such an approach has value for identifying wherever excesses occurred among highly exposed populations, it is not precise enough to conduct a quantitative dose-response analysis.
Conclusion	The lack of individual-level exposure data precludes quantitative dose-response modeling using these data.

Table B-13. Pesatori et al., 2003—All cancer sites combined, site-specific analyses

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Mortality was ascertained from 1977−1996, and, as reported in other related manuscripts, appears to be well captured from the vital statistics registries in the region (99% complete). Cancer incidence data was available from 1977−1991.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Individual-level data on potential confounders (i.e., age, calendar period, and gender) were adjusted for.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. Although risk of all cancer mortality was not associated with zone of residence, increased risk of cancer incidence was observed in Zone A. Among men, excess lymphatic and hematopoietic cancer incidence was observed in Zone A (primarily to non-Hodgkin's lymphoma). Soft tissues sarcoma cancer incidence was also associated with residence in Zone R among males, but not the more highly exposed zones (A and B). Among females living in Zones A and B, higher rates were observed for multiple myeloma (RR = 4.9, 95% CI = 1.5−16.1), cancer of the vagina (RR = 5.5, 95% CI = 1.3−23.8), and cancer of the biliary tract (RR = 3.0, 95% CI = 1.1−8.2).

4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration not satisfied. Subjects were assigned to one of the zones (A, B, R, or reference) based on official residence on the day of the accident or at entry into the area. Exposure misclassification is likely and lack of individual-level data precludes an examination of this source of error.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied for some endpoints, although several of the cancer specific mortality results among women were based on very small number of deaths (i.e., <5).
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Occup Env Med, 1998; 55:126–131. Authors discuss limitations such as residency-based exposure assignment, absence of smoking, differential and death certification in exposed versus nonexposed areas.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. Individual-level exposure data are unavailable. Exposure based on place of residence at time of the explosion. Soil sampling performed indicated considerable variability in TCDD levels within each region. In addition, place of residency at time of explosion does not ensure individuals were at their home around the time of the accident.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria not satisfied. An ecological measure of exposure (region of residency at time of accident) was used to categorize individuals according to their possible exposure. Latencies were considered. While such an approach has value for identifying wherever excesses occurred among highly exposed populations, it is not precise enough to conduct a quantitative dose-response analysis.
Conclusion	No dose-response patterns evident in the study, and the study lacked quantifiable measures of TCDD at an individual-level basis. The data are not well suited for dose-response analysis.

Table B-14. Consonni et al., 2008—All cancer sites combined, site-specific analyses

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Mortality appears to be well captured from the vital statistics registries in the region (99% complete). Both cancer and noncancer mortality evaluated, although diagnostic accuracy of death certificates is likely low. Ideally, would have evaluated incident rather than decedent outcomes for cancer.

2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Individual-level data on potential confounders (i.e., age, calendar period, and gender) were adjusted for. Comparison of cancer mortality rates before the time of the accident between the regions also revealed no differences. Information from other independent surveys suggests similarity between smoking behaviors across the regions.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied for some outcomes. For all cancer sites combined, no evidence of dose-response was observed relative to general population across Zones A, B and R. Only statistically significant excess found in Zone A was for chronic rheumatic disease but based on only three deaths. Higher cancer excesses were found in Zone A after a latency period was incorporated; however, no dose-response relationship observed with this latency period. Evidence of an exposure-response relationship was detected for lymphatic and hematopoietic tissues by zone of residence.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration not satisfied. Subjects were assigned to one of the zones (A, B, R, or reference) based on official residence on the day of the accident or at entry into the area. Exposure misclassification is likely and lack of individual-level data precludes an examination of this source of error.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. In total, 42, 244, and 1,848 cancer deaths were found among residents of Zones A, B, and R respectively.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Am J Epidemiol, 2008, 167:847–858. Authors discuss potential for selection bias, limitation of residential based measure of exposure, similarities of mortality ascertainment in exposed and referent populations, and multiple testing.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. Individual-level exposure data are unavailable. Exposure based on place of residence at time of the explosion. Soil sampling performed indicated considerable variability in TCDD levels within each region. In addition, place of residency at time of explosion does not ensure individuals were at their home around the time of the accident.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria not satisfied. An ecological measure of exposure (region of residency at time of accident) was used to categorize individuals according to their possible exposure. Latencies were considered. While such an approach has value for identifying wherever excesses occurred among highly exposed populations, it is not precise enough to conduct a quantitative dose-response analysis.

Conclusion	The lack of individual-level exposure data precludes quantitative dose-response modeling using these data.

Table B-15. Baccarelli et al., 2006—Site-specific analysis

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Polymerase chain reaction (PCR) methods were used to describe outcome measures. The prevalence of t(14; 18) was estimated as those individuals having a t(14; 18) positive blood sample divided by the t(14; 18) frequency (number of copies per million lymphocytes).
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Questionnaire data were used to collect information on cigarette smoking. Other potential confounders (age, smoking status, and duration of smoking). In addition, both exposure and outcome were objectively and accurately measured.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration was not satisfied. Associations were detected between the frequency of t(14; 18) and plasma TCDD levels as well as zone of residence at the time of the explosion. No association was detected for these exposure measures and prevalence of t(14; 18). A dose-response trend was detected for TCDD and the mean number of t(14;18) translocations/10^6 lymphocytes, however the relevance of t(14; 18) in lymphocytes to non-Hodgkin's lymphoma is uncertain.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. The authors highlight that exposure metrics represent both past and current body burdens. They employ several different exposure metrics of TCDD: place of residence (Zone A, B, R or reference), categorical serum measures, a linear term, log (base 10) transformed TCDD, and individuals with chloracne diagnosed after the accident.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. Analyses are made using 72 highly exposed, and 72 low exposed individuals.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Carcinogenesis, 2006, 27(10):2001–2007. The authors discuss the limitation of using t(14; 18) translocations as an outcome measure, and the uncertain role it plays in the development of non-Hodgkin's lymphoma.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.

Response	Criteria satisfied. A total of 144 subjects were included in the study. This included 72 subjects who had low exposures, and 72 who had high exposures based on serum concentrations.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. A variety of measures were employed including current TCDD levels, as well as surrogates of exposure at the time of the accident.
Conclusion	While an association was observed with the frequency of t(14; 18) translocation, it is uncertain whether this translates into an increased risk of non-Hodgkin's lymphoma. Given the speculative nature of this endpoint and lack of demonstrated adverse effect, dose-response analyses for this outcome were not conducted.

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3 **Table B-16. Warner et al., 2002—Breast cancer incidence**
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1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Diagnoses of incident breast cancer were based on interview and information from medical records appears thorough. Of the 15 cases of breast cancer, 13 were confirmed by pathology and the remaining 2 by surgery report only. Three cases of breast cancer were excluded which represents a large proportion of the total cases identified. This would reduce sample size and could result in bias if the exclusion was association with TCDD exposure.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Information was collected on an extensive series of risk factors by using an interviewer administered questionnaire. Participation rates for the survey were fairly good (80%).
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Limited evidence (not statistically significant) of a dose-response when TCDD was analyzed as a categorical variable; only one breast cancer case was in the referent exposure category. In the analysis of TCDD as a continuous measure ($\log_{10}$TCDD), the hazard ratio associated with a 10-fold increase in TCDD serum levels was 2.1 (95% CI: 1.0–4.6).
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Different exposure metrics were considered in these analyses (categorical, continuous, measures on a log-scale). Exposure data are of high quality as they are based on serum samples taken among women near the time of the accident. As such, exposure assignment is not dependent on as many assumption as used in occupational cohorts were back-extrapolation for many years had to be performed.

5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration somewhat satisfied. Inadequate follow-up for cancer limited the number of cases available. Sample size also limited the conclusions draw from the categorical analysis based on very few cases for some exposure categories.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Paper published in Environ Health Perspect, 2002 Jul, 110(7):625–628. A major limitation of the study is the small number of incident cases of breast cancer (n = 15), important strengths of the study include characterization of TCDD using serum collected near the time of the accident.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum was used to estimate TCDD levels in 981 of 1271 eligible women who had lived in either of the two contaminated sites in 1976. Data represent an objective measure of TCDD near the time of the exposure. Data obtained near the time of exposure which minimized the potential for exposure misclassification.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. Exposure characterized using serum measures obtained close to the time of the accident.
Conclusion	While characterization of exposure and availability of other risk factor data at an individual-level basis are important strengths of this study, small sample size (n = 15 cases) based on inadequate follow-up is a key limitation. Quantitative dose-response analyses were conducted using this study, but continued follow-up of the study population or consideration of all cancer outcomes would be valuable.

B.1.5. The Chapaevsk Study

Table B-17. Revich et al., 2001—All cancer sites combined, and site-specific analyses

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration cannot be evaluated. Insufficient details are provided in the paper to gauge the completeness and coverage of the cancer registry and mortality data. Health outcomes were studied on the basis of information in the official medical statistics.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. Given that this is an ecological study, bias may be present.

3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration cannot be evaluated. Dose-response was not evaluated as exposure was based on residency in the region vs. no residency.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration not satisfied. No individual-level exposure estimates were used.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. A total of 476 cancer deaths were observed among males, and 376 cancer deaths observed among females. The precision of the SMRs is demonstrated with fairly narrow confidence intervals for many causes of death.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria not satisfied. Published in Chemosphere, 2001, 43(4–7):951–966. Authors do not address the completeness of the mortality follow-up, and whether there are differences in death registrations between regions. The authors do acknowledge, however, that new investigations being undertaken would characterize exposure using serum-based measures.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. It is a cross-sectional study that compares mortality rates between regions. No individual-level exposure data available.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria not satisfied. No individual-level exposure estimates were used in the study.
Conclusion	These cancer data are cross-sectional in nature and not appropriate for a dose-response analysis.

B.1.6. The Air Force Health ("Ranch Hands") Study

Table B-18. Akhtar et al., 2004—All cancer sites combined and site-specific analyses

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Cancer incidence and mortality based on information from repeated medical examinations, medical records and death certificate.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.

Response	Consideration not satisfied. The risk estimates were adjusted for a number of factors measured on an individual level including smoking. However, analyses are unable to distinguish between exposure to TCDD and 2,4-D as both were used in equal parts in the formulation of Agent Orange.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. There is evidence of a dose-response for all cancers and for some site-specific cancers (i.e., malignant melanoma, and prostate cancer).
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. High quality exposure data for most veterans was collected, so extrapolation to other members of the cohort was not required. The serum dioxin measurements also correlated well with reported skin exposure to herbicide in Vietnam, but collection of the samples 25 years later required back-extrapolation.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. In total, 117 incidence cancers identified in the Ranch Hands cohort. For those sites with a dose-response association, malignant melanoma and prostate cancer, there were 16 and 34 incident cases, respectively.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Published in J Occup Environ Med, 2004, 46(2):123–136. Authors highlight that this is only cancer incidence study in US veterans, and the lengthy interval of follow-up (35–40 years)—both important strengths of the study. They addressed potential bias from healthy-worker effect, and uncertainties surrounding the estimation of TCDD exposure (extrapolation 30 years after exposure), as well as exposure to other chemical exposures. Study uses incident outcomes for cancer.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Individual exposure estimates are based on measurements of dioxin serum lipid concentrations. They were available for 1,009 Ranch Hands and 1,429 in the comparison cohort.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. TCDD exposures at the end of duty were estimated by back-extrapolating 1987 serum values.
Conclusion	The major limitation of the study is the inability to isolate effects of TCDD from other chemicals used in the formulation of the herbicides. This limitation precludes dose-response modeling of the TCDD and cancer outcomes data.

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Table B-19. Michalek and Pavuk, 2008—All cancer sites combined

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Cancer incidence was ascertained through the use of medical records. Death certificate were used to identify some malignancies. Little data is provided on the number of individuals lost to follow-up, however the same mechanisms of case ascertainment were applied to both the comparison and Ranch Hand cohorts.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. Information collected from repeated physical examinations allowed for the adjustment of risk factors such as smoking. Agent Orange was a 50% mixture of 2,4-D and TCDD; therefore, potential for confounding by other coexposures is likely.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied for some comparisons. Statistically significant associations were noted with cancer incidence and TCDD when analyses were restricted to workers who served at most two years in Southeast Asia and those who sprayed more than 30 days before 1967.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Initial TCDD dose were estimated at the end of the tour of duty for the Ranch Hands. Individual-level serum dioxin measurements correlated well with correlated with days of spraying and calendar period of service, but collection of the samples roughly 20 years later required back-extrapolation.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. A total of 347 incident cases of cancer were used in the analyses. For stratified analyses, statistical power is more limited. For example, only 67 incident cancer in the subset of workers who spent less than 2 years in Southeast Asia, and sprayed for at least 30 days before 1967.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied J Occup Environ Med 2008; 50:330–340. The authors discuss issues related to exposure misclassification error, and suggest approaches for improving characterization of days of spraying. Congener specific data were unavailable, thereby not allowing for congener specific risks or adjustments to be made.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. TCDD data was available for 986 veterans in the Ranch Hand cohort, and 1,597 members of the comparison cohort.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.

Response	Criteria satisfied. TCDD exposures at the end of duty were estimated by back-extrapolating 1987 serum values.
Conclusion	Ranch Hand veterans were exposed to other contaminants in the herbicides that were mixed, thereby making it difficult to determine independent effects of TCDD on cancer. In particular, 2,4-D has been shown to be associated with some cancers, notable cancer of the prostate. This limitation precludes dose-response modeling of TCDD and cancer using data from this cohort.

B.1.7. Other Studies of Potential Relevance to Dose-Response Modeling

Table B-20. 't Mannetje et al., 2005—All cancer sites combined, site specific analyses

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. National records for death registrations through the New Zealand Health Information Service (NZHIS). Subjects not registered as having died during the study period were confirmed to be actually alive and resident in New Zealand using the New Zealand Electoral Roll, drivers' license, and social security records.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. Seventeen percent of workers were lost to follow up but it is unclear if bias resulted. The dichotomous exposure measure was based on exposure to TCDD, chlorinated dioxins and phenoxy herbicides, so confounding is a possibility by these coexposures.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Dose-response evidence for duration of employment and elevated mortality noted only in synthesis workers.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Exposure measures were limited to duration of employment and exposed/unexposed.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. For all cancer sites combined, there were 43 cancer deaths among the production workers, and 35 such deaths among the sprayers. Site-specific cancer analyses are limited by small sample sizes.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria not satisfied Occup Env Med, 2005; 62:34–40. A high percentage of the cohort was lost to follow-up (17%). The authors fail to mention this important limitation in this paper.

2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. This study used duration of exposure, at an individual level, as a surrogate measure of TCDD.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria not satisfied. Exposure was defined according to duration, and not concentrations of TCDD. Latency intervals were not evaluated.
Conclusion	Overall, quantitative exposure data are lacking for TCDD and limited dose-response relationships were observed across duration of exposure categories. Furthermore, confounding by coexposures is a possibility. Taken together, these data are not suitable for inclusion in a dose-response analysis

Table B-21. McBride et al., 2009b—All cancer sites combined, site-specific analysis

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. The New Zealand Health Information Service Mortality Collection and the Registrar-General's Index to Deaths. Additional searches were based on the last known address from the work record; the electoral roll and the habitation index; the telephone book; the internet; and Terranet property information database. An additional search was carried out through the Births, Deaths, and Marriages office of the New Zealand Department of Internal Affairs. Lastly, automated personnel and pension records were also used to locate past New Plymouth workers and identify some deaths.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. Considerable amount of workers were lost to follow up (22%), but it is unclear if bias resulted. The dichotomous exposure measure was based on exposure to TCDD, chlorinated dioxins and phenoxy herbicides, so confounding is a possibility by these coexposures.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. There was no examination of dose-response effects.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Dichotomous exposure (exposed/unexposed) and duration of employment were examined from job exposure classification assessed via occupational history records industrial hygienists/factory personnel knowledge and questionnaires. Authors discuss limitations in the assignment of exposure among cohort members.

5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration not satisfied. A low number of deaths (n = 76) may have limited ability to detect effects small in magnitude and exposure-response relationships.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Published in Occup Medicine, 2009; 59(4):255–263. The authors highlight cohort lost to follow-up, the limited size of the cohort, differences in cohort definitions between sprayers and producers, and the potential for other exposures during employment at the plant.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. TCDD exposures were not quantified.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria not satisfied. Effective dose could not be estimated given the lack of individual-level exposure data.
Conclusion	The study lacks the quantification of exposures at an individual level, precluding dose-response analysis. This study is not considered further in the dose-response modeling analysis.

Table B-22. McBride et al., 2009a—All cancer sites combined, site-specific analysis

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. The New Zealand Health Information Service Mortality Collection and the Registrar-General's Index to Deaths were used to identify deaths. Additional searches were based on the last known address from the work record; the electoral roll and the habitation index; the telephone book; the internet; and several other public databases in New Zealand. An additional search was carried out through the Births, Deaths, and Marriages office of the New Zealand Department of Internal Affairs. Lastly, automated personnel and pension records were also used to locate past New Plymouth workers and identify some deaths.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Workers lost to follow-up were an unlikely source of bias especially for internal analyses. Confounding by other coexposures (e.g., 2,4,6-TCP) unlikely to have resulted in bias, due to presumed poor correlation with TCDD.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.

Response	Consideration not satisfied. The linear test for trend for TCDD exposure was not statistically significant for all cancer sites (combined), as well as lung cancer mortality. Dose-response relationships were not apparent across quartiles of TCDD exposure for all cancer sites combined, digestive cancers, lung cancer, soft tissue sarcomas or non-Hodgkin's Lymphoma.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Cumulative exposure to TCDD as a time-dependent metric was estimated for each worker from serum samples, but the authors did not examine a continuous measure of TCDD exposure (lagged or unlagged).
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Published in J Occup Environ Med 51:1049−1056. This paper discussed the 22% of the cohort lost to follow-up, differences in cohort definitions between sprayers and producers, and the potential for other exposures during employment at the plant.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum measures available for 346 workers were used to derive TCDD exposures for the entire cohort using the area under the curve approach.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria satisfied. Effective dose could be estimated from serum-derived cumulative exposure estimates.
Conclusion	Given that no dose-response associations were found, the data are not suited to dose-response analysis.

Table B-23. Hooiveld et al., 1998—All cancer sites combined, site-specific analysis

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Outcomes were mortality. Few deaths expected to be missed since only 5% of the cohort was lost to follow-up or had emigrated.

2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. Although dioxin-like compounds (PCDDs, PCDFs, and PCBs) were measured in the serum samples, these were not incorporated into the analysis. Therefore, confounding cannot be ruled out as an explanation of the reported association.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. A dose-response pattern was observed for internal cohort comparison for all cancer mortality, with RRs of 5.0 and 5.6 for the medium and high exposure, respectively. Dose-response patterns evident for lung cancer as well.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Detailed occupational histories to assign dichotomous exposures (exposed/unexposed) based on maximum exposure levels. Although serum data also collected for TCDD and other coexposures (PCDDs, PCDFs, and PCBs), study only presents data for TCDD exposure. TCDD exposures at time of maximum exposure were extrapolated from measured serum.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration not satisfied for internal cohort comparisons in either men or women. Among men, only 7 cancer deaths were observed among those in the unexposed part of the cohort, and 51 among exposed workers. For external cohort comparisons, a total of 20 deaths were observed.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Am J Epidemiol, 1998, 147:891–901. The authors address potential limitations of estimating TCDD exposure from a subsample of surviving workers, lack of smoking data, the healthy worker effect, and relevance of other occupational exposures.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum samples were obtained from 94 of 144 subjects who were asked to participate in serum measurement study. Of these, a further 44 excluded due to absence due to holiday or work (n = 22), and nonexposed workers excluded because matching exposed worker not participating (n = 20). TCDD levels were extrapolated to the time of maximum exposure.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined.
Response	Criteria not satisfied. Exposures assigned based on levels at maximum exposure. Assignment of exposure based on nonrepresentative sample of 50 survivors among the occupational cohort.
Conclusion	The small number of identified cancer deaths, limitations in terms of the exposure assignment (based on nonrepresentative sample, and maximum exposure level) and concern over potential confounding by coexposures preclude using these data for a dose-response analysis.

B.2. EVALUATION OF NONCANCER STUDIES

B.2.1. NIOSH Cohort

Table B-24. Steenland et al., 1999—Mortality (noncancer)

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. The study evaluated mortality from all cancer sites (combined). As described in the paper, the sources of vital status and cause of death information were received from the Social Security death files, the National Death Index, and the Internal Revenue Service. Vital status was known for 99.4% of the cohort members, cause of death information is available for 98% of the decedents.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. External comparisons for all-cause and cardiovascular mortality do not appear to be affected by the "healthy worker effect" as similar patterns were observed with internal cohort comparisons. Nonetheless, internal cohort comparisons are unable to adjust for many of the individual-level risk factors for cardiovascular disease.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. A dose-response relationship was observed with ischemic heart disease (linear test for trend $p = 0.05$), and with TCDD on a log-transformed scale the p-value was <0.001.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. The study conducted detailed sensitivity analyses and evaluated different assumptions regarding latency, log-transformed TCDD exposures, and half-life values for TCDD. Associations were stronger for log-transformed values, and latency intervals of 15 years.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. This is the largest of the occupational cohorts with exposures to TCDD. The cohort consisted of 5,132 male workers and a total of 456 deaths from ischemic heart disease. This permits characterization of risk for all cancer sites (combined).
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied Journal of the National Cancer Institute, 1999, 91(9):779–786. The authors discussed the potential for bias from smoking, and other occupational exposures for which data for both were lacking at an individual basis.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.

Response	Criteria not satisfied. Exposure scores assigned at an individual level based on job-exposure matrix (JEM). The JEM was based on estimated factor of contact with TCDD in each job, level of TCCD contamination of materials at each plant over time, and proportion of day worker could be in contact with materials. These factors were multiplied together to derive a daily exposure score, which was accumulated over the working history of each worker to obtain a cumulative measure of TCDD.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. The follow-up of the cohort extended from 1942 until the end of 1993. Greater than 25 years of follow-up have accrued in cohort allowing for latency to be examined. Different assumptions on the half-life of TCDD were evaluated and produced similar results. Latency intervals were incorporated, with strongest associations noted no lag. Suggests mechanisms occur at the same time as exposure. However, noncancer mortality is not a viable endpoint to consider for further dose-response analysis.
Conclusion	TCDD exposures were quantified in this study, and a dose-response relationship was observed with ischemic heart disease mortality. The sample size was sufficient, and the follow-up interval was lengthy. However, no individual-level data were available for cardiovascular conditions, and the inability to adjust for these exposures introduces considerable uncertainty into the risk estimates. Furthermore, noncancer mortality is not considered a viable endpoint for dose-response analysis.

Table B-25. Collins et al., 2009—Mortality (noncancer)

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Vital status complete for all but two workers.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. No information collected on smoking status, but no excess in lung cancer or nonmalignant respiratory diseases noted. Analyses took into account potential for exposure to pentachlorophenol. External cohort comparisons should be interpreted cautiously due to healthy worker effect, but internal cohort comparisons should not be influence by this bias.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. No statistically significant mortality excess for any noncancer mortality outcome evaluated. This included ischemic heart disease, stroke, nonmalignant respiratory disease, ulcers, cirrhosis, and external causes of death (accidents). Modeling of continuous measure of TCDD was not related to diabetes, ischemic heart disease, or nonmalignant respiratory mortality.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.

Response	Consideration satisfied. The authors used these serum from 280 former TCP workers to estimate historical exposure levels of TCDD, furans, and polychlorinated biphenyls for all 1,615 workers. Exposure assessment included detailed work history, industrial hygiene monitoring, and the presence of chloracne cases among groups of workers. This data was integrated into a 1-compartment, first-order pharmacokinetic to determine the average TCDD dose associated with jobs in each group, after accounting for the presence of background exposures estimated from the residual serum TCDD concentration in the sampled individuals. The authors did not evaluate departures from linearity, or examine skewness at higher exposures. No presentation of exposure levels was provided.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. A total of 662 deaths were observed. Of these, 218 were from ischemic heart disease, and 16 from diabetes (two outcomes for which associations have been noted elsewhere).
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Published in Am J Epidemiol, 2009, 170(4):501–506. The authors discuss potential for exposure misclassification, large size of the cohort, lengthy follow-up interval, and large number of workers who provided serum from which TCDD exposures were estimated.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. This study has the greatest number of serum samples obtained from a specific plant.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. Noncancer mortality is not a viable endpoint to consider for further dose-response analysis.
Conclusions	No dose-response associations were noted for noncancer mortality outcomes. The data are, therefore, not suited for dose-response modeling.

B.2.2. BASF Cohort

Table B-26. Ott and Zober, 1996—Mortality (noncancer)

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Mortality ascertainment appeared to be fairly complete.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.

Response	Consideration satisfied. Information was collected on smoking status, body mass index, and other occupational exposures, however a large portion of the cohort was firefighters who may have been exposed to other occupational carcinogens. However, the recruitment of survivors may results in under-ascertainment of mortality.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. For external cohort comparisons across the three TCDD exposure categories, there was no dose-response pattern observed for any of the noncancer causes of death. Cox regression risk estimates for all cause or circulatory disease mortality when TCDD was modeled as a continuous variable were not statistically significant.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Cumulative measure of TCDD expressed was derived from serum measures. Exposure was also estimated by chloracne status of the cohort members. The authors have not addressed the potential implication of deriving TCDD exposure estimates for the whole cohort using sera data that were available for only about half of the cohort.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. For all causes of death, there were 92 deaths, while 37 circulatory deaths. Many of the cause-specific death had less than 5 deaths in the upper exposure category.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Occup Environ Med, 1996, 53:606–612. A large component of the cohort was assembled by actively seeking out workers who were alive in the mid 1980s. As a result, it is likely a number of deaths were missed. This is supported by much lower SMRs in this component of the cohort published in earlier studies of the cohort. This underascertainment of mortality results in biased SMR statistics (underestimated). The authors do highlight the value of the serum based measures to estimate TCDD exposure
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum samples, taken in 1989, were available for 138 surviving workers out of 254 and allowed for cumulative TCDD levels to be estimated using regression techniques in the remainder of the cohort.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. Exposure assignment took into the affect that body mass index had on TCDD half-lives. TCDD levels estimates through back-extrapolation of serum levels based on half-life estimates obtained from previous studies. Latency was considered with stronger association observed in external comparisons incorporating a latency of 20 years. The follow-up of the cohort was lengthy (>50 years). However, noncancer mortality is not a viable endpoint to consider for further dose-response analysis.

Conclusion	No associations noted with any noncancer deaths. External comparisons should be treated cautiously especially for cardiovascular mortality which is recognized to often be biased by the healthy-worker effect. In the absence of any outcome with an association with TCDD exposure, dose-response analyses of these data were not undertaken.

B.2.3. Hamburg Cohort

Table B-27. Flesch-Janys et al., 1995; Flesch-Janys et al., 1996 erratum—Mortality (noncancer)

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Medical records used to identify deaths over the period 1952−1992.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Similarity in smoking rates between control cohort and the exposed workers was similar based on independent surveys. Occupational exposures to benzene, and dimethyl sulfate were unlikely to bias dose-response pattern observed as these exposures occurred in production departments with low to medium levels of TCDD exposure.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Dose-response relationship observed for all-cause mortality, cardiovascular mortality, and ischemic heart disease mortality across 6 exposure categories, with the cohort of gas supply workers used as the referent. The linear tests for trend for these three outcomes were all statistically significant ($p < 0.05$).
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. The exposure measures was an integrated TCDD concentration over time estimate that back-calculated TCDD exposures to the end of the employment. Categorical and continuous TCDD exposures were examined in relation to the health outcome. These efforts improve the exposure assessment of earlier studies.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. For all causes of death combined, there were 414 deaths in the exposed cohort, and 943 in the cohort of gas supply workers. A total of 157 and 76 deaths from cardiovascular disease, and ischemic heart disease were noted. The corresponding number in the cohort of gas supply workers was 459, and 205, respectively.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Am J Epidemiol, 1995, 1442:1165–1175. The authors discuss the potential role of other occupational exposures (i.e., dimethyl sulfate, solvents, benzene), smoking, and suitability of the comparison cohort of gas supply workers.

2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum and adipose tissues were used to estimate TCDD exposure in 190 workers. A one-compartment first-order kinetic model was used to estimate exposure at end of exposure for these workers. Regression methods were then used to estimates TCDD exposures for all workers.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. Exposure based on half-life estimates from individuals with repeated serum measures. Other dioxin-like compounds were considered with the TOTTEQ exposure metric. Noncancer mortality, however, is not a viable endpoint to consider for further dose-response analysis.
Conclusion	Although, the exposure data used within this study are well-suited to a dose-response analysis for all-cause and cardiovascular mortality given the associations observed, use of noncancer mortality endpoint is not amenable for further dose-response analysis.

B.2.4. The Seveso Women's Health Study

Table B-28. Eskenazi et al., 2002a—Menstrual cycle characteristics

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Information was also obtained from medical records for all obstetric and gynecologic conditions. Information on menstrual cycles was obtained from questionnaires. Women were asked about length of cycles, regularity, how many days flow lasted, and heaviness of menstrual flow (scanty, moderate, or heavy). Measurement error is likely for the subjective nature of self-reported menstrual parameters but specificity and sensitivity is difficult to ascertain due to lack of validation data for these measures.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Detailed risk factor information was collected from questionnaire, allowing for the potential confounding influence of many risk factors to be controlled for. The length of cycle study findings may have been affected by the presence of a few outliers.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. A positive dose-response relationship was found with TCDD among women who were premenarcheal at time of the explosion and longer menstrual cycle. Increased TCDD resulted in a reduced odds of scanty menstrual flow. No association was noted with these two outcomes among postmenarcheal women. A decreased risk of irregular cycles was observed with higher TCDD levels.

4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Criteria satisfied. Serum concentrations of TCDD offer improved exposure assessment, although delineating the critical exposure window is challenging given the nature of the very high initial exposure.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. Cohort was large enough as analyses were conducted on 301 women.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Am J Epidemiol, 2002; 156(4) 383−392. Limitations included an inability to assess affects on menstrual cycle at time body burdens were the highest (at time of the accident). Also, TCDD was estimated for 1976, not concurrent with their cycles in the previous year, and a large number of women were excluded due to intrauterine device or oral contraceptive use. Strengths included population-based nature of study, with characterization of exposure using serum, and levels of other polychlorinated dibenzo-*p*-dioxins and dibenzofurans were at background levels. Findings for length of menstrual cycle may be unduly influenced by the presence of some outliers.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. The study population was based on 301 women as those who were over the age of 44 were excluded, as well as women with surgical of natural menopause, women with Turner's syndrome, those who had been pregnant or breastfed in the past year, and those who had used an intrauterine device or oral contraceptives. For 272 women, TCDD levels were based on serum data provided in 1976; TCDD levels were back-extrapolated to 1976 levels for the other 29 women.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response had to be a nonfatal endpoint.
Response	Criteria satisfied. Ideally, TCDD exposures would be concurrent with reporting of cycle characteristics. Herein, TCDD exposures were based on levels in 1976; however, given the long half-life of TCDD and the same follow-up interval for all women, TCDD exposures in 1976 should correlate well with levels near the time of interview. Further, the critical window of exposure can be estimated for the women that were premenarcheal at the time of the accident (13 years).
Conclusion	This study meets all of the criteria and considerations for further dose-response analysis. The determination of the relevant time interval over which TCDD dose should be considered is uncertain .

Table B-29. Eskenazi et al., 2002b—Endometriosis

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration not satisfied. Results of a pilot study showed that ultrasounds had excellent specificity and sensitivity for ovarian endometriosis.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. More than half of the women were classified as 'uncertain' with respect to endometriosis disease status.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. While an increased risk of endometriosis was observed across the 3 TCDD categories, these risks were not statistically significant relative to the lowest exposure category. The test for trend based on a continuous measure (log_{10}TCDD) was also not statistically significant.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Criteria satisfied. Serum concentrations of TCDD offer improved exposure assessment, although delineating the critical exposure window is challenging given the nature of the very high initial exposure.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration not satisfied. Only a total of 19 cases of endometriosis were identified.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Environ Health Perspect 2002; 110(7) 629–634. Author's highlight that this is the first study to examine the relationship between TCDD and endometriosis, and the availability of sera data to estimate TCDD levels. Limitations included the small number of women with endometriosis, and inability to confirm disease status using laparoscopy. Finally, young women may have been underrepresented due to cultural difficulties in examining women who had never been sexually active.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Eligible study subjects were women between 1 month and 40 years of age at time of accident. These analyses excluded virgins, those with Turner's syndrome, and women who refused the examination of ultrasound. Serum data were available for the 601 participants on which the analyses are based. Of these, 559 had serum measures taken in 1976/77, 25 between 1978 and 1981, and 17 women in 1996.

3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. TCDD exposure was estimated at the time of "conception attempt" using serum measures, with extrapolation from 1976 levels using half-life assumptions. It is difficult to identify the relevant time interval over which TCDD dose should be considered for dose-response analysis. The critical window of exposure is unknown.
Conclusion	The lack of a statistically significant association coupled with a large number of women for which endometriosis disease status was "uncertain", precludes the use of these data to conduct dose-response analysis.

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3 **Table B-30. Eskenazi et al., 2003—Birth outcomes**
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1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration not satisfied. Outcomes were identified through self-reported questionnaires. Women were found to over-report birth weight, and have a tendency to underreport birth defects in children. As a large number of women in Seveso underwent voluntary abortion in the first year after the explosion, an awareness bias may have contributed to differential reporting of pregnancy histories.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. See above.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. There was no association between spontaneous abortions and $\log_{10}$TCDD, or with births small for gestational age. An inverse association with birth weight was noted in first eight years following the accident as were the number of births small for gestational age; however, none achieved statistical significance at $p < 0.05$.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Criteria satisfied. Serum concentrations of TCDD offer improved exposure assessment, although delineating the critical exposure window is challenging given the nature of the very high initial exposure.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. For spontaneous abortions there were 769 pregnancies. Fetal growth and gestational age analysis was carried out on 608 singleton births that occurred post-explosion.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.

Response	Criteria satisfied. Environ Health Perspect, 2003, 111(7):947–953. The authors highlight potential limitation of reliance on self-reported data to ascertain pregnancy outcomes. They also address the relevance of paternal exposures to TCDD on the developing fetus—such exposure data were not considered in this study.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. A total of 745 women in the SWHS had reported getting pregnant, of these 510 women were pregnant after the explosion (888 pregnancies). Analyses of spontaneous abortions based on 476 women (excludes those with voluntary abortion, ectopic pregnancy, or molar pregnancy). TCDD measured for 413 women in 1976/77, 12 women between 1978 and 1981, and 1996 for 19 women.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. TCDD exposures were extrapolated to 1976 values. However, it is difficult to identify the relevant time interval over which TCDD dose should be considered for dose-response analysis.
Conclusion	The findings of the study are somewhat limited due to the reliance on self-reported information for pregnancy outcomes, and lack of paternal exposures. The findings were not statistically significant. Considered together, quantitative dose-response analyses for this study population were not undertaken.

Table B-31. Warner et al., 2004—Age at menarche

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. In this study age at menarche was based on retrospective recall 5 to 19 years before the interview. Previous work suggests moderate to high correlations between actual and recalled menarche, misclassification of outcome would bias risk estimates towards the null (assuming nondifferential misclassification).
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Data collected from self-reported questionnaires allow for the potential confounding influence of many risk factors to be taken into account. Some misclassification of outcome may bias risk estimates towards the null.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. There was no association between TCDD levels and the age at menarche with either the continuous or categorical measures of TCDD.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.

Response	Criteria satisfied. Serum concentrations of TCDD offer improved exposure assessment, although delineating the critical exposure window is challenging given the nature of the very high initial exposure.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. Cohort was large enough as analyses were performed using 282 women who were premenarcheal at the time of the explosion.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Environ Health Perspect, 2004, 112:1289–1292. Authors discuss use of pooled serum from residents of the unexposed zone, and that those in lowest exposure group had high exposures relative with contemporary levels for the area. Strengths of study include use of serum to estimate TCDD exposure.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. The SWHS included women between 1 month and 40 years of age at time of accident who attempted to get pregnant after the explosion (n = 463). This study is restricted to those who were premenarcheal at the time of the explosion (n = 282). Serum was collected for these women, primarily in 1976–1977 (n = 257), between 1978 and 1981 for 23, and in 1996–1997 for the 2 remaining women.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. TCDD exposures in 1976 were estimated by extrapolation serum levels obtained after this date using the Filser model. Both categorical and continuous measures of exposure were modeled. In utero measures of exposure are likely most relevant exposure based on findings from animal studies.
Conclusion	No association between TCDD levels and age at menarche was found. There may be some misclassification of age at menarche based on self-report, and biologically, the most relevant dose as suggested by animal studies occurs in utero. Additionally, it is difficult to identify the relevant time interval over which TCDD dose should be considered for dose-response analysis. For these reasons, these data are not suited to a dose-response analysis.

Table B-32. Eskenazi et al., 2005—Age at menopause

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Outcome measures were obtained based on self-reported data collected from questionnaires. Studies have shown that self-reports of age at menopause are reported with accuracy and reliability, and among women with surgical menopause, the self-reported age correlated well with that on the medical records.

2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Data obtained from the questionnaire allow for the potential confounding influence of several potential confounders to be controlled for.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. Although risks of earlier menopause increased in the first four quintiles, with a statistically significant trend, no increased risk was noted in the highest exposure category (hazard ratio = 1.0 relative to lowest exposure group). Study authors suggest this is due to the "inverted U" dose response often seen with hormonally active compounds. Additionally, no statistically significant association was noted with $\log_{10}$TCDD for the individual quintiles.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Criteria satisfied. Serum concentrations of TCDD offer improved exposure assessment, although delineating the critical exposure window is challenging given the nature of the very high initial exposure.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. The study included 616 women. Of these, 260 were premenopausal, 169 classified as natural menopause, 83 as surgical menopause, 24 as impending menopause, 33 as premenopausal, and 58 in an "other" category.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Environ Health Perspect, 113:858–862 (2005). Authors highlight this is first study to look at relationship between dioxin and age at menopause. Other limitations of the study include lowest exposure group ($\leq$ 20.4 ppt) includes exposures level that are far higher than background, and age at menopause was based on retrospective recall. Strength of study is ability to characterize TCDD using serum measures.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. The Seveso Women's Health Study collected serum sample which allowed TCDD exposures to be characterized. Those women (n = 616) who had not reached natural menopause at the time of the accident were included in the study. Serum measures collected in 1976/77 were available for 564 women, for 28 women, sera was collected between 1978 and 1981, while for 24 women, sera was collected in 1996/97.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. TCDD levels were estimated at the time of the explosion using available information on TCDD half-life. However, it is difficult to identify the relevant time interval over which TCDD dose should be considered for dose-response analysis. The critical window of exposure can be estimated but is large and highly uncertain.

Conclusion	The findings do not provide strong support for a dose-response relationship. As such, they are not well suited to a quantitative dose-response analysis.

Table B-33. Warner et al., 2007—Ovarian function

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Ovarian cyst analysis based on women who underwent ultrasound (n = 310). Ovarian follicle analysis based on self-report on menstrual cycle and done in women in preovulatory cycle (n = 96) at time of ultrasound. Hormonal analysis based on women in last 14 days of cycle (n = 129).
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Data collected from self-reported questionnaires allow for the potential confounding influence of many risk factors to be taken into account. Some misclassification of outcome based on self-reports of menstrual cycle may bias risk estimates towards the null.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. There was no association between serum TCDD levels and the number or size of ovarian follicles. TCDD was also not associated wit the odds of ovulation.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Criteria satisfied. Serum concentrations of TCDD offer improved exposure assessment, although delineating the critical exposure window is challenging given the nature of the very high initial exposure.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. Cohort was large enough as analyses were performed using 129 women for ovulation outcome, and hormone analyses based on 87 women in luteal, and 55 in midluteal phases.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Environ Health Perspect, 2007,115:336–340. An important limitation cited by the authors was that women may not have been exposed at critical period (prenatally). Phases of the cycle may also have been misclassified as this was based on self-reported data. Strength, first study to have examined ovarian function and TCDD exposures.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. The SWHS included women between 1 month and 40 years of age at time of accident who were between 20–40 years of age and not using oral contraceptives at follow-up (n = 363).Of these, serum was collected for 330 women between 1976 and 1977, between 1978 and 1982 for 25 women, and between 1996 and 1997 for 8 women.

3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. The women may not have been exposed at critical period (prenatally).
Conclusion	No association between TCDD levels and ovarian function was found. There may be some misclassification of period of the cycle based on self-report, and biologically, the most relevant dose as suggested by animal studies occurs in utero. For these reasons, these data are not suited to a dose-response analysis.

Table B-34. Eskenazi et al., 2007—Uterine leiomyoma

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Outcomes were determined using two definitions: current fibroids, or past diagnosis of fibroids. For past diagnosis of fibroids, self-reported data and medical records were used to determine whether women were previously diagnosed with fibroids, these were confirmed with medical records. A total of 25 women indicated they had never been diagnosed with fibroids. Medical records indicate a past diagnosis for these women, and they were classified as such. For current fibroids, this was determined at the time of the interview for 634 women using transvaginal ultrasound examinations.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. In the SWHS questionnaires were administered to the participants and detailed data for reproductive characteristics, smoking, body mass index, and alcohol use were collected so risks could readily be adjusted for these covariates.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied, but inversely. An inverse dose-response pattern with the percentage of women diagnosed (current and past history—combined) with fibroids across 3 categories of exposure. Namely, the percentages of women with fibroids in the ≤20, 20.1–75.0, and >75.0 ppt categories were 41.1%, 26.8%, and 20.0%, respectively.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. A variety of different exposure metrics were considered including linear, categorical, splines, and $\log_{10}$TCDD.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. A total of 251 women were found to have fibroids, and there were 62, 110, and 79 women with fibroids diagnosed in the 3 TCDD exposure categories.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.

Response	Criteria satisfied. Am J Epidemiol, 2007, 166:79–87. In this study, the authors found an inverse association between TCDD and uterine leiomyoma risk. The authors highlighted strengths of the study that included the longitudinal design, serum measures taken at an individual-level basis and most taken within 2 years of the accident, ability to include outcomes among those who did not take an ultrasound by using an adapted statistical approach. An important limitation that was the differences in risk by the stage of development could not be assessed as all women were exposed postnatally, and only 4 cases were observed among those who were premenarcheal at the time of exposure.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Final sample consisted of 956 women in the Seveso Women's Health Study without a history of fibroids. For 872 of these women, serum was collected in 1976 and 1977. For 56 women, TCDD was measured in women between 1978 and 1981, and for 28 women the serum was collected in 1996.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. TCDD exposures were back extrapolated to expected levels in 1976 (at the time of the accident). However, it is difficult to identify the relevant time interval over which TCDD dose should be considered for dose-response analysis. The critical window of exposure is unknown.
Conclusion	The data suggest an inverse (protective) effect between fibroids and exposure to TCDD. As such, these data are not suited to further dose-response analyses.

B.2.5. Other Seveso Noncancer Studies

Table B-35. Mocarelli et al., 2008—Semen quality

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Serum levels of TCDD were measured on an individual basis for men in exposed areas; pooled samples from men in uncontaminated areas were measured to assess background TCDD exposure levels.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. While compliance rates may have introduced some possible bias, this does not seem likely as different effects noted between the 22–31 and 32–39 year old age groups. Information collected for other risks factors, which have been used as adjustment factors in the models.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.

Response	Consideration satisfied. Figure 3 suggests dose-response relationship among those aged 1–9 at the time of the accident for sperm concentration and motility.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Serum concentrations of TCDD offer improved exposure assessment, although delineating the critical exposure window is challenging.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. Analyses are based on 135 males exposed to TCDD.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Environmental Health Perspective s, 2008, 116(1):70–77. The authors describe strengths associated with characterization of exposure (using serum samples), and representativeness of study population. Limitation of study includes low compliance (but high for semen sample studies), namely, 60% among a group of healthy men. The compliance rate was higher among exposed group (69%).
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Involved males, < 16 years old at time of accident.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria satisfied. TCDD exposures were based on serum samples. Serum samples were drawn (in 1997/1998) from participants whose 1976 samples were above 15 ppt. Pooled samples obtained in 1997/98 were used to describe background TCDD levels in uncontaminated areas. The associated between TCDD exposure and semen quality was found statistically significant for the boys with 1 and 9 years of age at the time of the accident. This provides a critical window of exposure to estimate TCDD concentration.
Conclusion	Health outcomes are exposures are well characterized using serum data. However, the men exposed between the ages of 1 and 9 to elevated TCDD levels had reduced semen quality 22 years later. It is difficult to discern whether this effect is a consequence of the initial high exposure between 1 and 9 years of age or a function of the cumulative exposure for this entire exposure window beginning at the early age. Nonetheless, quantitative dose-response analyses for this outcome were conducted.

Table B-36. Mocarelli et al., 2000—Sex ratio

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Birth records examined for those who lived in parents who lived in the area and who provided serum samples.

2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Paternal TCDD exposures were associated with an increased probability of female births ($p = 0.008$).
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Serum samples were used to estimate maternal and paternal TCDD levels. No discussion of exposure levels in reference population.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. Statistically significant findings achieved.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria not satisfied. The Lancet, 2000, 355:1858–1863. There is no discussion on the strengths and limitations of this study.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum levels of TCDD were obtained from parents using samples provided in 1976/77. Serum measures available for 296 mothers and 239 fathers.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. Serum based measures of TCDD were obtained shortly after the accident. TCDD levels were also extrapolated to the time of conception. However, it is difficult to identify the relevant time interval over which TCDD dose should be considered for dose-response analysis. The critical window of exposure is unknown.
Conclusion	The data from this study demonstrate a positive dose-response relationship with paternal TCDD levels at the time of the accident and increased likelihood for female births. However, It is difficult to identify the relevant time interval over which TCDD dose should be considered; specifically, it is difficult to discern whether this effect is a consequence of the initial high exposure during childhood or a function of the cumulative exposure for this entire exposure window beginning at the early age. Using the initial exposures in a dose-response model would yield LOAELs that are too high to be relevant to factor into the RfD calculation. Dose-response analysis for this outcome is, therefore, was not conducted.

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Table B-37. Baccarelli et al., 2008—Neonatal thyroid function

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Measures of b-TSH are taken using a standardized protocol 72 hours after birth. These b-TSH measures are taken on all newborns born in the region of Lombardy of which Seveso if a part of.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied for component of the study based on plasma dioxin measures. For the comparisons involving place of residence at the time of the accident, exposure misclassification is likely given variability in soil TCDD exposure levels within these areas.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Mean neonatal b-TSH was 0.98μU/ml [0.90–1.08] in the reference area, 1.35μU/ml [1.22–1.49] in zone B, and 1.66μU/ml [1.19–2.31] in zone A ($p < 0.001$). The plotted frequency distributions have similar shapes, but have shifted to the right for areas of higher exposures. Neonatal b-TSH was correlated with current maternal plasma TCDD (β-0.47, $p < 0.001$) in the 51 newborns for which individual maternal serum TCDD values were available.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. TEQs were measured among the 38 women for which serum samples were available and were defined for a mixture of dioxin-like compounds. Maternal mean total TEQs (PCDDs, PCDFs, coplanar PCBs, and noncoplanar PCBs) was 41.8 ppt. Two measures of exposure included place of residence at time of accident and plasma samples obtained from mothers at the time of delivery. Similarities in positive dose-response relationships give stronger weight to the findings.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied for exposure metric that was based on 'place of residence'. For plasma based estimate of maternal TCDD there were only 51 mother-child pairs. Only seven children in total were found to have b-TSH levels in excess of 5 uU/ml; this implies limited statistical power involving this health outcome.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. PLOS Medicine 2008; 5(7)1133–1142. The authors discuss the strength of the study related to characterization of exposure using serum sampling, and ability to adjust for factors related to b-TSH or TCDD levels (gender, birth weight, birth order, maternal age, hospital and type of delivery). They also highlight that a limitation of study was that the influence of mother-child dioxin transfer through colostrum could not be assessed because no information on breastfeeding before b-TSH measurement was available.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.

Response	Criteria satisfied. In the population-based study, eligible women who resided in zones A and B at the time of the accident (n = 1,772) were matched to nonexposed women. In the study based on plasma dioxin measurements, participants were the 51 children born to 38 women from zones A, B, R, or a reference zone for which plasma dioxin measurements were available.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria satisfied. Maternal TCDD levels were estimated at the time of delivery based on plasma samples, and the critical window of exposure can be defined as the 9 month gestation period.
Conclusion	The data provide an opportunity for quantitative dose-response analyses.

Table B-38. Alaluusua et al., 2004—Oral hygiene

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Ascertainment of dental health was done blind to place of residence, used standard protocol for caries developed by the WHO, and the clinical examination supplemented by radiographic examination.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Additional risk factor information was collected on questionnaires. These factors were considered as adjustment factors. Findings potentially susceptible to participation biases.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Increased prevalence of developmental enamel effects found with increased TCDD serum measures. Namely, prevalence in unexposed region was 26%, whereas in the low, middle, and high TCCD groups the prevalence was 10, 40, and 60%, respectively.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. TCDD exposure level based on serum lipids. No discussion of exposure levels in reference population.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Criteria satisfied. Despite small numbers, statistically significant findings were achieved.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.

Response	Criteria satisfied. Environmental Health Perspectives, 2004, 112(13)1313–1318. Authors mention two important strength of the study: characterization of TCDD exposure using serum collected shortly after the time of the accident, and the fact that developmental defects are permanent in nature. Therefore, they represent a health outcome can evaluated years later. Little discussion was made of the impact of differential compliance rates between the exposed (74%) and nonexposed (58%) groups. Authors mention two important strength of the study: characterization of TCDD exposure using serum collected shortly after the time of the accident, and the fact that developmental defects are permanent in nature. Therefore, they represent a health outcome can evaluated years later. Little discussion was made of the impact of differential compliance rates between the exposed (74%) and nonexposed (58%) groups.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum levels of TCDD could be estimated for children in exposed areas. No serum levels were available for reference group of children, and assumption of zero exposure was made. This seems reasonable.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria satisfied. It is difficult to discern whether this effect is a consequence of the initial high exposure during childhood or a function of the cumulative exposure of the entire exposure window beginning at early age. However, assumptions can be made regarding the critical window of exposure and the relevant dose can be calculated.
Conclusion	The considerations for conducting a dose-response analysis have been satisfied with the study population of only those subjects who lived in the ABR zone at the time of the accident; exposure data are unavailable for those in the referent area. While is difficult to identify the relevant time interval over which TCDD dose should be considered, quantitative dose-response analysis for this outcome was conducted.

Table B-39. Bertazzi et al., 2001—Mortality (noncancer)

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied for some causes of death, but not others. Mortality appears to be well captured from the vital statistics registries in the region (99% complete). Some health outcomes (e.g., diabetes) are subject to misclassification using death certificate data.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Although individual-level data for individual risk factors are not available, the potential for confounding is likely minimal. For e.g., independent surveys suggests similarity between smoking behaviors across the regions. Exposure misclassification based on place of residency likely to bias risk estimates towards the null.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.

Response	Consideration not satisfied for most causes of death. An exception was the dose-response relationship was observed for chronic obstructive pulmonary disease across Zones A, and B.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration not satisfied. Exposure classification was based on the address of the residence on the date of the accident or when the person first entered the area. Although TCDD blood levels were also measured, these were not examined with respect to health outcomes. The lack of individual-level data also precluded an examination of these uncertainties.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. A total of 494 noncancer deaths were found among residents of Zones A, and B, respectively. This allowed examined of gender-specific effects.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Am J Epidemiol, 2001, 153:1031–1044. Authors discuss lack of individual-level exposure data and other risk factors (e.g., smoking), difficulties in extrapolating to background levels, diagnostic accuracy of using death certificates. Strengths included similarities between exposed and comparison population for several risk factors, completeness of follow-up, and consistent methods to identify mortality outcomes in the exposed and comparison populations.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. Individual-level exposure data are unavailable. Exposure based on place of residence at time of the explosion. Soil sampling performed indicated considerable variability in TCDD levels within each region. In addition, place of residency at time of explosion does not ensure individuals were at their home around the time of the accident.
3. Critieria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. An ecological measure of exposure (region of residency at time of accident) was used to categorize individuals according to their possible exposure. Latencies were considered. While such an approach has value for identifying whether excesses occurred among highly exposed populations, it is not precise enough to conduct a quantitative dose-response analysis. Furthermore, noncancer mortality is not a viable endpoint to consider for further dose-response analysis.
Conclusion	Study is not suitable for dose-response analysis due to mortality as endpoint and lack of individual-level exposure data.

Table B-40. Consonni et al., 2008—Mortality (noncancer)

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied for some causes of death, but not others. Mortality appears to be well captured from the vital statistics registries in the region (99% complete). Some health outcomes (e.g., diabetes) are subject to misclassification using death certificate data.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Although individual-level data for individual risk factors are not available, the potential for confounding is likely minimal. For e.g., information from other independent surveys suggests similarity between smoking behaviors across the regions. Exposure misclassification based on place of residency is likely to bias risk estimates towards the null.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. Statistically significant association noted in most highly exposed area for chronic rheumatic disease and chronic obstructive pulmonary disease. Dose-response pattern noted across Zones A, B and R for circulatory disease mortality 5–9 years after the accident.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration not satisfied. Lack of individual-level data precludes an examination of these uncertainties.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied for some causes of death but not others. For example, only three deaths from diabetes occurred among residents of Zone A. The limitation related to statistical power is exacerbated for stratified analyses carried out by number of years since the accident.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Am J Epidemiol, 2008, 167:847–858. Authors discuss potential for selection bias, limitation of residential based measure of exposure, similarities of mortality ascertainment in exposed and referent populations, and multiple testing.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. Individual-level exposure data are unavailable. Exposure based on place of residence at time of the explosion. Soil sampling performed indicated considerable variability in TCDD levels within each region. In addition, place of residency at time of explosion does not ensure individuals were at their home around the time of the accident.

3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. An ecological measure of exposure (region of residency at time of accident) was used to categorize individuals according to their possible exposure. Latencies were considered. While such an approach has value for identifying whether excesses occurred among highly exposed populations, it is not precise enough to conduct a quantitative dose-response analysis. Furthermore, noncancer mortality is not a viable endpoint to consider for further dose-response analysis.
Conclusion	Study is not suitable further dose-response evaluation due to noncancer morality endpoint.

Table B-41. Baccarelli et al., 2005—Chloracne

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Chloracne cases identified using standardized criteria.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Plasma TCDD was associated with an increased risk of chloracne. The odds ratios increased in a dose-response pattern across zone of residence.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Authors discussed implications of differential elimination rates by age and body growth.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. A total of 101 chloracne cases were identified, and 211 controls were selected. Statistically significant findings were observed in several comparisons.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. British Journal of Dermatology, 2005, 152, 459–465. The authors detail the limited statistical power they had available in the study. They also highlight a strength of the study that included uniqueness of age and sex distribution of chloracne cases, characterization of TCDD that could be done using sera samples, and availability of both clinical and epidemiological data.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.

Response	Criteria satisfied. TCDD was estimated in both chloracne cases and control using serum measures.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria satisfied. Serum based measures of TCDD were obtained shortly after the accident. Chloracne is thought to be caused by the initial high exposure.
Conclusion	Exposure to TCDD at sufficiently high levels is recognized to cause chloracne. This study provides limited relevance to dose-response modeling of TCDD as exposure levels typically observed in the general population are much lower.

Table B-42. Baccarelli et al, 2002 and 2004—Immunological effects

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Common methods were used to describe blood levels of plasma immunoglobulins (IgA, IgG, and IgM) and complement components (C3 and C4).
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Both exposure and outcome were objectively and accurately measured.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. Plasma IgG levels were inversely related with TCDD.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Both categorical (quintiles) and continuous measures of TCDD were examined in the dose-response analysis.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. Analyses are made using 72 highly exposed, and 72 low exposed individuals.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Toxicology letters, 2004, 149:287–293 and Environ Health Perspect, 2002, 110(12):1169–1173. The authors highlight that few studies have looked at immunological effects of TCDD in humans, that the current study was able to exclude those with concurrent medical conditions, and the ability to characterize exposure using serum measures. Limitations addressed were the uncertainty about the clinical relevance of the dose-response pattern found, and the relatively small size of the study population.

2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. A total of 120 subjects were included in the study. This included 62 randomly selected from the high exposed zone, and 58 selected from the reference area.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. Dose-response relationships were examined using current TCDD levels. However, it is difficult to identify the relevant time interval over which TCDD dose should be considered for dose-response analysis.
Conclusion	An inverse dose-response association between IgG and TCDD was observed, however, because the relationship can not be described in terms of clinical relevance with respect to a specific health outcome, it is our view that these data are not suited to dose-response modeling.

B.2.6. Chapaevsk Study

Table B-43. Revich et al., 2001—Mortality (noncancer) and reproductive health

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration cannot be evaluated. Insufficient details are provided in the paper to gauge the completeness and coverage of the cancer registry and mortality data. Health outcomes were studied on the basis of information in the official medical statistics
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. It is an ecological study.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration cannot be evaluated. Dose-response was not evaluated as exposure was based on residency in the region vs. no residency.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration not satisfied. No individual-level exposure estimates were used.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. Population-based data over several years were used to make ecological comparisons.

1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Published in Chemosphere, 2001, 43(4–7):951–966.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. It is a cross-sectional study that compares mortality rates between regions. No individual-level exposure data available.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. No exposure estimates were used in the study.
Conclusion	These cancer data are cross-sectional in nature and not appropriate for a dose-response analysis.

B.2.7. Air Force Health ("Ranch Hands") Study

Table B-44. Michalek and Pavuk, 2008—Diabetes

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. Prevalent diabetes identified from medical records from repeated medical check-ups. Preferred method of ascertaining outcome relative to use of death certificates.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. Adjustment was made for a number of risk factors related to diabetes (e.g., BMI, family history, smoking). However, Agent Orange was a 50% mixture of 2,4-D and TCDD; therefore, potential for confounding by other coexposures is likely.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration satisfied. The RR for an increase in 10 units was 1.29 ($p < 0.001$), and the risks across the background, low and high exposure categories, relative to the unexposed were 0.86, 1.45, and 1.68.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Initial TCDD dose were estimated at the end of the tour of duty for the Ranch Hands. Individual-level serum dioxin measurements correlated well with correlated with days of spraying and calendar period of service, but collection of the samples roughly 20 years later required back-extrapolation.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.

Response	Consideration satisfied. There were a total of 439 cases of diabetes identified.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. J Occup Environ Medicine, 2008, 50:330–340. The authors address strengths and limitations related to the accuracy of the one-compartment pharmacokinetic model, impact of the covariate time spent in Southeast Asia, and potential exposure misclassification on days sprayed.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. TCDD estimates were derived using serum samples. However, Ranch Hand veterans were exposed to other compounds in the herbicides, such as 2,4-D.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria satisfied. TCDD levels at the end of service were estimated. Extrapolation was done using a half-life of 7.6 years. Exposures were grouped into comparison, background, low and high. This allows for a shape of the dose-response curve to be evaluated. A continuous measure of TCDD was also examined ($\log_{10}$TCDD).
Conclusion	Ranch Hand veterans were exposed to other contaminants in the herbicides that were mixed, thereby making it difficult to determine independent effects of TCDD on diabetes. In our view, this limitation precludes dose-response modeling of TCDD and diabetes using data from this cohort.

B.2.8. Other Noncancer Studies of Dioxin

Table B-45. McBride et al., 2009a—Mortality (noncancer)

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. The New Zealand Health Information Service Mortality Collection and the Registrar-General's Index to Deaths were used to identify deaths. Additional searches were based on the last known address from the work record; the electoral roll and the habitation index; the telephone book; the internet; and Terranet property information database. An additional search was carried out through the Births, Deaths, and Marriages office of the New Zealand Department of Internal Affairs. Lastly, automated personnel and pension records were also used to locate past New Plymouth workers and identify some deaths.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration satisfied. Workers lost to follow-up were an unlikely source of bias especially for internal analyses. Confounding by other coexposures (e.g., 2,4,6-TCP) unlikely to have resulted in bias, due to presumed poor correlation with TCDD.

3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. There was no cause of death among those considered for which a dose-response trend was observed across four exposure categories of TCDD.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Dichotomous exposure (exposed/unexposed) and duration of employment were examined from job exposure classification assessed via occupational history records industrial hygienists/factory personnel knowledge and questionnaires.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration not satisfied.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Published in J Occup Environ Med, 2009, 51:1049–1056. The other studies in the cohort highlight the 22% of the cohort lost to follow-up, the limited size of the cohort tissue sarcomas, differences in cohort definitions between sprayers and producers, and the potential for other exposures during employment at the plant.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria satisfied. Serum measures available for 346 workers were used to derive TCDD exposures for the entire cohort using the area under the curve approach.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. Dichotomous exposure assessment did not allow individual estimates of dose to be developed. However, noncancer mortality is not a viable endpoint to consider for further dose-response analysis.
Conclusion	A considerable portion of the cohort was lost to follow-up, and no dose-response associations noted. As a result, the data are not suited to dose-response analysis.

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Table B-46. McBride et al., 2009b—Mortality (noncancer)

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration satisfied. The New Zealand Health Information Service Mortality Collection and the Registrar-General's Index to Deaths were used to identify deaths. Additional searches were based on the last known address from the work record; the electoral roll and the habitation index; the telephone book; the internet; and Terranet property information database. An additional search was carried out through the Births, Deaths, and Marriages office of the New Zealand Department of Internal Affairs. Lastly, automated personnel and pension records were also used to locate past New Plymouth workers and identify some deaths.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. Considerable amount of workers were lost to follow up (22%), but it is unclear if bias resulted. The dichotomous exposure measure was based on exposure to TCDD, chlorinated dioxins and phenoxy herbicides, so confounding is a possibility by these coexposures.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. Because no individual exposure estimates were available for these analyses, dose-response could not be evaluated.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration satisfied. Consideration satisfied. Dichotomous exposure (exposed/unexposed) and duration of employment were examined from job exposure classification assessed via occupational history records industrial hygienists/factory personnel knowledge and questionnaires. Authors discuss limitations in the assignment of exposure among cohort members.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.
Response	Criteria satisfied. Published in Occup Medicine, 2009, 59(4):255–263. The authors highlight cohort lost to follow-up, the limited size of the cohort, differences in cohort definitions between sprayers and producers, and the potential for other exposures during employment at the plant.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. Exposures were not quantified. The dichotomous exposure measure was based on exposure to TCDD, chlorinated dioxins and phenoxy herbicides.

3. Critiera	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Effective dose could not be estimated given the lack of individual-level exposure data. Noncancer mortality is not a viable endpoint to consider for further dose-response analysis.
Conclusion	The study lacks the quantification of exposures at an individual level, and a considerable portion of the cohort was lost to follow-up. As a result, the data are not suited to dose-response analysis.

Table B-47. Ryan et al., 2002—Sex ratio

1. Consideration	Methods used to ascertain health outcomes identified were unbiased, highly sensitive, and specific.
Response	Consideration not satisfied. Company records were used to identify births, the date of birth, and the sex of the child. No information was provided on the expected completeness of identifying births in this manner. Moreover, the study was expanded to include workers who heard about the study in a public forum. Therefore, the study could be influenced by participation bias.
2. Consideration	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.
Response	Consideration not satisfied. See above.
3. Consideration	Study demonstrates an association between TCDD and adverse health effect with evidence of an exposure-response relationship.
Response	Consideration not satisfied. The study compared birth ratios among men and women employed at the plant to the general population. No categories of exposure were examined.
4. Consideration	Exposure assessment methodology is clear and adequately characterizes individual-level exposures. The limitations and uncertainties in the exposure assessment are considered.
Response	Consideration not satisfied. This is not relevant as no analyses were done in relation to exposure levels.
5. Consideration	Study size and follow-up are large enough to yield precise estimates of risk and ensure adequate statistical power.
Response	Consideration satisfied. For the categories of exposure used (yes/no), and the stratified analyses by sex and subcohort, the study allows for the birth ratios to be estimated with sufficient precision.
1. Criteria	Study is published in the peer-reviewed scientific literature and has an appropriate discussion of the strengths and limitations.

Response	Criteria not satisfied. Published in Environ Health Perspect, 2002, 110(11):A699–A701. The authors discussed the limitations of using serum collected many years after they stopped working to estimate TCDD exposures when the preferred metric would be TCDD levels at the time of conception. They did not address issues about the representativeness of the study participants to the entire cohort of workers, nor did they address the limitation of not being able to conduct dose-response analyses using individual-level TCDD data.
2. Criteria	Exposure must be primarily TCDD and is properly quantified so that dose-response relationships can be assessed.
Response	Criteria not satisfied. While serum measures were available for 84 of the 198 participants of the study, birth ratios were compared between the cohort of 2,4,5-T and 2,4,5-trichlorphgenol workers relative to the city of Ufa. There was no attempt to derive birth ratios in relation to exposure levels. The serum data were only used to demonstrate that these workers, on average, had TCDD levels 30 times higher than Ufa residents.
3. Criteria	The effective dose and oral exposure can be reasonably estimated and the measures of exposure are consistent with the current biological understanding of dose. The reported dose is consistent with a toxicologically relevant dose. Latency and appropriate window(s) of exposure examined. Response has to be a nonfatal endpoint.
Response	Criteria not satisfied. TCDD exposures were based on serum measures taken in some cases many years after children were born; no attempt was made to back-extrapolate to the time of conception.
Conclusion	The data are not suitable for dose-response modeling. Risk estimates have not been derived in relation to TCDD exposure levels. There exist uncertainties about the representativeness of the participants in relation to the cohort as a whole, and insufficient details are provided to evaluate the extent in which all births were identified. While these data should not be used for quantitative dose-response modeling, the much lower M/F birth ratio among exposed fathers is consistent with the finding by Mocarelli et al, and lends support to those findings.

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APPENDIX C

Kinetic Modeling

NOTICE

THIS DOCUMENT IS AN EXTERNAL REVIEW DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

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APPENDIX C. KINETIC MODELING

C.1. LITERATURE SEARCH STRATEGY AND RESULTS—IDENTIFYING RECENT PUBLICATIONS FOR UPDATING TCDD TOXICOKINETIC MODEL INPUT PARAMETERS

The purpose of this literature search was to identify recent publications that address the input parameters for the physiologically based pharmacokinetic (PBPK) models Aylward and colleagues (described in articles published in 2005 and 2009) and Emond and colleagues (described in articles published in 2004, 2005, and 2006). This literature search was part of the U.S. Environmental Protection Agency (EPA)'s preparation of a response to the National Academy of Sciences' review (*Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment*, NAS, 2006]) of EPA *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* (U.S. EPA, 2003), herein called the "2003 Reassessment." English-only references from 2003 to May 2009 were searched using bibliographic data bases relevant to health effects and toxicology of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The search focused on toxicokinetic data that could be used to update the dynamic disposition of 2,3,7,8-TCDD in mice, rats, guinea pigs, monkeys, and humans.

In the primary search, EPA identified 775 distinct citations based on the literature search criteria described below. EPA also performed an independent supplemental search to avoid missing key studies. EPA identified 28 papers for further analysis that appeared on first review to report data to update the input parameters of the Aylward and Emond PBPK models; considerations for selection are described in Section C.1.3.

C.1.1. Data Bases Searched

EPA used the following DIALOG bibliographic data bases in the primary search. Brief descriptions of the DIALOG data bases searched are provided in Section C.1.5.

1. File 6: NTIS
2. File 41: Pollution Abstracts
3. File 55: Biosis
4. File 153: IPA Toxicology

5. File 155: MedLine
6. File 156: ToxFile
7. File 157: Biosis Toxicology
8. File 159: CancerLit
9. File 336: RTECS

The PUBMED data base was used for the supplemental search.

C.1.2. Literature Search Strategy and Approach

The primary search used a tiered key-word approach, as documented below. The principal search term was the Chemical Abstract Service Registry Number (CASRN) or specific chemical name, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or 2,3,7,8-TCDD. The next tier of search terms was species, and finally toxicokinetic keywords, as listed below. The period of the search was 2003 through May 2009, and articles were limited to English language.

The supplemental PUBMED search was limited to the most recent five years (2004 to present) and used four combinations of key words:

- TCDD + pharmacokinetic + humans,
- TCDD + toxicokinetic + humans,
- TCDD + pharmacokinetic + animals, and
- TCDD + toxicokinetic + animals.

C.1.2.1. *Chemical Search Terms—DIALOG Search*

- CASRN: 1746-01-6
- 2,3,7,8-tetrachlorodibenzo-*p*-dioxin
- 2,3,7,8-TCDD

C.1.2.2. *Primary Search Terms (Species)—DIALOG Search*

- Guinea pig(s)
- Human(s)
- Monkey(s)
- Mouse

- Mice
- Rodent(s)
- Rat(s)

C.1.2.3. *Secondary Search Terms (Toxicology)—DIALOG Search*

* = truncated
1w = terms are within 1 word of each other and in the order specified (see search term 32)

1. Absor*
2. ADME
3. Aryl hydrocarbon receptor
4. AhR
5. Bioavail*
6. Biliar*
7. Biotransform*
8. Cytochrome
9. CYP*
10. CYP1A1
11. CYP1A2
12. Diet, dietary, diets
13. Disposit*
14. Distrib*
15. Drink*
16. Elimin*
17. Excret*
18. Epidemiolog*
19. Feces
20. Feed*
21. First order kinetics
22. Food*
23. Gastro*
24. Gavage*
25. Half-life
26. Induct*
27. Ingest*
28. In silico
29. Kinetic*
30. Liver
31. Lymph*
32. Mechanism (1w) action
33. Metabo*
34. Oral*
35. P450
36. Partition coefficient
37. PBPK
38. Pharmacodynamic*
39. Pharmacokinetic*
40. Physiologically based
41. pharmacokinetic
42. Protein bind*
43. Toxicokinetic*
44. Urin*

ADME = absorption, distribution, metabolism, elimination; AhR = aryl hydrocarbon receptor; CYP = cytochrome P450.

C.1.3. Citation Screening Procedures and Results

Initial DIALOG searches resulted in a very large number of citation hits. Therefore, some title and key word restrictions were applied iteratively to screen out less relevant citations (e.g., requiring some search terms in title, requiring 2,3,7,8-TCDD rather than just TCDD). Then, using reference management software, pooled information obtained from the various DIALOG data bases was screened to remove duplicates. Citations then were numbered

sequentially (as a unique identifier). Information retrieved included the following (when available): author(s), publication year, title, source document name, volume, and page numbers.

The DIALOG search and duplicate removal procedure produced 775 unique citations. In the next step, all 775 citations were screened for potential applicability to updating parameters in the Aylward and Emond PBPK models. Of these 775 citations, 26 were selected for more detailed review to determine their potential applicability, and full publications were retrieved. Two citations were added from the supplemental search, giving a total of 28 articles identified for further review.

Bibliographic information for the 28 articles selected for full review is provided in the reference list at the end of this section. Table C-1 summarizes the model input parameters potentially addressed by the selected articles.

During 2003 to May 2009, the authors of the two kinetic models under consideration published several articles. For the Emond model, which was first published in 2004 (Emond et al., 2004), two subsequent papers have been published (Emond et al., 2005, 2006). The Aylward model, which originated from the 1995 papers by Carrier et al. (1995a, b), was later updated by the same group (Aylward et al., 2005a, b). The major change implemented in the last two papers was the description of a desorption process in the digestive tract. The transfer rate described is slow, but for a low body burden of TCDD, this process remains significant. This concept was reported in 2002 by Moser and McLachlan (2002). The major modifications expected to update the Emond model are (1) consideration of the desorption process in the gastrointestinal tract and (2) rearrangement of the elimination constant, which will have a negligible impact on the simulation. These changes are motivated by plausible observations reported in the literature.

Because of the body burden found in humans and the importance of selecting an appropriate dose metric in human risk assessment, the physiological model is an important tool for assessing the kinetics following exposure to TCDD (Kim et al., 2003). Based on the literature identified in this search, the major contributions that should be reviewed with respect to the Aylward and Emond kinetic models are not modes of action or pharmacokinetic mechanisms, but rather information for verifying or improving the accuracy of some model parameters.

Pharmacokinetics typically refers to four distinct steps including absorption, distribution, metabolism, and excretion. Physiologically-based models consider each step. In the model each

step is parameterized to reflect better predictions of the real observations. Occasionally, reviewing these models is essential to determine if any key processes or parameters might be described with better accuracy. This perspective underlies the review of the literature described here. The review indicates TCDD disposition has become recognized as relatively significant since the publication of the Emond and Aylward models. The literature that provides information related to improving these models, however, is limited. For the benefit of this exercise, EPA selected the literature that would likely contribute significantly to model response, or to clarify or confirm different key issues driving the model results. Regarding the two TCDD models, the two major issues that should be evaluated with respect to the recent literature identified are the elimination profile and the induction of CYP1A2.

Reviewing the elimination variation in different species and testing variable elimination with a data set appears to be appropriate. The literature reports that various factors might influence elimination rate. Recent publications report the influence of diverse predictors such age, body fat, or smoking habit on the elimination half-life (Milbrath et al., 2009; Kerger et al., 2006, 2007). Determining whether using the Milbrath et al. information would help account for intraspecies variability in elimination rate in the Emond and Aylward kinetic models would be useful. In 2006, Emond et al. reviewed the influence of body fat mass and CYP1A2 induction on the pharmacokinetics of TCDD. These two factors appear to contribute significantly to elimination and their influences seem to be driven by TCDD body burden. Mullerova and Kopecky (2007) discussed the influence of adipose tissue and the "yo-yo" effects on various diseases that might be influenced by persistent organic pollutant distribution. One group explored the importance of variable elimination and compared these predictions to first-order elimination using the Aylward and Emond models and supported these approaches for risk assessment (Heinzl et al., 2007). Two groups of authors considered a one-compartment model to derive the elimination half-life (Aylward et al., 2009; Nadal et al., 2008). Comparing the half-life they obtained using this approach for a range of body burden to the variable elimination half-life would be interesting.

The second important mechanism driving the distribution and elimination of TCDD is the induction of CYP1A2, identified as the major ligand protein in liver (Diliberto et al., 1997). For that process, authors suggested different aspects that should be investigated, including the importance of the dose metrics in the target tissue and the inducible level of CYP1A2 (Wilkes

et al., 2008; Staskal et al., 2005). Other papers address the intraspecies variability of lethal potency in mature species versus the developing fetus (Kransler et al., 2007; Korkalainen et al., 2004). Still others point out pronounced differences among species (namely, guinea pigs, hamsters, mice, and rats) (Bohonowych and Denison, 2007), as observed in studies of long-term effects of low TCDD dose in liver and in studies comparing hepatic accumulation and clearance of TCDD (Korenaga et al., 2007; Boverhof et al., 2005). The interspecies variation of the binding affinity constant of AhR also has been reported (Connor and Aylward, 2006; Nohara et al., 2006).

The articles identified in this literature review should be adequate to update the Aylward and Emond models, which need to be evaluated according to the same structure of compartments described in the literature by the two model authors.

C.1.4. References Selected for More Detailed Review for Updating the PBPK Models

Aylward, LL; Brunet, RC; Carrier, G; et al. (2004). Concentration-dependent TCDD elimination kinetics in humans: toxicokinetic modeling for moderately to highly exposed adults from Seveso, Italy, and Vienna, Austria, and impact on dose estimates for the NIOSH cohort. J Expo Anal Environ Epidemiol 15(1):51−65.

Aylward, LL; Brunet, RC; Starr, TB; et al. (2005). Exposure reconstruction for the TCDD-exposed NIOSH cohort using a concentration- and age-dependent model of elimination. Risk Anal 25(4):945−956.

Aylward, LL; Bodner, KM; Collins, JJ; et al. (2009). TCDD exposure estimation for workers at a New Zealand 2,4,5-T manufacturing facility based on serum sampling data. J Expo Sci Environ Epidemiol. doi: 10.1038/jes.2009.31.

Bohonowych, JE; Denison, MS. (2007). Persistent binding of ligands to the aryl hydrocarbon receptor. Toxicol Sci 98(1):99-109.

Boverhof, DR; Burgoon, LD; Tashiro, C; et al. (2005). Temporal and dose-dependent hepatic gene expression patterns in mice provide new insights into TCDD-mediated hepatotoxicity. Toxicol Sci 85(2):1048−1063.

Connor, KT; Aylward, LL. (2006). Human response to dioxin: aryl hydrocarbon receptor (AhR) molecular structure, function, and dose-response data for enzyme induction indicate an impaired human AhR. J Toxicol Environ Health B 9(2):147−171.

Heinzl, H; Mittlback, M; Edler, L. (2007). On the translation of uncertainty from toxicokinetic to toxicodynamic models - the TCDD example. Chemosphere 67(9):S365−S374.

Irigaray, P; Mejean, L; Laurent, F. (2005). Behaviour of dioxin in pig adipocytes. Food Chem Toxicol 43(3):457−460.

Kerger, BD; Leung, HW; Scott, P; et al. (2006). Age- and concentration-dependent elimination half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Seveso children. Environ Health Perspect 114(10):1596−1602.

Kerger, BD; Leung, HW; Scott, PK; et al. (2007). Refinements on the age-dependent half-life model for estimating child body burdens of polychlorodibenzodioxins and dibenzofurans. Chemosphere 67(9):S272−S278.

Kim, AH; Kohn, MC; Nyska, A; et al. (2003). Area under the curve as a dose metric for promotional responses following 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure. Toxicol Appl Pharmacol 191(1):12−21.

Korenaga, T; Fukusato, T; Ohta, M; et al. (2007). Long-term effects of subcutaneously injected 2,3,7,8-tetrachlorodibenzo-p-dioxin on the liver of rhesus monkeys. Chemosphere 67(9):S399−S404.

Korkalainen, M; Tuomisto, J; Pohjanvirta, R. (2004). Primary structure and inducibility by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) of aryl hydrocarbon receptor repressor in a TCDD-sensitive and a TCDD-resistant rat strain. Biochem Biophys Res Communications 315(1):123−131.

Kransler, KM; McGarrigle, BP; Olson, JR. (2007). Comparative developmental toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the hamster, rat and guinea pig. Toxicology 229(3):214−225.

Maruyama, W; Yoshida, K; Tanaka, T; et al. (2002). Determination of tissue-blood partition coefficients for a physiological model for humans, and estimation of dioxin concentration in tissues. Chemosphere 46(7):975−985.

Maruyama, W; Yoshida, K; Tanaka, T; et al. (2003). Simulation of dioxin accumulation in human tissues and analysis of reproductive risk. Chemosphere 53(4):301-313.

Maruyama, W; Aoki, Y. (2006). Estimated cancer risk of dioxins to humans using a bioassay and physiologically based pharmacokinetic model. Toxicol Appl Pharmacol 214(2):188−198.

Milbrath, MO; Wenger, Y; Chang, C-W; et al. (2009). Apparent Half-Lives of Dioxins, Furans, and Polychlorinated Biphenyls as a Function of Age, Body Fat, Smoking Status, and Breast-Feeding. Environ Health Perspect 117(3):417−425.

Moser, GA; McLachlan, MS. (2002). Modeling digestive tract absorption and desorption of lipophilic organic contaminants in humans. Environ Sci Technol 36(15):3318−25.

Mullerova, D; Kopecky, J. (2007). White adipose tissue: storage and effector site for environmental pollutants. Physiol Res 56(4):375−381.

Nadal, M; Perello, G; Schuhmacher, M; et al. (2008). Concentrations of PCDD/PCDFs in plasma of subjects living in the vicinity of a hazardous waste incinerator: Follow-up and modeling validation. Chemosphere 73(6):901−906.

Nohara, K; Ao, K; Miyamoto, Y; et al. (2006). Comparison of the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced CYP1A1 gene expression profile in lymphocytes from mice, rats, and humans: Most potent induction in humans. Toxicology 225(2-3):204−213.

Olsman, H; Engwall, M; Kammann, U; et al. (2007). Relative differences in aryl hydrocarbon receptor-mediated response for 18 polybrominated and mixed halogenated dibenzo-p-dioxins and -furans in cell lines from four different species. Environ Toxicol Chem 26(11):2448−2454.

Saghir, SA; Lebofsky, M; Pinson, DM; et al. (2005). Validation of Haber's Rule (doseX time=constant) in rats and mice for monochloroacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin under conditions of kinetic steady state. Toxicology 215(1−2):48−56.

Schecter, A; Pavuk, M; Popke, O; et al. (2003). Dioxin, dibenzofuran, and coplanar PCB Levels in Laotian blood and milk from Agent Orange-sprayed and nonsprayed areas, 2001. J Toxicol Environ Health A 66(21):2067−2075.

Staskal, DF; Diliberto, JJ; Devito, MJ; et al. (2005). Inhibition of human and rat CYP1A2 by TCDD and dioxin-like chemicals. Toxicol Sci 84(2):225−231.

Toyoshiba, H; Walker, NJ; Bailer, AJ; et al. (2004). Evaluation of toxic equivalency factors for induction of cytochromes P450 CYP1A1 and CYP1A2 enzyme activity by dioxin-like compounds. Toxicol Appl Pharmacol 194(2):156−168.

Wilkes, JG; Hass, BS; Buzatu, DA; et al. (2008) . Modeling and assaying dioxin-like biological effects for both dioxin-like and certain non-dioxin-like compounds. Toxicol Sci 102(1):187−195.

C.1.5. Brief Descriptions of DIALOG Bibliographic Data Bases Searched

The National Technical Information Service (NTIS) database comprises summaries of U.S. government-sponsored research, development, and engineering, plus analyses prepared by federal agencies, their contractors, or grantees. It is the means through which unclassified, publicly available, unlimited distribution reports are made available for sale from 240 agencies. Additionally, some state and local government agencies contribute summaries of their reports to the database. NTIS also provides access to the results of government-sponsored research and development from countries outside the United States. Organizations that currently contribute to the NTIS database include but are not limited to the following: the Japan Ministry of International Trade and Industry (MITI); laboratories administered by the United Kingdom

Department of Industry; the German Federal Ministry of Research and Technology (BMFT); and the French National Center for Scientific Research (CNRS).

Pollution Abstracts provides access to environmental information that combines information on scientific research and government policies in a single resource. Topics of growing concern are extensively covered from the standpoints of atmosphere, emissions, mathematical models, effects on people and animals, and environmental action in response to global pollution issues. This database also contains material from conference proceedings and hard-to-find summarized documents along with information from primary journals in the field of pollution.

BIOSIS Previews® contains citations from Biological Abstracts® (BA) and Biological Abstracts/Reports, Reviews, and Meetings® (BA/RRM) (formerly BioResearch Index®), the major publications of BIOSIS®. These publications constitute the major English-language service providing comprehensive worldwide coverage of research in the biological and biomedical sciences. Biological Abstracts includes approximately 350,000 accounts of original research yearly from nearly 5,000 primary journal and monograph titles. BA/RRM includes an additional 200,000+ citations a year from meeting abstracts, reviews, books, book chapters, notes, letters, and selected reports.

IPA Toxicology provides focused toxicology information on all phases of the development and use of drugs and on professional pharmaceutical practice. The scope of the database ranges from the clinical and practical to the theoretical aspects of toxicology literature. A unique feature of abstracts reporting clinical studies is the inclusion of the study design, number of patients, dosage, dosage forms, and dosage schedule.

Medical Literature, Analysis, and Retrieval System Online (MEDLINE®), produced by the U.S. National Library of Medicine (NLM), is NLM's premier bibliographic database. It contains more than 15 million references to journal articles in life sciences with a concentration on biomedicine. The broad coverage of the database includes basic biomedical research and the clinical sciences since 1950, including nursing, dentistry, veterinary medicine, pharmacy, allied health, and pre-clinical sciences. MEDLINE® also covers life sciences that are vital to biomedical practitioners, researchers, and educators, including some aspects of biology, environmental science, marine biology, and plant and animal science, as well as biophysics and chemistry. MEDLINE® is indexed using NLM's controlled vocabulary, Medical Subject

Headings (MeSH®). Approximately 400,000 records are added per year, of which more than 76 percent are in English. MEDLINE® contains AIDSLINE, HealthSTAR, Toxline, In Process (formerly known as Pre-MEDLINE®), In Data Review, and POPLINE.

ToxFile covers the toxicological, pharmacological, biochemical, and physiological effects of drugs and other chemicals. Adverse drug reactions, chemically induced diseases, carcinogenesis, mutagenesis, teratogenesis, environmental pollution, waste disposal, radiation, and food contamination are typical areas of coverage. The databases Environmental Mutagen Information Center (EMIC), Developmental and Reproductive Toxicology (DART), and Toxic Substances Control Act Test Submissions (TSCATS) are included in ToxFile. It is not clearly stated whether the Chemical Carcinogenesis Research Information System (CCRIS), Hazardous Substances Data Bank (HSDB), or Genetic Toxicology Data Bank (GENE-TOX) are included in ToxFile. Consequently, a separate, on-line search was conducted to ensure that these databases were searched.

BIOSIS® Toxicology contains citations from BA and BA/RRM (formerly BioResearch Index®), the major publications of BIOSIS®, that focus on toxicology and related topics. Records are drawn from journal articles, conference papers, monographs and book chapters, notes, letters, and reports, as well as original research. U.S. patent records are also included.

CANCERLIT® is produced by the International Cancer Research DataBank Branch (ICRDB) of the U.S. National Cancer Institute. The database consists of bibliographic records referencing cancer research publications dating from 1963 to 2002. Most records contain abstracts, and all records contain citation information and additional descriptive fields such as document type and language. Beginning with the June 1983 CANCERLIT update, records from the MEDLINE® database dealing with cancer topics have been added to CANCERLIT.

The Registry of Toxic Effects of Chemical Substances (RTECS®) is a comprehensive database of basic toxicity information for over 150,000 chemical substances including prescription and non-prescription drugs, food additives, pesticides, fungicides, herbicides, solvents, diluents, chemical wastes, reaction products of chemical waste, and substances used in both industrial and household situations. Reports of the toxic effects of each compound are cited. In addition to toxic effects and general toxicology reviews, data on skin and/or eye irritation, mutation, reproductive consequences and tumorigenicity are provided. Federal standards and regulations, National Institute for Occupational Safety and Health (NIOSH)

recommended exposure limits and information on the activities of EPA, NIOSH, National Toxicology Program (NTP), and Occupational Safety and Health Administration (OSHA) regarding the substance are also included. The toxic effects are linked to literature citations from both published and unpublished governmental reports, and published articles from the scientific literature. The database corresponds to the print version of the RTECS®, formerly known as the Toxic Substances List, which was started in 1971. Originally prepared by the NIOSH, the RTECS® database is now produced and distributed by Symyx Technologies, Inc.

C.2. TOXICOKINETIC MODELING CODE (EMOND ET AL., 2005)

C.2.1. Human Standard Model

C.2.1.1. *Model Code*

PROGRAM: 'Three Compartment PBPK Model for TCDD in Human: Standard Model (Non-Gestation)'

```
!HUM_NON_GEST_ICF_F083109.csl
!*************************************************

INITIAL  !INITIALIZATION OF PARAMETERS

     !SIMULATION PARAMETERS ====
CONSTANT EXP_TIME_ON       =     0.          ! TIME AT WHICH EXPOSURE BEGINS
(HOUR)
CONSTANT EXP_TIME_OFF      =     6.132e5   ! TIME AT WHICH EXPOSURE ENDS
(HOUR)
CONSTANT DAY_CYCLE         =     24.0      ! NUMBER OF HOURS BETWEEN DOSES
(HOUR)
CONSTANT BCK_TIME_ON       =     6.132e5      ! TIME AT WHICH BACKGROUND
EXPOSURE BEGINS (HOUR)
CONSTANT BCK_TIME_OFF      =     6.132e5      ! TIME AT WHICH BACKGROUND
EXPOSURE ENDS (HOUR)

     !EXPOSURE DOSES
CONSTANT  MSTOTBCKGR       =     0.0    ! ORAL BACKGROUND EXPOSURE DOSE
(NG/KG)
CONSTANT  MSTOT            =     1.0E-7       ! ORAL EXPOSURE DOSE (NG/KG)
CONSTANT  DOSEIV           =     0.0            ! INJECTED DOSE (NG/KG)
CONSTANT  MW             =     322.0        ! MOLECULAR WEIGHT (G/MOL)
 MSTOT_NM = MSTOT/MW                        ! CONVERTS THE DOSE TO NMOL/KG
 MSTOT_NMBCKGR = MSTOTBCKGR/MW  !CONVERTS THE BACKGROUND DOSE TO NMOL/KG
  DOSEIV_NM = DOSEIV/MW                     ! CONVERTS THE INJECTED DOSE TO
NMOL/KG

     !INITIAL GUESS OF THE FREE CONCENTRATION IN THE LIGAND (COMPARTMENT
INDICATED BELOW) ====
CONSTANT CFLLI0        =    0.0                  ! LIVER  (NMOL/L)
```

```
1
2       !BINDING CAPACITY (AhR) FOR NON LINEAR BINDING (COMPARTMENT INDICATED
3   BELOW) ===
4   CONSTANT LIBMAX         =    0.35            ! LIVER  (NMOL/L)
5
6       ! PROTEIN AFFINITY CONSTANTS (1A2 OR AhR, COMPARTMENT INDICATED BELOW)
7   ===
8   CONSTANT KDLI           =       0.1             ! LIVER (AhR) (NMOL/L) WANG
9   ET AL.. 1997
10  CONSTANT KDLI2          =       40.0          ! LIVER (1A2) (NMOL/L) EMOND ET
11  AL. 2004
12
13      !EXCRETION AND ABSORPTION CONSTANTS
14  CONSTANT KST            =       0.01            ! GASTRIC RATE CONSTANT (HR-
15  1), EMOND ET AL., 2005
16  CONSTANT KABS           =       0.06         ! INTESTINAL ABSORPTION CONSTANT
17  (HR-1), EMOND ET AL. 2005
18
19      !ELIMINATION CONSTANTS
20  CONSTANT CLURI          =      4.17D-8       ! URINARY CLEARANCE (L/HR), EMOND
21  ET AL., 2005
22  CONSTANT KELV           =     1.1e-3           ! INTERSPECIES VARIABLE
23  ELIMINATION CONSTANT (1/HOUR)
24
25      !CONSTANT TO DIVIDE THE ABSORPTION INTO LYMPHATIC AND PORTAL FRACTIONS
26  CONSTANT A              =       0.7                   ! LYMPHATIC FRACTION,
27  WANG ET AL. (1997)
28
29     !PARTITION COEFFICIENTS
30  CONSTANT PF             =       1.0e2               ! ADIPOSE TISSUE/BLOOD,
31  WANG ET AL. 1997
32  CONSTANT PRE            =       1.5                 ! REST OF THE BODY/BLOOD,
33  WANG ET AL. 1997
34  CONSTANT PLI            =       6.0                   ! LIVER/BLOOD, WANG ET
35  AL. 1997
36
37      !PARAMETERS FOR INDUCTION OF CYP1A2
38  CONSTANT PAS_INDUC      =       1.0           ! INCLUDE INDUCTION? (1 = YES, 0
39  = NO)
40  CONSTANT CYP1A2_1OUTZ   =   1.6e3       ! DEGRADATION CONCENTRATION CONSTANT
41  OF 1A2 (NMOL/L)
42  CONSTANT CYP1A2_1A1     =       1.6e3       ! BASAL CONCENTRATION OF 1A1
43  (NMOL/L)
44  CONSTANT CYP1A2_1EC50   =       1.3e2     ! DISSOCIATION CONSTANT TCDD-CYP1A2
45  (NMOL/L)
46  CONSTANT CYP1A2_1A2     =       1.6e3       ! BASAL CONCENTRATION OF 1A2
47  (NMOL/L)
48  CONSTANT CYP1A2_1KOUT   =       0.1       ! FIRST ORDER RATE OF DEGRADATION
49  (H-1)
50  CONSTANT CYP1A2_1TAU    =       0.25       ! HOLDING TIME (H)
51  CONSTANT CYP1A2_1EMAX   =       9.3e3   ! MAXIMUM INDUCTION OVER BASAL EFFECT
52  (UNITLESS)
53  CONSTANT HILL           =       0.6      !HILL CONSTANT; COOPERATIVELY LIGAND
54  BINDING EFFECT CONSTANT (UNITLESS)
55      ! DIFFUSIONAL PERMEABILITY FRACTION
56  CONSTANT PAFF           =      0.12                  ! ADIPOSE (UNITLESS)
```

```
CONSTANT PAREF          =      0.03                 ! REST OF BODY (UNITLESS)
CONSTANT PALIF          =      0.35                   ! LIVER (UNITLESS)

     !TISSUE BLOOD FLOW EXPRESSED AS A FRACTION OF CARDIAC OUTPUT  =========
CONSTANT QFF            =       0.05      ! ADIPOSE TISSUE BLOOD FLOW FRACTION
(UNITLESS), KRISHNAN 2008
CONSTANT QLIF           =       0.26      ! LIVER (UNITLESS), KRISHNAN 2008

     !COMPARTMENT TISSUE BLOOD EXPRESSED AS A FRACTION OF THE TOTAL
COMPARTMENT VOLUME =========
CONSTANT WFB0           =       0.050    ! ADIPOSE TISSUE, WANG ET AL. 1997
CONSTANT WREB0       =       0.030    ! REST OF THE BODY, WANG ET AL. 1997
CONSTANT WLIB0          =       0.266    ! LIVER, WANG ET AL. 1997

     !EXPOSURE SCENARIO FOR UNIQUE OR REPETITIVE WEEKLY OR MONTHLY EXPOSURE
     !NUMBER OF EXPOSURES PER WEEK
CONSTANT WEEK_LACK      =       0.0          ! DELAY BEFORE EXPOSURE ENDS
(WEEK)
CONSTANT WEEK_PERIOD    =       168.0     ! NUMBER OF HOURS IN THE WEEK
(HOURS)
CONSTANT WEEK_FINISH    =       168.0      ! TIME EXPOSURE ENDS (HOURS)
    !NUMBER OF EXPOSURES PER MONTH
CONSTANT MONTH_LACK     =       0.0        ! DELAY BEFORE EXPOSURE BEGINS
(MONTH)

     !SET FOR BACKGROUND EXPOSURE===========
     !TIME CONSTANT FOR BACKGROUND EXPOSURE==========
CONSTANT Day_LACK_BG      =    0.0          ! DELAY BEFORE EXPOSURE BEGINS
(HOUR)
CONSTANT Day_PERIOD_BG    =    24.0       ! LENGTH OF EXPOSURE (HOUR)

     !TIME CONSTANT FOR WEEKLY EXPOSURE
CONSTANT WEEK_LACK_BG     =    0.0       ! DELAY BEFORE BACKGROUND EXPOSURE
BEGINS (WEEK)
CONSTANT WEEK_PERIOD_BG   =    168.0     !  NUMBER OF HOURS IN THE WEEK
(HOURS)
CONSTANT WEEK_FINISH_BG   =    168.0       ! TIME EXPOSURE ENDS (HOURS)

     ! CONSTANT USED IN CARDIAC OUTPUT EQUATION
CONSTANT QCC            =    15.36                 ! (L/KG-H),  EMOND ET AL.
2004

     ! COMPARTMENT LIPID EXPRESSED AS THE FRACTION OF TOTAL LIPID
     !Data from Emonds Thesis 2001
CONSTANT F_TOTLIP         =    0.8000              ! ADIPOSE TISSUE
(UNITLESS)
CONSTANT B_TOTLIP         =    0.0057             ! BLOOD (UNITLESS)
CONSTANT RE_TOTLIP        =    0.0190            ! REST OF THE BODY
(UNITLESS)
CONSTANT LI_TOTLIP        =    0.0670              ! LIVER (UNITLESS)
CONSTANT MEANLIPID        =    974.0

END  ! END OF THE INITIAL SECTION


DYNAMIC ! DYNAMIC SIMULATION SECTION
```

```
   !
ALGORITHM  IALG          =      2         ! GEAR METHOD
CINTERVAL  CINT          =      10.0        ! COMMUNICATION INTERVAL
MAXTERVAL  MAXT          =    1.0e+10     !MAXIMUM INTERVAL CALCULATION
MINTERVAL  MINT          =    1.0E-10     !MINIMUM INTERVAL CALCULATION
VARIABLE   T             =      0.0
CONSTANT   TIMELIMIT     =     1.752e5  !SIMULATION LIMIT TIME (HOUR)
CONSTANT      Y0           =       0.0      ! AGE (YEARS) AT BEGINNING OF
SIMULATION
CONSTANT   GROWON        =        1.0    ! INCLUDE BODY WEIGHT AND HEIGHT
GROWTH? (1 = YES, 0 = NO)
  CINTXY  = CINT
  PFUNC   = CINT

  DAY=T/24.0                                    ! TIME IN DAYS
  WEEK =T/168.0                                ! TIME IN WEEKS
  MONTH =T/730.0                              ! TIME IN MONTHS
  YEAR=Y0+T/8760.0                          ! TIME IN YEARS
  GYR =Y0 + growon*T/8760.0        ! TIME FOR USE IN GROWTH EQUATION (YEARS)

DERIVATIVE ! PORTION OF CODE THAT SOLVES DIFFERENTIAL EQUATIONS

      ! CHRONIC OR SUBCHRONIC EXPOSURE SCENARIO =======
      ! NUMBER OF EXPOSURES PER DAY
 DAY_LACK     = EXP_TIME_ON        ! DELAY BEFORE EXPOSURE BEGINS (HOURS)
 DAY_PERIOD   = DAY_CYCLE          ! EXPOSURE PERIOD (HOURS)
 DAY_FINISH   = CINTXY             ! LENGTH OF EXPOSURE (HOURS)
 MONTH_PERIOD = TIMELIMIT          ! EXPOSURE PERIOD (MONTHS)
 MONTH_FINISH = EXP_TIME_OFF       ! LENGTH OF EXPOSURE (MONTHS)


      ! NUMBER OF EXPOSURES PER DAY AND MONTH
 DAY_FINISH_BG   = CINTXY
 MONTH_LACK_BG   = BCK_TIME_ON    !DELAY BEFORE BACKGROUD EXPOSURE BEGINS
(MONTHS)
 MONTH_PERIOD_BG = TIMELIMIT       ! BACKGROUND EXPOSURE PERIOD (MONTHS)
 MONTH_FINISH_BG = BCK_TIME_OFF    ! LENGTH OF BACKGROUND EXPOSURE (MONTHS)

 B = 1.0-A ! FRACTION OF DIOXIN ABSORBED IN THE PORTAL FRACTION OF THE LIVER

      !HUMAN BODY WEIGHT GROWTH EQUATION========
       ! POLYNOMIAL REGRESSION EXPRESSION WRITTEN
!APRIL 10 2008, OPTIMIZED WITH DATA OF PELEKIS ET AL. 2001
! POLYNOMIAL REGRESSION EXPRESSION WRITTEN WITH
!HUH AND BOLCH 2003 FOR BMI CALCULATION

  ! BODY WEIGHT CALCULATION
  WT0 = (0.0006*GYR**3 - 0.0912*GYR**2 + 4.32*GYR + 3.652)

   ! BODY MASS INDEX CALCULATION
     BH =  -2D-5*GYR**4+4.2D-3*GYR**3.0-0.315*GYR**2.0+9.7465*GYR+72.098

   !HEIGHT EQUATION FORMULATED FOR USE FROM 0 TO 70 YEARS
     BHM= (BH/100.0)               !HUMAN HEIGHT IN METERS (BHM)
     HBMI= WT0/(BHM**2.0) ! HUMAN BODY MASS INDEX (BMI)

```

```
    ! ADIPOSE TISSUE FRACTION
    WT0GR= WT0*1.0e3    ! BODY WEIGHT IN GRAMS
    WF0= -6.36D-20*WT0GR**4.0 +1.12D-14*WT0GR**3.0 -5.8D-10*WT0GR**2.0 +1.2D-
5*WT0GR+5.91D-2

    ! LIVER,VOLUME,
    ! APPROACH BASED ON LUECKE (2007)
    WLI0= (3.59D-2 -(4.76D-7*WT0GR)+(8.50D-12*WT0GR**2.0)-(5.45D-
17*WT0GR**3.0))

 WRE0 = (0.91 -(WLIB0*WLI0+WFB0*WF0+WLI0+WF0))/(1.0+WREB0)
                                    !REST OF THE BODY FRACTION; UPDATED FOR
EPA ASSESSMENT
 QREF = 1.0-(QFF+QLIF)                !REST OF BODY BLOOD FLOW
 QTTQF = QFF+QREF+QLIF               ! SUM MUST EQUAL 1

   !COMPARTMENT VOLUME (L OR KG) =========
 WF  =  WF0  * WT0                    ! ADIPOSE
 WRE =  WRE0 * WT0                    ! REST OF THE BODY
 WLI =  WLI0 * WT0                    ! LIVER
 WB=0.075*WT0                          ! BLOOD

   !COMPARTMENT TISSUE BLOOD (L OR KG) =========
 WFB  =  WFB0  * WF                    ! ADIPOSE
 WREB =  WREB0 * WRE                   ! REST OF THE BODY
 WLIB =  WLIB0 * WLI                   ! LIVER
   !CARDIAC OUTPUT FOR THE GIVEN BODY WEIGHT
QC= QCC*(WT0**0.75)                    ! [L BLOOD/HOUR]

QF  = QFF*QC                           ! ADIPOSE TISSUE BLOOD FLOW RATE
[L/HR]
QLI = QLIF*QC                          ! LIVER TISSUE BLOOD FLOW RATE [L/HR]
QRE = QREF*QC                         !REST OF THE BODY BLOOD FLOW RATE [L/HR]

QTTQ = QF+QRE+QLI                  ! TOTAL FLOW RATE [L/HR]

   !PERMEABILITY ORGAN FLOW [L/HR]=======
PAF  = PAFF*QF                          ! ADIPOSE
PARE = PAREF*QRE                        ! REST OF THE BODY
PALI = PALIF*QLI                        ! LIVER TISSUE

   ! ABSORPTION SECTION
   ! INTRAVENOUS
 IV        =    DOSEIV_NM * WT0          !AMOUNT IN NMOL
 MSTTBCKGR =    MSTOT_NMBCKGR *WT0       !AMOUNT IN (NMOL)
 MSTT      =    MSTOT_NM * WT0           !AMOUNT IN NMOL

     !REPETITIVE ORAL BACKGROUND EXPOSURE SCENARIOS
DAY_EXPOSURE_BG   = PULSE(DAY_LACK_BG,DAY_PERIOD_BG,DAY_FINISH_BG)
WEEK_EXPOSURE_BG  = PULSE(WEEK_LACK_BG,WEEK_PERIOD_BG,WEEK_FINISH_BG)
MONTH_EXPOSURE_BG = PULSE(MONTH_LACK_BG,MONTH_PERIOD_BG,MONTH_FINISH_BG)

MSTTCH_BG = (DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG)*MSTTBCKGR
MSTTFR_BG = MSTTBCKGR/CINT

CYCLE_BG =DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG
```

```
1
2
3       ! CONDITIONAL ORAL EXPOSURE (BACKGROUND EXPOSURE)
4   IF (MSTTCH_BG.EQ.MSTTBCKGR) THEN
5       ABSMSTT_GB= MSTTFR_BG
6   ELSE
7       ABSMSTT_GB = 0.0
8   END IF
9
10
11      !REPETITIVE ORAL MAIN EXPOSURE SCENARIO
12  DAY_EXPOSURE   = PULSE(DAY_LACK,DAY_PERIOD,DAY_FINISH)
13  WEEK_EXPOSURE  = PULSE(WEEK_LACK,WEEK_PERIOD,WEEK_FINISH)
14  MONTH_EXPOSURE = PULSE(MONTH_LACK,MONTH_PERIOD,MONTH_FINISH)
15
16  MSTTCH = (DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE)*MSTT
17  CYCLE = DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE
18  MSTTFR=MSTT/CINT
19
20      !CONDITIONAL ORAL EXPOSURE
21  IF (MSTTCH.EQ.MSTT) THEN
22     ABSMSTT= MSTTFR
23  ELSE
24     ABSMSTT = 0.
25  END IF
26
27   CYCLETOT=INTEG(CYCLE,0.0)
28
29        ! MASS Balance CHANGE IN THE LUMEN
30  RMSTT= -(KST+KABS)*MST+ABSMSTT +ABSMSTT_GB ! RATE OF CHANGE (NMOL/H)
31   MST = INTEG(RMSTT,0.)                      !AMOUNT REMAINING IN GI TRACT
32  (NMOL)
33
34        ! ABSORPTION IN LYMPH CIRCULATION
35  LYRMLUM = KABS*MST*A
36   LYMLUM = INTEG(LYRMLUM,0.0)
37
38        ! ABSORPTION IN PORTAL CIRCULATION
39  LIRMLUM = KABS*MST*B
40    LIMLUM = INTEG(LIRMLUM,0.0)
41
42        ! PERCENT OF DOSE REMAINING IN THE GI TRACT
43  PRCT_remain_GIT  = 100.0*MST/(MSTT+1E-30)
44
45        !IV ABSORTPION SCENARIO ---------
46   IVR= IV/PFUNC ! RATE FOR IV INFUSION IN BLOOD
47   EXPIV= IVR * (1.0-STEP(PFUNC))
48   IVDOSE = integ(EXPIV,0.0)
49
50        !SYSTEMIC BLOOD COMPARTMENT
51        ! MODIFICATION OCT 8 2009
52  CB=(QF*CFB+QRE*CREB+QLI*CLIB+EXPIV+LYRMLUM)/(QC+CLURI) !
53   CA = CB                                  !CONCENTRATION (NMOL/L)
54
55      !CB=(QF*CFB+QRE*CREB+QLI*CLIB+EXPIV+LYRMLUM-RAURI)/QC !
56      ! CA = CB                             ! CONCENTRATION (NMOL/L)
```

```
1
2          !URINARY EXCRETION BY KIDNEY
3          ! MODIFICATION OCT 8 2009
4   RAURI = CLURI *CB
5     AURI = INTEG(RAURI,0.0)
6
7
8          !CONCENTRATION UNIT
9     PRCT_B = 100.0*CB/(MSTT+1E-30)          ! PERCENT OF DOSE
10    CBSNGKGLIADJ = CB*MW/(0.55*B_TOTLIP) !serum concentration in lipid adjust
11  (PG/G LIPID=PPT)
12         CBPPT = CBSNGKGLIADJ
13    CBNGKG = CB*MW
14
15  CBpptRH = CB*MW*10000/(0.55*MEANLIPID) !SERUM CONCENTRATION IN LIPID ADJUST
16  (PG/G LIPID=PPT)
17
18      AUC_CBSNGKGLIADJ=INTEG(CBSNGKGLIADJ,0.0)
19
20         !ADIPOSE TISSUE COMPARTMENT
21  RAFB= QF*(CA-CFB)-PAF*(CFB-CF/PF)         !(NMOL/HR)
22   AFB = INTEG(RAFB,0.0)                     !(NMOL)
23   CFB = AFB/WFB                            !(NMOL/KG)
24         !TISSUE SUBCOMPARTMENT
25  RAF = PAF*(CFB-CF/PF)                     !(NMOL/HR)
26   AF = INTEG(RAF,0.0)                      !(NMOL)
27   CF  = AF/WF                              !(NMOL/KG)
28
29         !POST SIMULATION UNIT CONVERSION
30  CFTOTAL = (AF + AFB)/(WF + WFB) ! TOTAL CONCENTRATION NMOL/ML
31  PRCT_F = 100.0*CFTOTAL/(MSTT+1E-30)
32  CFNGKG =CFTOTAL*MW
33
34         !REST OF THE BODY COMPARTMENT========
35  RAREB= QRE*(CA-CREB)-PARE*(CREB-CRE/PRE) !(NMOL/HR)
36   AREB = INTEG(RAREB,0.0)                  !(NMOL)
37   CREB = AREB/WREB                         !(NMOL/KG)
38         !TISSUE SUBCOMPARTMENT
39  RARE = PARE*(CREB-CRE/PRE)                !(NMOL/HR)
40   ARE = INTEG(RARE,0.0)                   !(NMOL)
41   CRE  = ARE/WRE                           !(NMOL/KG)
42
43         !POST SIMULATION UNIT CONVERSION
44  CRETOTAL = (ARE + AREB)/(WRE + WREB) ! TOTAL CONCENTRATION IN NMOL/ML
45  PRCT_RE = 100.0*CRETOTAL/(MSTT+1E-30) ! PERCENT OF DOSE
46
47         !LIVER COMPARTMENT
48         !TISSUE BLOOD SUBCOMPARTMENT
49  RALIB = QLI*(CA-CLIB)-PALI*(CLIB-CFLLIR)+LIRMLUM          !(NMOL/HR)
50    ALIB = INTEG(RALIB,0.0)                                  !(NMOL)
51   CLIB = ALIB/WLIB
52         !TISSUE SUBCOMPARTMENT
53   RALI =  PALI*(CLIB-CFLLIR)-REXCLI                        !(NMOL/HR)
54    ALI = INTEG(RALI,0.0)                 !(NMOL)
55    CLI  = ALI/WLI                  !(NMOL/KG)
56
```

```
1
2         !FREE TCDD IN LIVER
3         ! MODIFICATION OCTOBER 8 2009
4   CFLLI= IMPLC(CLI-(CFLLIR*PLI+(LIBMAX*CFLLIR/(KDLI+CFLLIR)) &
5           +((CYP1A2_1O3*CFLLIR/(KDLI2+CFLLIR)*PAS_INDUC)))-CFLLI,CFLLI0) !
6   CONCENTRATION OF FREE TCDD IN LIVER
7       CFLLIR=DIM(CFLLI,0.0)
8
9   !MODIFIED FROM:
10        !PARAMETER (LIVER_1RMN = 1.0E-30)
11        ! CFLLI= IMPLC(CLI-(CFLLIR*PLI+(LIBMAX*CFLLIR/(KDLI+CFLLIR &       !
12  +LIVER_1RMN))+((CYP1A2_1O3*CFLLIR/(KDLI2+CFLLIR &
13        !         +LIVER_1RMN)*PAS_INDUC)))-CFLLI,CFLLI0)
14        !    CFLLIR=DIM(CFLLI,0.0)
15
16
17  CBNDLI= LIBMAX*CFLLIR/(KDLI+CFLLIR) !CONC OF TCDD BOUDN TO AhR
18
19  !CBNDLI= LIBMAX*CFLLIR/(KDLI+CFLLIR+LIVER_1RMN) !CONC BIND
20
21        !POST SIMULATION UNIT CONVERSION
22  CLITOTAL = (ALI + ALIB)/(WLI + WLIB)       ! TOTAL CONCENTRATION IN NMOL/ML
23  PRCT_LI = 100.0*CLITOTAL/(MSTT+1.0E-30)
24  rec_occ_AHR= 100.0*CFLLIR/(KDLI+CFLLIR+1.0)  ! PERCENT  BOUND TO AhR
25  OCCUPANCY
26  PROT_occ_1A2= 100.0*CFLLIR/(KDLI2+CFLLIR)    ! PERCENT  BOUND TO 1A2
27  OCCUPANCY
28  CLINGKG= CLITOTAL*MW                        ![NG TCDD/KG]
29  CBNDLINGKG = CBNDLI*MW
30
31      !FRACTION INCREASE OF INDUCTION OF CYP1A2
32  fold_ind=CYP1A2_1OUT/CYP1A2_1A2
33  VARIATIONOFAC =(CYP1A2_1OUT-CYP1A2_1A2)/CYP1A2_1A2
34
35      !VARIABLE ELIMINATION BASED ON THE CYP1A2
36  KBILE_LI_T = Kelv*VARIATIONOFAC!
37
38   REXCLI = KBILE_LI_T*CFLLIR*WLI ! DOSE-DEPENDENT RATE OF BILLIARY EXCRETION
39  OF DIOXIN
40      EXCLI = INTEG(REXCLI,0.0)  !TOTAL AMOUNT OF DIOXIN EXCRETED
41
42      !CHEMICAL IN CYP450 (1A2) COMPARTMENT
43      !PARAMETER FOR INDUCTION OF CYP1A2
44
45  CYP1A2_1KINP = CYP1A2_1KOUT*CYP1A2_1OUTZ ! BASAL RATE OF CYP1A2 PRODUCTION
46  SET EQUAL TO BASAL RATE OF DEGRDATION AT STEADY STATE
47
48      ! MODIFICATION OCTOBER 8 2009
49  CYP1A2_1OUT =INTEG(CYP1A2_1KINP * (1.0 + CYP1A2_1EMAX *(CBNDLI+1.0e-30)**HILL
50  &
51       /(CYP1A2_1EC50**HILL + (CBNDLI+1.0e-30)**HILL)) &
52        - CYP1A2_1KOUT*CYP1A2_1OUT, CYP1A2_1OUTZ) ! LEVELS OF CYP1A2
53  ! MODEIFIED FROM:
54  !PARAMETER (CYP1A2_1RMN = 1e-30)
55  !CYP1A2_1OUT =INTEG(CYP1A2_1KINP * (1 + CYP1A2_1EMAX *(CBNDLI &
56  !     +CYP1A2_1RMN)**HILL/(CYP1A2_1EC50 + (CBNDLI + CYP1A2_1RMN)**HILL) &
```

```
!     +CYP1A2_1RMN) - CYP1A2_1KOUT*CYP1A2_1&
!     OUT, CYP1A2_1OUTZ)

! EQUATIONS INCORPORATING DELAY OF CYP1A2 PRODUCTION (NOT USED IN
SIMULATIONS)
CYP1A2_1RO2 = (CYP1A2_1OUT - CYP1A2_1O2)/ CYP1A2_1TAU
    CYP1A2_1O2 =INTEG(CYP1A2_1RO2, CYP1A2_1A1)
 CYP1A2_1RO3 = (CYP1A2_1O2 - CYP1A2_1O3)/ CYP1A2_1TAU
    CYP1A2_1O3 =INTEG(CYP1A2_1RO3, CYP1A2_1A2)

     !CHECK MASS BALANCE
  BDOSE= LYMLUM+LIMLUM+IVDOSE
  BMASSE = EXCLI+AURI+AFB+AF+AREB+ARE+ALIB+ALI
      BDIFF = BDOSE-BMASSE
     ! BODY BURDEN IN TERMS OF CONCENTRATION (NG/KG)
  BBNGKG = (AFB+AF+AREB+ARE+ALIB+ALI)*MW/WT0       !

     !COMMAND END OF THE SIMULATION
TERMT (T.GE. TIMELIMIT, 'Time limit has been reached.')

END   ! END OF THE DERIVATIVE SECTION
END   ! END OF THE DYTNAMIC SECTION
END   ! END OF THE PROGRAM
```

C.2.1.2. *Input File*

```
% base file name = "TESTJULY2009.m"
%clear @variable
output @clear
prepare @clear year T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG  CBNDLINGKG CBNGKG
%output @all
% PARAMETERS FOR SIMULATION
CINT = 1 %0.5
EXP_TIME_ON  =   0.       % TIME AT WHICH EXPOSURE BEGINS (HOUR)
EXP_TIME_OFF = 613200     %324120     % HOUR/YEAR !TIME AT WHICH EXPOSURE
ENDS (HOUR)
DAY_CYCLE    = 24         % NUMBER OF HOURS BETWEEN DOSES (HOUR)
BCK_TIME_ON  = 613200     %324120     % TIME AT WHICH BACKGROUND EXPOSURE
BEGINS (HOUR)
BCK_TIME_OFF = 613200     %324120     % TIME AT WHICH BACKGROUND EXPOSURE
ENDS (HOUR)
TIMELIMIT    = 613200     %324120       %324120     % SIMULATION TIME LIMIT
(HOUR)
MSTOTBCKGR   =   0.       % ORAL BACKGROUND EXPOSURE DOSE (UG/KG)

% oral dose oral dose oral dose
MSTOT        = 9.97339283634997E-07       % ORAL DAILY EXPOSURE DOSE (NG/KG)
DOSEIV       =   0          %NG/KG
% oral dose oral dose oral dose

MEANLIPID    = 730        %
PAS_INDUC= 1          % INDUCTION INCLUDED? (1=YES, 0=NO)
```

C.2.2. Human Gestational Model

C.2.2.1. *Model Code*

PROGRAM: 'Three Compartment PBPK Model for TCDD in Human (Gestation)'

```
! Parameters were change may 16, 2002
! Come from {8MAI_CHR_PRE-EXP_GD}
! Come from {12_Mouse_GD}file
!*****************************************
!{{IMPORTANT-IMPORTANT-IMPORTANT-IMPORTANT}}
! REDUCTION OF MOTHER AND FETUS COMPARTMENT
! 2M_R_TCDD_JULY2002 ////(JULY 18,2002)////
!TCDD_RED_4Species_2003_4        ////(APR  8 ,2003)////
!TCDD_RED_4Species_2003_9        ////(APR  17 ,2003)////
!TCDD_RED_4Species_2003_12        ////(APR  17 ,2003)////
!************************************************
!APRIL 18 2003
!TCDD_4C_4SP_2003        ////(APR  18 ,2003)////
! was ''Gest 4 species 1.csl''   but update July 2009

!GEST_HUM_0_45Y_4_ICF_afterKKfix_v3_humangestational.csl
!HUM_GESTATIONAL_ICF_F083109.csl
!HUM_GESTATIONAL_ICF_F100709.csl
!************************************************

 !Legend/Legend/Legend/Legend/Legend/Legend/Legend/Legend/
 !Legend for this PBPK model
 !Mating: control the tenure of exchange between fetus and
    !Mother and also control imitated tissue growth
    !Control: WTFE, WPLA0, QPLAF
    !(for rat, mouse, human, and monkey)
 !Control transfer from mother to fetus and fetus to mother by TRANSTIME_ON
    !SWITCH_trans = 0 NO TRANSFER
    !SWITCH_trans = 1 TRANSFER OCCURS
 ! These switches are also controlled by mating parameters

INITIAL  !

     !SIMULATION PARAMETERS
CONSTANT PARA_ZERO          = 1e-30
CONSTANT EXP_TIME_ON     = 0.0       !TIME AT WHICH EXPOSURE BEGINS (HOURS)
CONSTANT EXP_TIME_OFF    = 530.0      !TIME AT WHICH EXPOSURE ENDS (HOURS)
CONSTANT DAY_CYCLE       =  24.0     !NUMBER OF HOURS BETWEEN DOSES (HOURS)
CONSTANT BCK_TIME_ON     = 0.0       !TIME AT WHICH BACKGROUND EXPOSURE
BEGINS (HOURS)
CONSTANT BCK_TIME_OFF    = 0.0       !TIME AT WHICH BACKGROUND EXPOSURE ENDS
(HOURS)
CONSTANT TRANSTIME_ON    = 0.0       !CONTROL TRANSFER FROM MOTHER TO FETUS
AT 9 WEEKS OR 1512 HOURS OF GESTATION

     ! INTRAVENOUS SEQUENCY
CONSTANT IV_LACK         = 0.0
CONSTANT IV_PERIOD       = 0.0

     !PREGNANCY PARAMETER
```

```
CONSTANT MATTING         = 0.0       !BEGINNING OF MATING (HOUR)
CONSTANT PFETUS          = 4.0       !PARTITION COEFFICIENT
CONSTANT CLPLA_FET       = 1.0e-3    !CLEARANCE TRANSFER FOR MOTHER TO FETUS
(L/HR)

     !CONSTANT EXPOSURE CONTROL
     !ACUTE, SUBCHRONIC, CHRONIC EXPOSURE =====
     !OR BACKGROUND EXPOSURE (IN THIS CASE 3 TIMES A DAY)===
CONSTANT MSTOTBCKGR      = 0.0       ! ORAL BACKGROUND EXPOSURE DOSE (NG/KG)
CONSTANT MSTOT           = 0.0       ! ORAL EXPOSURE DOSE (NG/KG)

     !ORAL ABSORPTION
     ! MSTT= MSTOT/1000 *WT0 *1/322*1000 !AMOUNT IN NMOL
  MSTOT_NM = MSTOT/MW              !CONVERTS THE DOSE TO NMOL/KG

     !INTRAVENOUS ABSORPTION
CONSTANT  DOSEIV         = 0.0       ! INJECTED DOSE (NG/KG)
  DOSEIV_NM = DOSEIV/MW            ! CONVERTS THE INJECTED DOSE TO NMOL/KG
CONSTANT DOSEIVLATE = 0.0            !INJECTED DOSE LATE (UG/KG)
  DOSEIVNMlate = DOSEIVLATE/MW     !AMOUNT IN NMOL/G

     !INITIAL GUESS OF THE FREE CONCENTRATION IN THE LIGAND (COMPARTMENT
INDICATED BELOW)====
CONSTANT CFLLI0          =  0.0      !LIVER    (NMOL/L)
CONSTANT CFLPLA0         =  0.0      !PLACENTA (NMOL/L)

     !BINDING CAPACITY (AhR) FOR NON LINEAR BINDING (COMPARTMENT INDICATED
BELOW) (NMOL/L) ===
CONSTANT LIBMAX          = 0.35     ! LIVER  (NMOL/L)
CONSTANT PLABMAX         = 0.2      !TEMPORARY PARAMETER

     !PROTEIN AFFINITY CONSTANTS (1A2 OR AhR, COMPARTMENT INDICATED BELOW)
(NMOL/ML)===
CONSTANT KDLI            = 0.1      !LIVER (AhR) (NMOL/L), WANG ET AL. 1997
CONSTANT KDLI2           = 40.0       !LIVER (1A2) (NMOL/L), EMOND ET AL.
2004
CONSTANT KDPLA           = 0.1      !ASSUME IDENTICAL TO KDLI (AhR)

     !EXCRETION AND ABSORPTION CONSTANT
CONSTANT KST             = 0.01     ! GASTRIC RATE CONSTANT (HR-1), EMOND ET
AL. 2005
CONSTANT KABS            = 0.06     ! INTESTINAL ABSORPTION CONSTANT (HR-1),
EMOND ET AL. (2005)

     !INTERSPECIES ELIMINATION CONSTANT
     !TEST ELIMINATION VARIABLE, EMOND ET AL. 2005
CONSTANT KELV          =     1.1e-3 !4.0D-3          ! INTERSPECIES VARIABLE
ELIMINATION CONSTANT (1/HOUR)

     ! ELIMINATION CONSTANTS
CONSTANT CLURI           =  4.17e-8 ! URINARY CLEARANCE (L/HR), EMOND ET AL.
2005

     ! CONSTANT TO DIVIDE THE ABSORPTION INTO LYMPHATIC AND PORTAL FRACTIONS
CONSTANT A               = 0.7        ! LYMPHATIC FRACTION, WANG ET AL. 1997
```

```
     !PARTITION COEFFICIENTS
CONSTANT PF              = 1.0e2     ! ADIPOSE TISSUE/BLOOD, WANG ET AL. 1997
CONSTANT PRE             = 1.5      ! REST OF THE BODY/BLOOD, WANG ET AL.
1997
CONSTANT PLI             = 6.0       ! LIVER/BLOOD, WANG ET AL. 1997
CONSTANT PPLA            = 1.5      ! TEMPORARY PARAMETER NOT CONFIGURED,
WANG ET AL. 1997

    !PARAMETER FOR INDUCTION OF CYP 1A2, WANG ET AL. 1997
CONSTANT PAS_INDUC       = 1.0        ! INCLUDE INDUCTION? (1 = YES, 0 = NO)
CONSTANT CYP1A2_1OUTZ    = 1.6e3     ! DEGRADATION CONCENTRATION CONSTANT OF
1A2 (NMOL/L)
CONSTANT CYP1A2_1A1      = 1.6e3     ! BASAL CONCENTRATION OF 1A1 (NMOL/L)
CONSTANT CYP1A2_1EC50    = 1.3e2    ! DISSOCIATION CONSTANT TCDD-CYP1A2
(NMOL/L)
CONSTANT CYP1A2_1A2      = 1.6e3     !BASAL CONCENTRATION OF 1A2 (NMOL/ML)
CONSTANT CYP1A2_1KOUT    = 0.1      ! FIRST ORDER RATE OF DEGRADATION (H-1)
CONSTANT CYP1A2_1TAU     = 0.25     !HOLDING TIME (H)
CONSTANT CYP1A2_1EMAX    = 9.3e3     ! MAXIMUM INDUCTION OVER BASAL EFFECT
(UNITLESS)
CONSTANT HILL            = 0.6      !HILL CONSTANT; COOPERATIVELY LIGAND
BINDING EFFECT CONSTANT (UNITLESS)

   !DIFFUSIONAL PERMEABILITY FRACTION, WANG ET AL (1997)
CONSTANT PAFF            = 0.12     ! ADIPOSE (UNITLESS)
CONSTANT PAREF           = 0.03     ! REST OF THE BODY (UNITLESS)
CONSTANT PALIF           = 0.35     ! LIVER (UNITLESS)
CONSTANT PAPLAF          = 0.3      ! OPTIMIZED PARAMETER

!TISSUE BLOOD FLOW EXPRESSED AS A FRACTION OF CARDIAC OUTPUT, KRISHNAN 2007
CONSTANT QFF             = 0.05     ! ADIPOSE TISSUE BLOOD FLOW FRACTION
(UNITLESS), KRISHNAN 2008
CONSTANT QLIF            = 0.26     ! LIVER (UNITLESS), KRISHNAN 2008

!===FRACTION OF TISSUE BLOOD WEIGHT Wang et al . (1997)
CONSTANT WFB0            = 0.050    !ADIPOSE TISSUE, WANG ET AL. 1997
CONSTANT WREB0           = 0.030    !REST OF THE BODY, WANG ET AL. 1997
CONSTANT WLIB0           = 0.266    !LIVER, WANG ET AL. 1997
CONSTANT WPLAB0          = 0.500    !ASSUME HIGHLY VASCULARIZED

! EXPOSURE SCENARIO FOR UNIQUE OR REPETITIVE WEEKLY OR MONTHLY EXPOSURE
! NUMBER OF EXPOSURES PER WEEK
CONSTANT WEEK_LACK       = 0.0       !DELAY BEFORE EXPOSURE ENDS (WEEK)
CONSTANT WEEK_PERIOD     = 168.0      ! NUMBER OF HOURS IN THE WEEK (HOURS)
CONSTANT WEEK_FINISH     = 168.0      ! TIME EXPOSURE ENDS (HOURS)

! NUMBER OF EXPOSURES PER MONTH
CONSTANT MONTH_LACK      = 0.0       !DELAY BEFORE EXPOSURE BEGINS (MONTHS)

!======= CONSTANT FOR BACKGROUND EXPOSURE===========
CONSTANT Day_LACK_BG     = 0.0       ! DELAY BEFORE EXPOSURE BEGINS (HOURS)
CONSTANT Day_PERIOD_BG   = 24.0       !LENGTH OF EXPOSURE (HOURS)

! NUMBER OF EXPOSURES PER WEEK
CONSTANT WEEK_LACK_BG    = 0.0      !DELAY BEFORE BACKGROUD EXPOSURE BEGINS
(WEEK)
```

```
CONSTANT WEEK_PERIOD_BG  = 168.0      ! NUMBER OF HOURS IN THE WEEK (HOURS)
CONSTANT WEEK_FINISH_BG  = 168.0      !TIME EXPOSURE ENDS (HOURS)


! CONSTANT USED IN CARDIAC OUTPUT EQUATION
CONSTANT QCC            =  15.36    ![L/KG-H], EMOND ET AL. 2004

! COMPARTMENT LIPID EXPRESSED AS THE FRACTION OF TOTAL LIPID
!Data from Emonds Thesis 2001
CONSTANT F_TOTLIP         =    0.8000       ! ADIPOSE TISSUE (UNITLESS)
CONSTANT B_TOTLIP         =    0.0057       ! BLOOD (UNITLESS)
CONSTANT RE_TOTLIP        =    0.0190       ! REST OF THE BODY (UNITLESS)
CONSTANT LI_TOTLIP        =    0.0670       ! LIVER (UNITLESS)
CONSTANT PLA_TOTLIP       =    0.019       ! PLACENTA (UNITLESS)
CONSTANT FETUS_TOTLIP     =    0.019     ! FETUS (UNITLESS)

CONSTANT MEANLIPID        =    974

END  ! END OF THE INITIAL SECTION

DYNAMIC ! DYNAMIC SIMULATION SECTION

ALGORITHM  IALG          =           2        ! GEAR METHOD
CINTERVAL  CINT          =         0.1        ! COMMUNICATION INTERVAL
MAXTERVAL  MAXT          =       1.0e+10      ! MAXIMUM CALCULATION INTERVAL
MINTERVAL  MINT          =       1.0E-10      ! MINIMUM CALCULATION INTERVAL
VARIABLE   T             =         0.0
CONSTANT   TIMELIMIT     =         100        !SIMULATION LIMIT TIME (HOUR)
CONSTANT      Y0           =          0.0      ! AGE (YEARS) AT BEGINNING OF
SIMULATION
CONSTANT   GROWON        =         1.0       ! INCLUDE BODY WEIGHT AND HEIGHT
GROWTH? (1=YES, 0=NO)

 CINTXY  = CINT
 PFUNC   = CINT

   !TIME TRANSFORMATION
 DAY= T/24.0
 WEEK  =T/168.0
 YEAR=Y0+T/8760.0                              ! TIME IN YEARS
  GYR =Y0 + growon*T/8760.0                    ! TIME FOR USE IN GROWTH
EQUATION

DERIVATIVE ! PORTION OF CODE THAT SOLVES DIFFERENTIAL EQUATIONS

!====== CHRONIC OR SUBCHRONIC EXPOSURE SCENARIO =======
! NUMBER OF EXPOSURES PER DAY

 DAY_LACK         = EXP_TIME_ON    ! DELAY BEFORE EXPOSURE BEGINS (HOURS)
 DAY_PERIOD       = DAY_CYCLE      ! EXPOSURE PERIOD (HOURS)
 DAY_FINISH       = CINTXY         ! LENGTH OF EXPOSURE (HOURS)
 MONTH_PERIOD     = TIMELIMIT      ! EXPOSURE PERIOD (MONTHS)
 MONTH_FINISH     = EXP_TIME_OFF   ! LENGTH OF EXPOSURE (MONTHS)


! NUMBER OF EXPOSURES PER DAY AND MONTH
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```
 DAY_FINISH_BG    = CINTXY
 MONTH_LACK_BG    = BCK_TIME_ON    !DELAY BEFORE BACKGROUND EXPOSURE BEGINS
(MONTHS)
 MONTH_PERIOD_BG  = TIMELIMIT      !BACKGROUND EXPOSURE PERIOD (MONTHS)
 MONTH_FINISH_BG  = BCK_TIME_OFF   !LENGTH OF BACKGROUND EXPOSURE (MONTHS)

! INTRAVENOUS LATE
 IV_FINISH = CINTXY
 B = 1-A ! FRACTION OF DIOXIN ABSORBED IN THE PORTAL FRACTION OF THE LIVER

! MOTHER BODY WEIGHT GROWTH EQUATION
! MODIFICATION TO ADAPT THIS MODEL AT HUMAN MODEL
! BECAUSE LINEAR DESCRIPTION IS NOT GOOD ENOUGH FOR MOTHER GROWTH
! MOTHER BODY WEIGHT GROWTH
! HUMAN BODY WEIGHT (0 TO 45 YEARS)
! POLYNOMIAL REGRESSION EXPRESSION WRITTEN
!APRIL 10 2008, OPTIMIZED WITH DATA OF PELEKIS ET AL. 2001
! POLYNOMIAL REGRESSION EXPRESSION WRITTEN WITH
!HUH AND BOLCH 2003 FOR BMI CALCULATION

! BODY WEIGHT CALCULATION.  UNIT IN KG FOR GESTATIONAL PORTION

     WT0 = (0.0006*GYR**3 - 0.0912*GYR**2 + 4.32*GYR + 3.652)

 !BODY MASS INDEX CALCULATION

     BH =  -2D-5*GYR**4+4.2D-3*GYR**3.0-0.315*GYR**2.0+9.7465*GYR+72.098
!HEIGHT EQUATION FORMULATED FOR USE FROM 0 TO 70 YEARS
     BHM= (BH/100.0)!HUMAN HEIGHT IN METER (BHM)
     HBMI= WT0/(BHM**2.0) ! HUMAN BODY MASS INDEX (BMI)


!MODIFICATION IN KG
RTESTGEST= T-MATTING ! STARTING TIME FOR FETAL GROWTH
TESTGEST=DIM(RTESTGEST,0.0)
! GROWTH OF FETAL TISSUE
GESTATTION_FE=((4d-15*TESTGEST**4 -3d-11*TESTGEST**3 +1d-7*TESTGEST**2 -8d-
5*TESTGEST +0.0608))
   WTFER= DIM(GESTATTION_FE,0.0) ! FETAL COMPARTMENT WEIGHT
 WTFE= WTFER

!////////////////////////////////////////////////////////////////////
! FAT GROWTH EXPRESSION LINEAR DURING PREGNANCY
! FROM O'FLAHERTY_1992
!////////////////////////////////////////////////////////////////////

WT0GR= WT0*1.0e3    ! MOTHER BODY WEIGHT IN G

WF0 =(-6.36D-20*WT0GR**4.0 +1.12D-14*WT0GR**3.0 &
           -5.8D-10*WT0GR**2.0+1.2D-5*WT0GR+5.91D-2) ! MOTHER FAT COMPARTMENT
GROWTH

!////////////////////////////////////////////////////////////////////
! WPLA PLACENTA GROWTH EXPRESSION, SINGLE EXPONENTIAL WITH OFFSET
! FROM O'FLAHERTY_1992  ! FOR EACH PUP
!////////////////////////////////////////////////////////////////////
```

```
!SAME EQUATION THEN THE FORST MODEL. BODY WEIGHT KEPT IN G
!A CORRECTION FOR THE BODY WEIGHT (WTO(KG)*1000 = WTOGR)

WPLA0N_HUMAN= (850*exp(-9.434*(exp(-5.23d-4*(TESTGEST)))))
 WPLA0R = WPLA0N_HUMAN/WT0GR
 WPLA0W = DIM(WPLA0R,0.0) ! PLACENTA WEIGHT
  WPLA0=WPLA0W

!////////////////////////////////////////////////////////////////////
! QPLA PLACENTA GROWTH EXPRESSION, DOUBLE EXPONENTIAL WITH OFFSET
! FROM O'FLAHERTY_1992
!////////////////////////////////////////////////////////////////////

QPLAF_HUMAN= SWITCH_trans*((1d-10*TESTGEST**3.0 -5D-7*TESTGEST**2.0
+0.0017*TESTGEST+1.1937)/QC)
  GEST_QPLAF=DIM(QPLAF_HUMAN,0.0) ! PLACENTA BLOOD FLOW RATE
  QPLAF =GEST_QPLAF

! LIVER,VOLUME  (HUMAN 0 TO 70 YEARS)
! APPROACH BASED ON LUECKE (2007)
 WLI0= (3.59D-2 -(4.76D-7*WT0GR)+(8.50D-12*WT0GR**2.0)-(5.45D-17*WT0GR**3.0))
! LIVER VOLUME IN GROWING HUMAN

! VARIABILITY OF REST OF THE BODY DEPENDS ON OTHER ORGAN
 WRE0 = (0.91-(WLIB0*WLI0+WFB0*WF0+ WPLAB0*WPLA0 + WLI0 + WF0 +
WPLA0))/(1+WREB0)
 QREF = 1-(QFF+QLIF+QPLAF)            !REST BODY BLOOD FLOW (ML/HR)
 QTTQF = QFF+QREF+QLIF+QPLAF          ! SUM MUST EQUAL 1

! COMPARTMENT TISSUE BLOOD VOLUME (L) =========
 WF  =  WF0  * WT0                    ! ADIPOSE TISSUE
 WRE =  WRE0 * WT0                    ! REST OF THE BODY
 WLI =  WLI0 * WT0                    ! LIVER
 WPLA=  WPLA0* WT0                    ! PLACENTA

! COMPARTMENT TISSUE VOLUME (L) =========
 WFB  =  WFB0  * WF                   ! ADIPOSE TISSUE
 WREB =  WREB0 * WRE                  ! REST OF THE BODY
 WLIB =  WLIB0 * WLI                  ! LIVER
 WPLAB = WPLAB0* WPLA                 ! PLACANTA

! TOTAL VOLUME OF COMPARTMENT (L)======
WFT =   WF                            ! TOTAL ADIPOSE TISSUE
WRET =  WRE                           ! TOTAL REST OF THE BODY
WLIT =  WLI                           ! TOTAL LIVER TISSUE
WPLAT=  WPLAB                         ! TOTAL PLACENTA TISSUE

! CONSTANT USED IN CARDIAC OUTPUT EQUATION

! UNIT CHANGED ON JULY 14 2009  (L/HR)
QC= QCC*(WT0)**0.75

QF  = QFF*QC                         ! ADIPOSE TISSUE BLOOD FLOW RATE (L/HR)
QLI = QLIF*QC                        ! LIVER TISSUE BLOOD FLOW RATE (L/HR)
QRE = QREF*QC                        !REST OF THE BODY BLOOD FLOW RATE (L/HR)
QPLA = QPLAF*QC                      !PLACENTA TISSUE BLOOD FLOW RATE (L/HR)
```

```
QTTQ = QF+QRE+QLI+QPLA     !TOTAL FLOW RATE (L/HR)

! ========= DIFFUSIONAL PERMEABILITY FACTORS FRACTION ORGAN FLOW =========
PAF  = PAFF*QF                        ! ADIPOSE TISSUE BLOOD FLOW RATE (L/HR)
PARE = PAREF*QRE                      ! REST OF THE BODY BLOOD FLOW RATE
(L/HR)
PALI = PALIF*QLI                      ! LIVER TISSUE BLOOD FLOW RATE (L/HR)
PAPLA = PAPLAF*QPLA                   ! PLACENTA TISSUE BLOOD FLOW RATE (L/HR)

!************************************
! ABSORPTION SECTION
! ORAL
! INTRAPERITONEAL
! SUBCUTANEOUS
! INTRAVENOUS
!************************************

!BACKGROUND EXPOSURE
!EXPOSURE FOR STEADY STATE CONSIDERATION
!REPETITIVE EXPOSURE SCENARIO

MSTOT_NMBCKGR = MSTOTBCKGR/322       !AMOUNT IN NMOL/G
MSTTBCKGR =MSTOT_NMBCKGR *WT0

DAY_EXPOSURE_BG   = PULSE(DAY_LACK_BG,DAY_PERIOD_BG,DAY_FINISH_BG)
WEEK_EXPOSURE_BG  = PULSE(WEEK_LACK_BG,WEEK_PERIOD_BG,WEEK_FINISH_BG)
MONTH_EXPOSURE_BG = PULSE(MONTH_LACK_BG,MONTH_PERIOD_BG,MONTH_FINISH_BG)

MSTTCH_BG = (DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG)*MSTTBCKGR
MSTTFR_BG = MSTTBCKGR/CINT

CYCLE_BG =DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG

! CONDITIONAL ORAL EXPOSURE (BACKGROUND EXPOSURE)

IF (MSTTCH_BG.EQ.MSTTBCKGR) THEN
    ABSMSTT_GB= MSTTFR_BG
ELSE
    ABSMSTT_GB = 0.0
END IF

CYCLETOTBG=INTEG(CYCLE_BG,0.0)

!************************************
!MULTIROUTE  EXPOSURE
!REPETITIVE EXPOSURE SCENARIO
!************************************
MSTT= MSTOT_NM * WT0                   !AMOUNT IN NMOL
DAY_EXPOSURE   = PULSE(DAY_LACK,DAY_PERIOD,DAY_FINISH)
WEEK_EXPOSURE  = PULSE(WEEK_LACK,WEEK_PERIOD,WEEK_FINISH)
MONTH_EXPOSURE = PULSE(MONTH_LACK,MONTH_PERIOD,MONTH_FINISH)

MSTTCH = (DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE)*MSTT

MSTTFR = MSTT/CINT

```

```
CYCLE = DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE

SUMEXPEVENT= INTEG (CYCLE,0.0) !NUMBER OF CYCLES GENERATED DURING SIMULATION

! CONDITIONAL ORAL EXPOSURE
IF (MSTTCH.EQ.MSTT) THEN
   ABSMSTT= MSTTFR
ELSE
   ABSMSTT = 0.0
END IF


 CYCLETOT=INTEG(CYCLE,0.0)

! MASS CHANGE IN THE LUMEN
 RMSTT= -(KST+KABS)*MST +ABSMSTT +ABSMSTT_GB ! RATE OF CHANGE (NMOL/H)
  MST = INTEG(RMSTT,0.0)                      !AMOUNT REMAINING IN DUODENUM
(NMOL)

! ABSORPTION IN LYMPH CIRCULATION
 LYRMLUM = KABS*MST*A
  LYMLUM = INTEG(LYRMLUM,0.0)

! ABSORPTION IN PORTAL CIRCULATION
 LIRMLUM = KABS*MST*B
  LIMLUM = INTEG(LIRMLUM,0.0)


    !IV ABSORPTION SCENARIO---------
 IV= DOSEIV_NM * WT0 !AMOUNT IN NMOL
 IVR= IV/PFUNC ! RATE FOR IV INFUSION IN BLOOD
 EXPIV= IVR * (1-STEP(PFUNC))
 IVDOSE = integ(EXPIV,0.0)

   !IV LATE IN THE CYCLE
   !MODIFICATION JANUARY 13 2004
 IV_RlateR = DOSEIVNMlate*WT0
 IV_EXPOSURE=PULSE(IV_LACK,IV_PERIOD,IV_FINISH)

 IV_lateT = IV_EXPOSURE *IV_RlateR
 IV_late = IV_lateT/CINT

SUMEXPEVENTIV= integ(IV_EXPOSURE,0.0) !NUMBER OF CYCLE GENERATE DURING
SIMULATION

      !SYSTEMIC BLOOD COMPARTMENT
      ! MODIFICATION OCT 8 2009
CB=(QF*CFB+QRE*CREB+QLI*CLIB+EXPIV+LYRMLUM+QPLA*CPLAB+IV_late)/(QC+CLURI) !
 CA = CB                                  ! CONCENTRATION (NMOL/L)

     !CB=(QF*CFB+QRE*CREB+QLI*CLIB+EXPIV+LYRMLUM+QPLA*CPLAB+IV_late-RAURI)/QC
!(NMOL/L)

    !URINARY EXCRETION BY KIDNEY
    ! MODIFICATION OCT 8 2009
RAURI = CLURI *CB
```

```
  AURI = INTEG(RAURI,0.0)

    !RAURI = CLURI * CRE
    !AURI = INTEG(RAURI,0.0)

    !UNIT CONVERSION POST SIMULATION
CONSTANT MW=322 !MOLECULAR WEIGHT (NG/NMOL)
CONSTANT SERBLO = 0.55
CONSTANT UNITCORR = 1.0e3

 CBSNGKGLIADJ = CB*MW/(0.55*B_TOTLIP) !NG SERUM LIPID ADJUSTED/KG
   AUCBS_NGKGLIADJ=integ(CBSNGKGLIADJ,0.)
CBNGKG= CB*MW   !NG/KG
PRCT_B = 100.0*CB/(MSTT+1E-30)        !PERCENT OF ORAL DOSE IN BLOOD
PRCT_BIV = 100.0*CB/(IV_RlateR+1E-30) ! PERCENT OF IV DOSE IN BLOOD

    !ADIPOSE COMPARMTENT
    !TISSUE BLOOD SUBCOMPARTMENT
RAFB= QF*(CA-CFB)-PAF*(CFB-CF/PF)     !(NMOL/H)
 AFB = INTEG(RAFB,0.0)                 !(NMOL)
 CFB = AFB/WFB                        !(NMOL/L)
    !TISSUE SUBCOMPARTMENT
RAF = PAF*(CFB-CF/PF)                 !(NMOL/H)
 AF = INTEG(RAF,0.0)                  !(NMOL)
 CF  = AF/WF                          !(NMOL/L)

    !UNIT CONVERSION POST SIMULATION
CFTOTAL= (AF + AFB)/(WF + WFB) ! TOTAL CONCENTRATION IN NMOL/ML
PRCT_F = 100.0*CFTOTAL/(MSTT+1E-30) !PERCENT OF ORAL DOSE IN FAT
PRCT_FIV = 100.0*CFTOTAL/(IV_RlateR+1E-30) !PERCENT OF IV DOSE IN FAT
CFNGKG=CFTOTAL*MW ! FAT CONCENTRATION IN NG/KG
AUCF_NGKGH=integ(CFNGKG,0.)


    !REST OF THE BODY COMPARTMENT
    !TISSUE BLOOD SUBCOMPARTMENT
RAREB= QRE *(CA-CREB)-PARE*(CREB-CRE/PRE)      !(NMOL/H)
 AREB = INTEG(RAREB,0.0)                        !(NMOL)
 CREB = AREB/WREB                              !(NMOL/L)
    !TISSUE SUBCOMPARTMENT
RARE = PARE*(CREB - CRE/PRE)                   !(NMOL/H)
 ARE = INTEG(RARE,0.0)                         !(NMOL)
 CRE  = ARE/WRE                                 !(NMOL/L)
ARETOT = ARE +AREB

    !POST SIMULATION UNIT CONVERSION
CRETOTAL= (ARE + AREB)/(WRE + WREB)            ! TOTAL CONCENTRATION (NMOL/L)
PRCT_RE = 100.0*CRETOTAL/(MSTT+1E-30) ! PERCENT OF ORAL DOSE IN REST OF BODY
PRCT_REIV = 100.0*CRETOTAL/(IV_RlateR+1E-30)   ![ PERCENT OF IV DOSE IN REST
OF BODY
CRENGKG=CRETOTAL*MW                            ! REST OF THE BODY
CONCENTRATION (NG/KG)


    !LIVER COMPARTMENT
    !TISSUE BLOOD SUBCOMPARTMENT
```

```
 RALIB = QLI*(CA-CLIB)-PALI*(CLIB-CFLLIR)+LIRMLUM ! (NMOL/HR)
  ALIB = INTEG(RALIB,0.0)                          !(NMOL)
 CLIB = ALIB/WLIB                                 !(NMOL/L)
    !TISSUE SUBCOMPARMTENT
 RALI = PALI*(CLIB - CFLLIR)-REXCLI               ! (NMOL/HR)
  ALI = INTEG(RALI,0.0)                                !(NMOL)
 CLI  = ALI/WLI                                   !(NMOL/L)

    !FREE TCDD CONCENTRATION IN LIVER
      ! MODIFICATION OCTOBER 8 2009
 CFLLI= IMPLC(CLI-(CFLLIR*PLI+(LIBMAX*CFLLIR/(KDLI+CFLLIR)) &
        +((CYP1A2_1O3*CFLLIR/(KDLI2+CFLLIR)*PAS_INDUC)))-CFLLI,CFLLI0)
    CFLLIR=DIM(CFLLI,0.0) ! FREE TCDD CONCENTRATION IN LIVER
!MODIFIED FROM:
!PARAMETER (LIVER_1RMN = 1.0E-30)
! CFLLI= IMPLC(CLI-(CFLLIR*PLI+(LIBMAX*CFLLIR/(KDLI+CFLLIR &
!+LIVER_1RMN))+((CYP1A2_1O3*CFLLIR/(KDLI2 + CFLLIR &
!+LIVER_1RMN)*PAS_INDUC)))-CFLLI,CFLLI0)
!CFLLIR=DIM(CFLLI,0.0)

! MODIFICATION OCTOBER 8 2009
CBNDLI= LIBMAX*CFLLIR/(KDLI+CFLLIR) !BOUND CONCENTRATION (NMOL/L)

    !POST SIMULATION UNIT CONVERSION
CLITOTAL= (ALI + ALIB)/(WLI + WLIB) ! TOTAL CONCENTRATION (NMOL/L)
PRCT_LI = 100.0*CLITOTAL/(MSTT+1E-30) ! PERCENT OF ORAL DOSE IN LIVER
PRCT_LIIV = 100.0*CLITOTAL/(IV_RlateR+1E-30) ! PERCENT OF IV DOSE IN LIVER
Rec_occ= CFLLIR/(KDLI+CFLLIR)
CLINGKG=CLITOTAL*MW ! LIVER CONCENTRATION IN NG/KG
  AUCLI_NGKGH=integ(CLINGKG,0.0)
CBNDLINGKG = CBNDLI*MW ! BOUND CONCENTRATION IN NG/KG
  AUCBNDLI_NGKGH =INTEG(CBNDLINGKG,0.0)

    !FRACTION INCREASE OF INDUCTION OF CYP1A2
fold_ind=CYP1A2_1OUT/CYP1A2_1A2
VARIATIONOFAC =(CYP1A2_1OUT-CYP1A2_1A2)/CYP1A2_1A2

!VARIABLE ELIMINATION BASED ON THE CYP1A2
! MODIFICATION OCTOBER 8 2009
KBILE_LI_T = Kelv*VARIATIONOFAC! ! DOSE-DEPENDENT EXCRETION RATE CONSTANT

 REXCLI = KBILE_LI_T*CFLLIR*WLI ! DOSE-DEPENDENT BILLIARY EXCRETION RATE
    EXCLI = INTEG(REXCLI,0.0)

!KBILE_LI_T =((CYP1A2_1OUT-CYP1A2_1A2)/CYP1A2_1A2)*Kelv !


!CHEMICAL IN CYP450 (1A2) COMPARTMENT

CYP1A2_1KINP = CYP1A2_1KOUT* CYP1A2_1OUTZ ! BASAL PRODCUTION RATE OF CYP1A2
SET EQUAL TO BASAL DEGREDATION RATE

    ! MODIFICATION OCTOBER 8 2009
CYP1A2_1OUT =INTEG(CYP1A2_1KINP * (1.0 + CYP1A2_1EMAX *(CBNDLI+1.0e-30)**HILL
&
     /(CYP1A2_1EC50**HILL + (CBNDLI+1.0e-30)**HILL)) &
```

```
      - CYP1A2_1KOUT*CYP1A2_1OUT, CYP1A2_1OUTZ)
!MODIFIED FROM:
!PARAMETER (CYP1A2_1RMN = 1E-30)
!CYP1A2_1OUT =INTEG(CYP1A2_1KINP * (1 + CYP1A2_1EMAX *(CBND&
!LI +CYP1A2_1RMN)**HILL/(CYP1A2_1EC50 + (CBNDLI + CYP1A2_1&
!RMN)**HILL) +CYP1A2_1RMN) - CYP1A2_1KOUT*CYP1A2_1&
!OUT, CYP1A2_1OUTZ)

! EQUATIONS INCORPORATING DELAY OF CYP1A2 PRODUCTION (NOT USED IN
SIMULATIONS)
CYP1A2_1RO2 = (CYP1A2_1OUT - CYP1A2_1O2)/ CYP1A2_1TAU
  CYP1A2_1O2 =INTEG(CYP1A2_1RO2, CYP1A2_1A1)

CYP1A2_1RO3 = (CYP1A2_1O2 - CYP1A2_1O3)/ CYP1A2_1TAU
  CYP1A2_1O3 =INTEG(CYP1A2_1RO3, CYP1A2_1A2)

    !PLACENTA COMPARTMENT
    !TISSUE BLOOD SUBCOMPARTMENT
RAPLAB= QPLA*(CA - CPLAB)-PAPLA*(CPLAB -CFLPLAR)    ! NMOL/HR)
 APLAB = INTEG(RAPLAB,0.0)                           ! (NMOL)
 CPLAB = APLAB/(WPLAB+1E-30)                        ! (NMOL/ML)
    !TISSUE SUBCOMPARTMENT
RAPLA = PAPLA*(CPLAB-CFLPLAR)-RAMPF + RAFPM         ! (NMOL/HR)
 APLA = INTEG(RAPLA,0.0)                             ! (NMOL)
 CPLA  = APLA/(WPLA+1e-30)                          ! (NMOL/ML)

    ! NEW EQUATION AUGUST 28  2009
PARAMETER (PARA_ZERO = 1.0E-30)
CFLPLA= IMPLC(CPLA-(CFLPLAR*PPLA +(PLABMAX*CFLPLAR/(KDPLA&
    +CFLPLAR+PARA_ZERO)))-CFLPLA,CFLPLA0)
CFLPLAR=DIM(CFLPLA,0.0)

    !POST SIMULATION UNIT CONVERSION
CPLATOTAL = ((APLAB+APLA)/(WPLAB+WPLA))
PRCT_PLA = (CPLATOTAL/(MSTT+1E-30))*100
PRCT_PLAIV = (CPLATOTAL/(IV_RlateR+1E-30))*100

    !FETUS COMPARTMENT
RAFETUS= RAMPF-RAFPM
 AFETUS=INTEG(RAFETUS,0.0)
CFETUS=AFETUS/(WTFE+1.0e-30)
CFETOTAL= CFETUS
CFETUS_v = CFETUS/PFETUS

    !POST SIMULATION UNIT CONVERSION
 CFETUSNGKG = CFETUS*MW                    !(NG/KG)
 PRCT_FE = 100.0*CFETOTAL/(MSTT+1E-30)
 PRCT_FEIV = 100.0*CFETOTAL/(IV_RlateR+1E-30)

    !TRANSFER OF DIOXIN FROM PLACENTA TO FETUS
    !FETAL EXPOSURE ONLY DURING EXPOSURE

IF (T.LT.TRANSTIME_ON) THEN
 SWITCH_trans = 0.0
ELSE
 SWITCH_trans = 1
```

```
END IF

    !TRANSFER OF DIOXIN FROM PLACENTA TO FETUS
    ! MODIFICATION 26 SEPTEMBER 2003

RAMPF = (CLPLA_FET*CPLA)*SWITCH_trans
  AMPF=INTEG(RAMPF,0.0)

    !TRANSFER OF DIOXIN FROM FETUS TO PLACENTA
RAFPM = (CLPLA_FET*CFETUS_v)*SWITCH_trans!
 AFPM = INTEG(RAFPM,0.0)

    !CHECK MASS BALANCE ----------
BDOSE= IVDOSE +LYMLUM+LIMLUM
BMASSE = EXCLI+AURI+AFB+AF+AREB+ARE+ALIB+ALI+APLA+APLAB+AFETUS !
BDIFF = BDOSE-BMASSE

    !BODY BURDEN (NMOL)
BODY_BURDEN = AFB+AF+AREB+ARE+ALIB+ALI+APLA+APLAB

    !BODY BURDEN CONCENTRATION (NG/KG)
 BBNGKG =(AFB+AF+AREB+ARE+ALIB+ALI+APLA+APLAB)*MW/WT0

! END SIMULATION COMMAND

TERMT (T.GE. TimeLimit, 'Time limit has been reached.')

END   ! END OF THE DERIVATIVE SECTION
END   ! END OF THE DYNAMIC SECTION
END   ! END OF THE PROGRAM
```

C.2.2.2. *Input File*

```
output @clear
prepare @clear T  year CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG  CBNDLINGKG CBNGKG

CINT = 1   %168 %100              %INTEGRATION TIME
   %EXPOSURE SCENARIO
EXP_TIME_ON       = 0          % TIME AT WHICH EXPOSURE BEGINS (HOUR)
EXP_TIME_OFF      = 401190     %TIME AT WHICH EXPOSURE ENDS (HOUR)
DAY_CYCLE         = 24        %NUMBER OF HOURS BETWEEN DOSES (HOUR)
BCK_TIME_ON       = 401190     %TIME AT WHICH BACKGROUND EXPOSURE BEGINS
(HOUR)
BCK_TIME_OFF      = 401190     %TIME AT WHICH BACKGROUND EXPOSURE ENDS (HOUR)
IV_LACK           = 401190
IV_PERIOD         = 401190
    %GESTATION CONTROL
MATTING           = 393120     % BEGINNING OF MATING (HOUR) AT 45 YEARS OLD
TIMELIMIT         = 399840     %SIMULATION TIME LIMIT (HOUR)
TRANSTIME_ON      = 394632     % TRANSFER FROM MOTHER TO FETUS AT 1512 HOURS
GESTATION
    %EXPOSURE DOSE
MSTOT             = 9.97339283634997E-07       % NG OF TCDD PER KG OF BW
MSTOTBCKGR        = 0.   %0.1      % ORAL BACKGROUND EXPOSURE DOSE (NG/KG)
DOSEIV            = 0.   %10
```

```
DOSEIVLATE        = 0.    %10

     % TRANFER MOTHER TO FETUS CLEARANCE
CLPLA_FET         = 0.001 % MOTHER TO FETUS TRANFER CLEARANCE (L/HR)
```

C.2.3. Rat Standard Model

C.2.3.1. *Model Code*

PROGRAM: 'Three Compartment PBPK Model in Rat: Standard Model (Non-Gestation)'

```
!Rat_Dioxin_3C June09_2clean_icf_afterKKfix_v3_ratnongest.csl
!RAT_NON_GEST_ICF_F083109.CSL
!RAT_NON_GEST_ICF_F100609.CSL
 !*************************************************

INITIAL  ! INITIALIZATION OF PARAMETERS

     !SIMULATION PARAMETERS
CONSTANT PARA_ZERO       =      1d-30
CONSTANT EXP_TIME_ON     =      0.0           ! TIME AT WHICH EXPOSURE BEGINS
(HOURS)
CONSTANT EXP_TIME_OFF    =     900.0           ! TIME AT WHICH EXPOSURE ENDS
(HOURS)
CONSTANT DAY_CYCLE       =     900.0            ! NUMBER OF HOURS BETWEEN
DOSES (HOURS)
CONSTANT BCK_TIME_ON     =      0.0           ! TIME AT WHICH BACKGROUND
EXPOSURE BEGINS (HOURS)
CONSTANT BCK_TIME_OFF    =      0.0           ! TIME AT WHICH BACKGROUND
EXPOSURE ENDS (HOURS)

CONSTANT MW=322 !MOLECULAR WEIGHT (NG/NMOL)
CONSTANT SERBLO = 0.55
CONSTANT UNITCORR = 1000


     !EXPOSURE DOSES
CONSTANT MSTOTBCKGR      =      0.0           !ORAL BACKGROUND EXPOSURE DOSE
(UG/KG)
CONSTANT MSTOT           =      10       !ORAL EXPOSURE DOSE (UG/KG)
CONSTANT MSTOTsc         =      0.0           !SUBCUTANEOUS EXPOSURE DOSE
(UG/KG)
CONSTANT DOSEIV          =      0.0           ! INJECTED DOSE (UG/KG)

     !ORAL DOSE
  MSTOT_NM               =   MSTOT/MW       !AMOUNT IN NMOL/G
  MSTOT_NMBCKGR          =   MSTOTBCKGR/MW  !AMOUNT IN NMOL/G

     !INTRAVENOUS DOSE
  DOSEIV_NM              =   DOSEIV/MW      !AMOUNT IN NMOL/G

     !INITIAL GUESS OF THE FREE CONCENTRATION IN THE LIGAND (COMPARTMENT
INDICATED BELOW)====
CONSTANT CFLLI0          =     0.0            !LIVER (NMOL/ML)
```

```
     !BINDING CAPACITY (AhR) FOR NON LINEAR BINDING (COMPARTMENT INDICATED
BELOW) (NMOL/ML) ===
CONSTANT LIBMAX          =    3.5e-4          ! LIVER (NMOL/ML), WANG ET AL.
1997

     ! PROTEIN AFFINITY CONSTANTS (1A2 OR AhR, COMPARTMENT INDICATED BELOW)
(NMOL/ML)===
CONSTANT KDLI            =    1.0e-4          ! LIVER (AhR) (NMOL/ML), WANG
ET AL. 1997
CONSTANT KDLI2           =    4.0e-2          !LIVER (1A2) (NMOL/ML), EMOND
ET AL. 2004

     !EXCRETION AND ABSORPTION CONSTANT [RAT]
CONSTANT KST             =    0.36            ! GASTRIC RATE CONSTANT (HR-1),
WANG ET AL. (1997)
CONSTANT KABS            =    0.48            !INTESTINAL ABSORPTION CONSTANT
(HR-1), WANG ET AL. 1997

     !URINARY ELIMINATION CLEARANCE (ML/HR)
CONSTANT CLURI           =     0.01           !URINARY CLEARANCE (ML/HR),
EMOND ET AL. 2004

     !INTERSPECIES VARIABLE ELIMINATION
CONSTANT KELV            =     0.15           ! INTERSPECIES VARIABLE
ELIMINATION CONSTANT (1/HOUR) (OPTIMIZED), EMOND ET AL. 2004

     ! CONSTANT TO DIVIDE THE ABSORPTION INTO LYMPHATIC AND PORTAL FRACTIONS
CONSTANT A               =     0.7            ! LYMPHATIC FRACTION, WANG ET
AL. 1997

     !PARTITION COEFFICIENTS
CONSTANT PF              =     100            ! ADIPOSE TISSUE/BLOOD, WANG ET
AL. 1997
CONSTANT PRE             =     1.5            ! REST OF THE BODY/BLOOD, WANG
ET AL. 1997
CONSTANT PLI             =     6.0              ! LIVER/BLOOD, WANG ET AL.
1997

     !PARAMETER FOR INDUCTION OF CYP 1A2 [MOUSE] ===
CONSTANT PAS_INDUC       =     1.0            ! INCLUDE INDUCTION? (1 = YES,
0 = NO)
CONSTANT CYP1A2_1OUTZ    =     1.6            ! DEGRADATION CONCENTRATION
CONSTANT OF 1A2 (NMOL/ML), WANG ET AL. 1997
CONSTANT CYP1A2_1A1      =     1.6            ! BASAL CONCENTRATION OF 1A1
(NMOL/ML), WANG ET AL. 1997
CONSTANT CYP1A2_1EC50    =     0.13           ! DISSOCIATION CONSTANT TCDD-
CYP1A2 (NMOL/ML) , WANG ET AL. 1997
CONSTANT CYP1A2_1A2      =     1.6            ! BASAL CONCENTRATION OF 1A2
(NMOL/ML) Wang et al (1997)
CONSTANT CYP1A2_1KOUT    =     0.1            ! FIRST ORDER RATE OF
DEGRADATION (H-1), WANG ET AL. 1997
CONSTANT CYP1A2_1TAU     =     0.25           ! HOLDING TIME (H), WANG ET AL.
1997
CONSTANT CYP1A2_1EMAX    =     600            ! MAXIMUM INDUCTION OVER BASAL
EFFECT (UNITLESS), WANG ET AL. 1997
```

```
CONSTANT HILL            =     0.6      !HILL CONSTANT; COOPERATIVELY LIGAND
BINDING EFFECT CONSTANT (UNITLESS)

   !TISSUE BLOOD FLOW EXPRESSED AS A FRACTION OF CARDIAC OUTPUT
CONSTANT QFF  = 0.069                          ! ADIPOSE TISSUE BLOOD FLOW
FRACTION (UNITLESS), WANG ET AL. 1997
CONSTANT QLIF = 0.183                          ! LIVER (UNITLESS), WANG ET AL.
1997

     !DIFFUSIONAL PERMEABILITY FRACTION
CONSTANT PAFF            = 0.0910              ! ADIPOSE (UNITLESS), WANG ET
AL. 1997
CONSTANT PAREF           = 0.0298              ! REST OF THE BODY (UNITLESS),
WANG ET AL. 1997
CONSTANT PALIF           = 0.35                ! LIVER (UNITLESS), WANG ET AL.
1997

     !FRACTION OF TISSUE VOLUME (UNITLESS)
CONSTANT WLI0            =  0.0360             ! LIVER, WANG ET AL. 1997
CONSTANT WF0             =  0.069              ! BLOOD, WANG ET AL. 1997

     !COMPARTMENT TISSUE BLOOD EXPRESSED AS A FRACTION OF THE TOTAL
COMPARTMENT VOLUME =========
CONSTANT WFB0            =  0.050              ! ADIPOSE TISSUE, WANG ET AL.
1997
CONSTANT WREB0           =  0.030              ! REST OF THE BODY, WANG ET AL.
1997
CONSTANT WLIB0           =  0.266              ! LIVER , WANG ET AL. 1997

     !EXPOSURE SCENARIO FOR UNIQUE OR REPETITIVE WEEKLY OR MONTHLY EXPOSURE
     ! NUMBER OF EXPOSURES PER WEEK
CONSTANT WEEK_LACK       = 0.0                  ! DELAY BEFORE EXPOSURE ENDS
(WEEK)
CONSTANT WEEK_PERIOD     = 168.0                ! NUMBER OF HOURS IN THE WEEK
(HOURS)
CONSTANT WEEK_FINISH     = 168.0                ! TIME EXPOSURE ENDS (HOURS)

     !NUMBER OF EXPOSURES PER MONTH
CONSTANT MONTH_LACK      = 0.0                  ! DELAY BEFORE EXPOSURE BEGINS
(MONTH)

     !SET FOR BACKGROUND EXPOSURE===========
     !CONSTANT FOR BACKGROUND EXPOSURE===========
CONSTANT Day_LACK_BG     = 0.0                  ! DELAY BEFORE EXPOSURE BEGINS
(HOURS)
CONSTANT Day_PERIOD_BG   = 24.0                 ! LENGTH OF EXPOSURE (HOURS)

     !NUMBER OF EXPOSURES PER WEEK
CONSTANT WEEK_LACK_BG    = 0.0                 ! DELAY BEFORE BACKGROUND
EXPOSURE (WEEK)
CONSTANT WEEK_PERIOD_BG  = 168.0                !NUMBER OF HOURS IN THE WEEK
(HOURS)
CONSTANT WEEK_FINISH_BG  = 168.0                ! TIME EXPOSURE ENDS (HOURS)

     !GROWTH CONSTANT FOR RAT
     !CONSTANT FOR MOTHER BODY WEIGHT GROWTH ======
```

```
CONSTANT BW_T0 = 250.0                          !CHANGED FOR SIMULATION

     ! CONSTANT USED IN CARDIAC OUTPUT EQUATION
CONSTANT QCCAR =311.4                          !CONSTANT (ML/MIN/KG), WANG ET
AL.

     ! COMPARTMENT LIPID EXPRESSED AS THE FRACTION OF TOTAL LIPID
CONSTANT F_TOTLIP        = 0.855               !ADIPOSE TISSUE (UNITLESS)
CONSTANT B_TOTLIP        = 0.0033              !BLOOD (UNITLESS)
CONSTANT RE_TOTLIP       = 0.019               !REST OF THE BODY (UNITLESS)
CONSTANT LI_TOTLIP       = 0.06                !LIVER (UNITLESS)

END      !END OF THE INITIAL SECTION

DYNAMIC  !DYNAMIC SIMULATION SECTION

ALGORITHM  IALG          =           2         ! GEAR METHOD
CINTERVAL  CINT          =         0.1         ! COMMUNICATION INTERVAL
MAXTERVAL  MAXT          =       1.0e+10       ! MAXIMUM CALCULATION INTERVAL
MINTERVAL  MINT          =       1.0E-10       ! MINIMUM CALCULATION INTERVAL
VARIABLE   T             =         0.0
CONSTANT   TIMELIMIT     =         900.0         !SIMULATION TIME LIMIT
(HOURS)
 CINTXY  = CINT
 PFUNC   = CINT

         !TIME CONVERSION
  DAY=T/24.0                                     ! TIME IN DAYS
  WEEK =T/168.0                                  ! TIME IN WEEKS
  MONTH =T/730.0                                 ! TIME IN MONTHS
  YEAR=T/8760.0                                  ! TIME IN YEARS


DERIVATIVE ! PORTION OF CODE THAT SOLVES DIFFERENTIAL EQUATIONS

        !CHRONIC OR SUBCHRONIC EXPOSURE SCENARIO =======
        !NUMBER OF EXPOSURES PER DAY
 DAY_LACK     = EXP_TIME_ON                  ! DELAY BEFORE EXPOSURE BEGINS
(HOURS)
 DAY_PERIOD   = DAY_CYCLE                    ! EXPOSURE PERIOD (HOURS)
 DAY_FINISH   = CINTXY                       ! LENGTH OF EXPOSURE (HOURS)
 MONTH_PERIOD = TIMELIMIT                    ! EXPOSURE PERIOD (MONTHS)
 MONTH_FINISH = EXP_TIME_OFF                 ! LENGTH OF EXPOSURE (MONTHS)

        !NUMBER OF EXPOSURES PER DAY AND MONTH
 DAY_FINISH_BG   = CINTXY                    ! LENGTH OF EXPOSURE (HOURS)
 MONTH_LACK_BG   = BCK_TIME_ON               ! DELAY BEFORE BACKGROUND
EXPOSURE BEGINS (MONTHS)
 MONTH_PERIOD_BG = TIMELIMIT                 ! BACKGROUND EXPOSURE PERIOD
(MONTHS)
 MONTH_FINISH_BG = BCK_TIME_OFF              ! LENGTH OF BACKGROUND EXPOSURE
(MONTHS)


  B = 1-A                                   ! FRACTION OF DIOXIN ABSORBED IN
THE PORTAL FRACTION OF THE LIVER
```

```
1
2           ! BODY WEIGHT GROWTH EQUATION========
3    PARAMETER (BW_RMN = 1.0E-30)
4    WT0= (BW_T0 *(1.0+(0.41*T)/(1402.5+T+BW_RMN)))
5
6           !VARIABILITY OF REST OF THE BODY DEPEND OTHERS ORGAN
7    WRE0 = (0.91 - (WLIB0*WLI0 + WFB0*WF0 + WLI0 + WF0))/(1.0+WREB0) !REST OF
8   THE BODY FRACTION; UPDATED FOR EPA ASSESSMENT
9    QREF = 1.0-(QFF+QLIF)                          !REST OF BODY BLOOD FLOW
10   QTTQF = QFF+QREF+QLIF                        ! SUM MUST EQUAL 1
11
12          !COMPARTMENT VOLUME (G) =========
13   WF  =  WF0  * WT0                            ! ADIPOSE
14   WRE =  WRE0 * WT0                            ! REST OF THE BODY
15   WLI =  WLI0 * WT0                            ! LIVER
16
17          !COMPARTMENT TISSUE BLOOD VOLUME (G) =========
18   WFB  =  WFB0  * WF                           ! ADIPOSE
19   WREB =  WREB0 * WRE                          ! REST OF THE BODY
20   WLIB =  WLIB0 * WLI                          ! LIVER
21
22          !CARDIAC OUTPUT FOR THE GIVEN BODY WEIGHT
23    QC= QCCAR*60.0*(WT0/UNITCORR)**0.75
24
25          ! COMPARTMENT BLOOD FLOW (ML/HR)
26    QF  = QFF*QC                                 ! ADIPOSE TISSUE BLOOD FLOW RATE
27    QLI = QLIF*QC                                ! LIVER TISSUE BLOOD FLOW RATE
28    QRE = QREF*QC                                ! REST OF THE BODY BLOOD FLOW
29   RATE
30    QTTQ = QF+QRE+QLI                    ! TOTAL FLOW RATE
31
32          !PERMEABILITY ORGAN FLOW (ML/HR)
33   PAF  = PAFF*QF                               ! ADIPOSE
34   PARE = PAREF*QRE                             ! REST OF THE BODY
35   PALI = PALIF*QLI                             ! LIVER TISSUE
36
37          !CONDITIONAL ORAL EXPOSURE (BACKGROUND EXPOSURE)
38          !EXPOSURE + !REPETITIVE EXPOSURE SCENARIO
39    IV= DOSEIV_NM * WT0  !AMOUNT IN NMOL
40    MSTT= MSTOT_NM * WT0 !AMOUNT IN NMOL
41    MSTTBCKGR =MSTOT_NMBCKGR *WT0
42
43          !REPETITIVE ORAL BACKGROUND EXPOSURE SCENARIOS
44    DAY_EXPOSURE_BG   = PULSE(DAY_LACK_BG,DAY_PERIOD_BG,DAY_FINISH_BG)
45    WEEK_EXPOSURE_BG  = PULSE(WEEK_LACK_BG,WEEK_PERIOD_BG,WEEK_FINISH_BG)
46    MONTH_EXPOSURE_BG = PULSE(MONTH_LACK_BG,MONTH_PERIOD_BG,MONTH_FINISH_BG)
47
48    MSTTCH_BG = (DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG)*MSTTBCKGR
49    MSTTFR_BG = MSTTBCKGR/CINT
50
51    CYCLE_BG =DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG
52
53   IF (MSTTCH_BG.EQ.MSTTBCKGR) THEN
54       ABSMSTT_GB= MSTTFR_BG
55   ELSE
56       ABSMSTT_GB = 0.0
```

```
END IF


        !REPETITIVE ORAL MAIN EXPOSURE SCENARIO
  DAY_EXPOSURE   = PULSE(DAY_LACK,DAY_PERIOD,DAY_FINISH)
  WEEK_EXPOSURE  = PULSE(WEEK_LACK,WEEK_PERIOD,WEEK_FINISH)
  MONTH_EXPOSURE = PULSE(MONTH_LACK,MONTH_PERIOD,MONTH_FINISH)

  MSTTCH = (DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE)*MSTT
  CYCLE = DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE
  MSTTFR = MSTT/CINT

  SUMEXPEVENT= integ (CYCLE,0.0) !NUMBER OF CYCLE GENERATE DURING SIMULATION


        !CONDITIONAL ORAL EXPOSURE
IF (MSTTCH.EQ.MSTT) THEN
   ABSMSTT= MSTTFR
ELSE
   ABSMSTT = 0.0
END IF

 CYCLETOT=INTEG(CYCLE,0.0)

        !MASS CHANGE IN THE LUMEN
 RMSTT  = -(KST+KABS)*MST+ABSMSTT +ABSMSTT_GB ! RATE OF CHANGE (NMOL/H)
   MST = INTEG(RMSTT,0.0)  !AMOUNT OF STAY IN DUODENUM (NMOL)

        !ABSORPTION IN LYMPH CIRCULATION
 LYRMLUM = KABS*MST*A
   LYMLUM = INTEG(LYRMLUM,0.0)

        !ABSORPTION IN PORTAL CIRCULATION
 LIRMLUM = KABS*MST*B
   LIMLUM = INTEG(LIRMLUM,0.0)

        !PERCENT OF DOSE REMAINING IN THE GI TRACT
 PRCT_remain_GIT  = (MST/(MSTT+PARA_ZERO))*100.0

        !ABSORPTION of Dioxin by IV route---------
 IVR= IV/PFUNC ! RATE FOR IV INFUSION IN BLOOD
 EXPIV= IVR * (1.0-STEP(PFUNC))
   IVDOSE = integ(EXPIV,0.0)

        !SYSTEMIC BLOOD COMPARTMENT
        ! MODIFICATION ON OCTOBER 6, 2009
CB=(QF*CFB+QRE*CREB+QLI*CLIB+EXPIV+LYRMLUM)/(QC+CLURI) !
   CA = CB

        !URINARY EXCRETION BY KIDNEY
        ! MODIFICATION ON OCTOBER 6, 2009
RAURI = CLURI *CB
  AURI = INTEG(RAURI,0.0)

        !CONVERSION EQUATION POST SIMULATION
 PRCT_B = (CB/(MSTT+PARA_ZERO))*100.0
```

```
 CBNGKG = CB*MW*UNITCORR ![NG/KG]


CBSNGKGLIADJ= (CB*MW*UNITCORR*(1.0/B_TOTLIP)*(1.0/SERBLO))![NG of TCDD
Serum/Kg OF LIPIP]

       !ADIPOSE TISSUE COMPARTMENT
       !TISSUE BLOOD SUBCOMPARTMENT
 RAFB = QF*(CA-CFB)-PAF*(CFB-CF/PF)                !(NMOL/HR)
   AFB = INTEG(RAFB,0.0)                            !(NMOL)
   CFB = AFB/WFB                                   !(NMOL/ML)
       !TISSUE SUBCOMPARTMENT
 RAF  = PAF*(CFB-CF/PF)                            !(NMOL/HR)
   AF  = INTEG(RAF,0.0)                             !(NMOL)
   CF  = AF/WF                                     !(NMOL/ML)

      !CONVERSION EQUATION POST SIMULATION
   CFTOTAL    = (AF + AFB)/(WF + WFB)       !TOTAL CONCENTRATION IN NMOL/ML
   PRCT_F  = (CFTOTAL/(MSTT+PARA_ZERO))*100.0 ! PRCENT OF DOSE IN FAT
   CFNGKG   = CFTOTAL*MW*UNITCORR           ! CONCENTRATION [NG/KG]

      !REST OF THE BODY COMPARTMENT
      ! TISSUE BLOOD SUBCOMPARTMENT
 RAREB= QRE*(CA-CREB)-PARE*(CREB-CRE/PRE)           !(NMOL/HR)
   AREB = INTEG(RAREB,0.0)                          !(NMOL)
   CREB = AREB/WREB                                !(NMOL/ML)
      ! TISSUE COMPARTMENT
 RARE = PARE*(CREB - CRE/PRE)                       !(NMOL/HR)
   ARE = INTEG(RARE,0.0)                               !(NMOL)
   CRE  = ARE/WRE                                  !(NMOL/ML)

   !CONVERSION EQUATION POST SIMULATION
   CRETOTAL= (ARE + AREB)/(WRE + WREB)              ! TOTAL CONCENTRATION IN
NMOL/ML
   PRCT_RE = (CRETOTAL/(MSTT+PARA_ZERO))*100.0
   CTREPGG= CRETOTAL*MW*UNITCORR !(PG/ML)
    AUC_REPPG = integ(CTREPGG,0.0)

   !LIVER COMPARTMENT
   !TISSUE BLOOD COMPARTMENT
 RALIB = QLI*(CA-CLIB)-PALI*(CLIB-CFLLIR)+LIRMLUM   !(NMOL/HR)
   ALIB = INTeg(RALIB,0.0)                          !(NMOL)
   CLIB = ALIB/WLIB
   !TISSUE COMPARTMENT
 RALI =  PALI*(CLIB-CFLLIR)-REXCLI                  !(NMOL/HR)
   ALI = integ(RALI,0.0)                               !(NMOL)
   CLI  = ALI/WLI                                  !(NMOL/ML)


PARAMETER (LIVER_1RMN = 1.0E-30)
CFLLI= IMPLC(CLI-(CFLLIR*PLI+(LIBMAX*CFLLIR/(KDLI+CFLLIR &
+LIVER_1RMN))+((CYP1A2_1O3*CFLLIR/(KDLI2+CFLLIR &
+LIVER_1RMN)*PAS_INDUC)))-CFLLIR,CFLLI0) ! FREE TCDD CONCENTRATION IN LIVER
CFLLIR=DIM(CFLLI,0.0)

  CBNDLI= LIBMAX*CFLLIR/(KDLI+CFLLIR+LIVER_1RMN) !BOUND CONCENTRATION
```

```
1
2         !CONVERSION EQUATION POST SIMULATION
3       CLITOTAL= (ALI + ALIB)/(WLI + WLIB)                ! TOTAL CONCENTRATION IN
4   NMOL/ML
5       PRCT_LI = (CLITOTAL/(MSTT+PARA_ZERO))*100.0
6       rec_occ_AHR= (CFLLIR/(KDLI+CFLLIR+1))*100.0          ! PERCENT OF AhR
7   OCCUPANCY
8       PROT_occ_1A2= (CFLLIR/(KDLI2+CFLLIR))*100.0          ! PERCENT OF 1A2
9   OCCUPANCY
10      CLINGKG =(CLITOTAL*MW*UNITCORR)
11      CBNDLINGKG = CBNDLI*MW*UNITCORR
12        AUCLI_NGKGH=INTEG(CLINGKG,0.0)
13      CLINGG=CLITOTAL*MW
14
15           !VARIABLE ELIMINATION HALF-LIFE BASED ON THE CONCENTRATION OF CYP1A2
16        KBILE_LI_T =((CYP1A2_1OUT-CYP1A2_1A2)/CYP1A2_1A2)*Kelv ! INDUCED BILIARY
17  EXCRETION RATE CONSTANT
18
19    REXCLI= (KBILE_LI_T*CFLLIR*WLI) ! DOSE-DEPENDENT BILIARY EXCRETION RATE
20      EXCLI = INTEG(REXCLI,0.0)
21
22        !CHEMICAL IN CYP450 (1A2) COMPARTMENT
23      !===PARAMETER FOR INDUCTION OF CYP1A2
24
25    CYP1A2_1KINP = CYP1A2_1KOUT* CYP1A2_1OUTZ ! BASAL RATE OF CYP1A2 PRODUCTION
26  SET EQUAL TO BASAL RATE OF DEGREDATION
27
28
29        ! MODIFICATION ON OCTOBER 6, 2009
30    CYP1A2_1OUT =INTEG(CYP1A2_1KINP * (1.0 + CYP1A2_1EMAX *(CBNDLI+1.0e-
31  30)**HILL &
32        /(CYP1A2_1EC50**HILL + (CBNDLI+1.0e-30)**HILL)) &-
33         - CYP1A2_1KOUT*CYP1A2_1OUT, CYP1A2_1OUTZ)
34
35  ! EQUATIONS INCORPORATING DELAY OF CYP1A2 PRODUCTION (NOT USED IN
36  SIMULATIONS)
37
38    CYP1A2_1RO2 = (CYP1A2_1OUT - CYP1A2_1O2)/ CYP1A2_1TAU
39       CYP1A2_1O2 =INTEG(CYP1A2_1RO2, CYP1A2_1A1)
40    CYP1A2_1RO3 = (CYP1A2_1O2 - CYP1A2_1O3)/ CYP1A2_1TAU
41       CYP1A2_1O3 =INTEG(CYP1A2_1RO3, CYP1A2_1A2)
42
43  ! ------------CHECK MASS BALANCE ----------
44      BDOSE= LYMLUM+LIMLUM+IVDOSE
45      BMASSE = EXCLI+AURI+AFB+AF+AREB+ARE+ALIB+ALI
46          BDIFF = BDOSE-BMASSE
47
48  !---------------BODY BURDEN-----------------------
49      BBNGKG =(((AFB+AF+AREB+ARE+ALIB+ALI)*MW)/(WT0/UNITCORR)) !
50  ! ------------ END OF THE SIMULATION COMMAND ----------
51
52  TERMT (T.GE. TimeLimit, 'Time limit has been reached.')
53
54  END   ! END OF THE DERIVATIVE SECTION
55  END   ! END OF THE DYNAMIC SIMULATION SECTION
56  END   ! END OF THE PROGRAM.
```

C.2.3.2. *Input Files*

C.2.3.2.1. *Cantoni et al. (1981).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

%Cantoni et al. 1981
%protocol:  oral exposure 1 dose/week for 45 weeks; female CD-COBS rats
%Rat_Dioxin_3C June09_2clean.csl
%RAT_NON_GEST_ICF_F083109.CSL  (now 09-11-09)
%dose levels: 0.01, 0.1, 1  ug/kg 1 dose/week for 45 weeks
%dose levels: 10, 100, 1000 ng/kg 1 dose/week for 45 weeks
%dose levels equivalent to: 1.43, 14.3 143 ng/kg 7 days/weeks for 45 weeks

MAXT              = 0.01
CINT              = 0.1
EXP_TIME_ON       = 0.              %delay before begin exposure (HOUR)
EXP_TIME_OFF      = 7560     %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE         = 168
BCK_TIME_ON       = 0.       %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF      = 0.       %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT         = 7560     %SIMULATION LIMIT TIME (HOUR)
BW_T0             = 125      % Body weight at the beginning of the simulation
(g)

%EXPOSURE DOSE SCENARIOS (UG/KG)
  %MSTOT          = 0.01     % exposure dose ug/kg
   %MSTOT        = 0.1      % exposure dose ug/kg
   MSTOT         = 1        % exposure dose ug/kg
```

C.2.3.2.2. *Chu et al. (2007).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% Chu et al. 2007
%protocol:  oral exposure daily for 28 days
%dose levels: 0.0025, 0.025, 0.250, 1.0 ug/kg every day for 28 days
% dose levels = 2.5, 25, 250, 1000 ng/kg every day for 28 days
MAXT            =  0.01
CINT            =  0.1
EXP_TIME_ON     =  0.            %delay before begin exposure (HOUR) 5 weeks
after start of experiment (age = 12 weeks)
EXP_TIME_OFF    =  672.            %TIME EXPOSURE STOP (HOUR); 30 doses, 1
every two weeks
DAY_CYCLE       =  24.            % once every two weeks
BCK_TIME_ON     =  0.              %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF    =  0.              %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT       =  672.           %SIMULATION LIMIT TIME (HOUR)
BW_T0           =  200.             % Body weight at the beginning of the
simulation (g); corresponds to 12 week old female

%EXPOSURE DOSE SCENARIOS (UG/KG)
```

```
  %MSTOT          = 0.0025        % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 0.025         % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 0.250          % ORAL EXPOSURE DOSE (UG/KG)
  MSTOT          = 1.0           % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.3.2.3. *Crofton et al. (2005).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% Crofton et al. 2005
%protocol:  oral exposure daily for 4 days
%dose levels: 0.0001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 ug/kg every
day for four days
%dose levels: 0.1, 3, 10, 30, 100, 300, 1000, 3000, and 10000 ng/kg every day
for four days

MAXT            =  0.01
CINT            =  0.1
EXP_TIME_ON     =  0.            %delay before begin exposure (HOUR) 5 weeks
after start of experiment (age = 12 weeks)
EXP_TIME_OFF    =  96.            %TIME EXPOSURE STOP (HOUR); 30 doses, 1
every two weeks
DAY_CYCLE       =  24.            % once every two weeks
BCK_TIME_ON     =  0.              %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF    =  0.              %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT       =  96.            %SIMULATION LIMIT TIME (HOUR)
BW_T0           =  250             % Body weight at the beginning of the
simulation (g); corresponds to 12 week old female

%EXPOSURE DOSE SCENARIOS (UG/KG)
  MSTOT           = 0.0001        % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 0.003         % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 0.01          % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 0.03          % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 0.1          % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 0.3          % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 1.          % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 3.          % ORAL EXPOSURE DOSE (UG/KG)
  MSTOT          = 10.          % ORAL EXPOSURE DOSE (UG/KG)


```

C.2.3.2.4. *Fattore et al. (2000).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% Fattore et al. 2000
%built and check in August 7 2009
%protocol:  oral exposure in diet for 13 weeks; SD rats
%dose levels:  0.02, 0.1, 0.2, 2 ug/kg 7 days/week for 13 weeks
%dose levels equivalent to: 20, 100, 200, 2000 ng/kg 7 days/week for 13 weeks

MAXT = 0.01
```

```
CINT  = 0.1
EXP_TIME_ON       = 0.              %TIME AT WHICH EXPOSURE BEGINS (HOUR)
EXP_TIME_OFF      = 2184     %TIME AT WHICH EXPOSURE ENDS (HOUR)
DAY_CYCLE         = 24
BCK_TIME_ON       = 0.       %TIME AT WHICH BACKGROUND EXPOSURE BEGINS (HOUR)
BCK_TIME_OFF      = 0.       %TIME AT WHICH BACKGROUND EXPOSURE ENDS (HOUR)
TIMELIMIT         = 2184     %SIMULATION TIME LIMIT (HOUR)
BW_T0             = 150      % BODY WEIGHT AT THE BEGINNING OF THE SIMULATION
(G)

%EXPOSURE DOSE SCENARIOS (UG/KG)
 %MSTOT            = 0.02              % EXPOSURE DOSE IN UG/KG
  %MSTOT          = 0.1               % EXPOSURE DOSE IN UG/KG
  %MSTOT          = 0.2               % EXPOSURE DOSE IN UG/KG
  MSTOT          = 2              % EXPOSURE DOSE IN UG/KG
```

C.2.3.2.5. *Franc et al. (2001). Sprague Dawley rats*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Franc et al. 2001
% Non-gestational rat model
% dose levels: 0.140, 0.420, and 1.400 ug/kg every 2 weeks for 22 weeks
% dose levels: 140, 420, and 1400 ng/kg every 2 weeks for 22 weeks
% dose levels equivalent to 10, 30, and 100 ng/kg/day

MAXT            =  0.01
CINT            =  0.1
EXP_TIME_ON     =  0.               %delay before begin exposure (HOUR)
EXP_TIME_OFF    =  3696.              %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE       =  336.
BCK_TIME_ON     =  0.                %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF    =  0.                %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT       =  3696.               %SIMULATION LIMIT TIME (HOUR)
BW_T0           =  200.              % Body weight at the beginning of the
simulation (g); corresponds to approximate weight of females 10 weeks old

%EXPOSURE DOSE SCENARIOS (UG/KG)
   %MSTOT         = 0.14     % ORAL EXPOSURE DOSE (UG/KG)
   %MSTOT         = 0.42      % ORAL EXPOSURE DOSE (UG/KG)
   MSTOT         = 1.4      % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.3.2.6. *Franc et al. (2001). Long-Evans rats*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Franc et al. 2001
% Non-gestational rat model
% dose levels: 0.140, 0.420, and 1.400 ug/kg every 2 weeks for 22 weeks
% dose levels: 140, 420, and 1400 ng/kg every 2 weeks for 22 weeks
% dose levels equivalent to 10, 30, and 100 ng/kg/day

```

```
MAXT            =  0.01
CINT            =  0.1
EXP_TIME_ON     =  0.               %delay before begin exposure (HOUR)
EXP_TIME_OFF    =  3696.              %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE       =  336.
BCK_TIME_ON     =  0.                %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF    =  0.                %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT       =  3696.              %SIMULATION LIMIT TIME (HOUR)
BW_T0           =  190.              % Body weight at the beginning of the
simulation (g); corresponds to approximate weight of females 10 weeks old

%EXPOSURE DOSE SCENARIOS (UG/KG)
   %MSTOT         = 0.14     % ORAL EXPOSURE DOSE (UG/KG)
   %MSTOT         = 0.42      % ORAL EXPOSURE DOSE (UG/KG)
   MSTOT         = 1.4      % ORAL EXP
```

C.2.3.2.7. *Franc et al. (2001). Hans Wistar rats*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Franc et al. 2001
% Non-gestational rat model
% dose levels: 0.140, 0.420, and 1.400 ug/kg every 2 weeks for 22 weeks
% dose levels: 140, 420, and 1400 ng/kg every 2 weeks for 22 weeks
% dose levels equivalent to 10, 30, and 100 ng/kg/day

MAXT            =  0.01
CINT            =  0.1
EXP_TIME_ON     =  0.               %delay before begin exposure (HOUR)
EXP_TIME_OFF    =  3696.              %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE       =  336.
BCK_TIME_ON     =  0.                %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF    =  0.                %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT       =  3696.              %SIMULATION LIMIT TIME (HOUR)
BW_T0           =  205.              % Body weight at the beginning of the
simulation (g); corresponds to approximate weight of females 10 weeks old

%EXPOSURE DOSE SCENARIOS (UG/KG)
   %MSTOT         = 0.14     % ORAL EXPOSURE DOSE (UG/KG)
   %MSTOT         = 0.42      % ORAL EXPOSURE DOSE (UG/KG)
   MSTOT         = 1.4      % ORAL EXP
```

C.2.3.2.8. *Hassoun et al. (2000).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Hassoun et al. 2000
%protocol:  oral exposure for 13 weeks; SD rats
%dose levels:  0.003, 0.010, 0.022, 0.046 0.1 ug/kg 5 days/weeks for 13 weeks
%dose levels equivalent to: 3, 10, 22, 46 100 ng/kg 5 days/weeks for 13 weeks
%dose levels equivalent to: 2.14, 7.14, 15.7, 32.9 71.4 ng/kg 7 days/weeks
for 13 weeks
```

```
MAXT             = 0.01
CINT             = 0.1
EXP_TIME_ON      = 0.          %delay before begin exposure (HOUR)
EXP_TIME_OFF     = 2184.       %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE        =  24.
WEEK_PERIOD      = 168.
WEEK_FINISH      = 119.
BCK_TIME_ON      = 0.          %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF     = 0.          %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT        = 2184.       %SIMULATION LIMIT TIME (HOUR)
BW_T0            = 215.         % Body weight at the beginning of the
simulation (g)

%EXPOSURE DOSE SCENARIOS (UG/KG)
     %MSTOT        = 0.003      % exposure dose ug/kg
     %MSTOT      = 0.010             % exposure dose ug/kg
     %MSTOT      = 0.022             % exposure dose ug/kg
     %MSTOT      = 0.046                 % exposure dose ug/kg
     MSTOT      = 0.1             % exposure dose ug/kg
```

C.2.3.2.9. *Hutt et al. (2008).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Hutt et al. 2008
% Non-gestational rat model
% dose levels: 0.050 ug/kg every week for 13 weeks
% dose levels: 50 ng/kg every week for 13 weeks
% dose levels equivalent to 7.14 ng/kg/day

MAXT             =  0.01
CINT             =  0.1
EXP_TIME_ON      =  0.               %delay before begin exposure (HOUR)
EXP_TIME_OFF     =  2184.               %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE        =  168.
BCK_TIME_ON      =  0.                %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF     =  0.                %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT        =  2184.               %SIMULATION LIMIT TIME (HOUR)
BW_T0            =  4.5             % Body weight at the beginning of the
simulation (g); corresponds to approximate weight of females 10 weeks old

%EXPOSURE DOSE SCENARIOS (UG/KG)
   MSTOT         = 0.05      % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.3.2.10. *Kitchin and Woods (1979)*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Kitchen and Woods 1979
%protocol: single oral gavage
```

```
%dose levels:  0.0006, 0.002, 0.004, 0.020, 0.060, 0.200, 0.600, 2.000,
5.000, 20.000 ug/kg single oral gavage
% dose levels = 0.6, 2, 4, 20, 60, 200, 600, 2000, 5000, 20000 ng/kg single
oral gavage
MAXT            =  0.001
CINT            =  0.1
EXP_TIME_ON     =  0.           %delay before begin exposure (HOUR)
EXP_TIME_OFF    =  24.           %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE       =  24.           % daily
BCK_TIME_ON     =  0.              %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF    =  0.              %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT       =  24.           %SIMULATION LIMIT TIME (HOUR)
BW_T0           =  225.             % Body weight at the beginning of the
simulation (g)

%EXPOSURE DOSE SCENARIOS (UG/KG)
  %MSTOT         = 0.0006        % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.002         % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.004          % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.020          % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.060          % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.200        % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.600         % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 2.000          % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 5.000          % ORAL EXPOSURE DOSE (UG/KG)
  MSTOT         = 20.000          % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.3.2.11. *Kociba et al. (1976) (13 weeks).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Kociba et al. 1976.
%built and check in August 7 2009
%protocol:  5 days/week exposure for 13 weeks; SD rats
%Rat_Dioxin_3C June09_2clean.csl
%RAT_NON_GEST_ICF_F083109.CSL  (now 09-11-09)
%dose levels:  0.001, 0.01, 0.1, 1 ug/kg 5 days/weeks for 13 weeks
%dose levels: 1, 10, 100, 1000 ng/kg 5 days/weeks for 13 weeks
%dose levels equivalent to: 0.714, 7.14, 71.4, 714 ng/kg/d (adj) 7 days/weeks
for 13 weeks

MAXT             = 0.001
CINT             = 0.1
EXP_TIME_ON      = 0.        %delay before begin exposure (HOUR)
EXP_TIME_OFF     = 2184      %TIME EXPOSURE STOP (HOUR)
WEEK_PERIOD      = 168
WEEK_FINISH      = 119
DAY_CYCLE        =  24
BCK_TIME_ON      = 0.        %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF     = 0.        %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT        = 2184      %SIMULATION LIMIT TIME (HOUR)
BW_T0            = 180       % Body weight at the begeniong of the
simulation (g)
```

```
%EXPOSURE DOSE SCENARIOS (UG/KG)
%MSTOT           = 0.001
 %MSTOT          = 0.01
 %MSTOT          = 0.1
 MSTOT          = 1
```

C.2.3.2.12. *Kociba et al. (1978) (female) (104 weeks).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% Kociba et al, 1978.
%built and check in August 7 2009
%protocol:  daily dietary exposure for 104 weeks; SD rats
%dose levels:  0.001, 0.01, 0.1 ug/kg 7 days/week for 104 weeks
%dose levels: 1, 10, 100 ng/kg 7 days/week for 104 weeks

MAXT            =  0.01
CINT            =  0.1
EXP_TIME_ON     =  0.              %TIME AT WHICH EXPOSURE BEGINS (HOUR)
EXP_TIME_OFF    =  17472           %TIME AT WHICH EXPOSURE ENDS (HOUR)
DAY_CYCLE       =  24
BCK_TIME_ON     =  0.              %TIME AT WHICH BACKGROUND EXPOSURE BEGINS
(HOUR)
BCK_TIME_OFF    =  0.              %TIME AT WHICH BACKGROUND EXPOSURE ENDS
(HOUR)
TIMELIMIT       =  17472           %SIMULATION TIME LIMIT (HOUR)
BW_T0           =  180             % BODY WEIGHT AT THE BEGINNING OF THE
SIMULATION (G)

%EXPOSURE DOSE SCENARIOS (UG/KG)
 %MSTOT          = 0.001             % EXPOSURE DOSE IN UG/KG
 %MSTOT         = 0.01             % EXPOSURE DOSE IN UG/KG
 MSTOT         = 0.1             % EXPOSURE DOSE IN UG/KG
```

C.2.3.2.13. *Kociba et al. (1978) (male) (104 weeks).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% Kociba et al, 1978.
%built and check in August 7 2009
%protocol:  daily dietary exposure for 104 weeks; SD rats
%dose levels:  0.001, 0.01, 0.1 ug/kg 7 days/week for 104 weeks
%dose levels: 1, 10, 100 ng/kg 7 days/week for 104 weeks

MAXT            =  0.01
CINT            =  0.1
EXP_TIME_ON     =  0.              %TIME AT WHICH EXPOSURE BEGINS (HOUR)
EXP_TIME_OFF    =  17472           %TIME AT WHICH EXPOSURE ENDS (HOUR)
DAY_CYCLE       =  24
BCK_TIME_ON     =  0.              %TIME AT WHICH BACKGROUND EXPOSURE BEGINS
(HOUR)
```

```
BCK_TIME_OFF    =  0.              %TIME AT WHICH BACKGROUND EXPOSURE ENDS
(HOUR)
TIMELIMIT       =  17472           %SIMULATION TIME LIMIT (HOUR)
BW_T0           =  250             % BODY WEIGHT AT THE BEGINNING OF THE
SIMULATION (G)

%EXPOSURE DOSE SCENARIOS (UG/KG)
 %MSTOT          = 0.001             % EXPOSURE DOSE IN UG/KG
 %MSTOT         = 0.01             % EXPOSURE DOSE IN UG/KG
 MSTOT         = 0.1             % EXPOSURE DOSE IN UG/KG
```

C.2.3.2.14. *Latchoumycandane and Mathur (2002).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Latchoumycandane and Mathur 2002.
%built and check in August 7 2009
%protocol:  1 time per day for 45 days oral gavage
%Rat_Dioxin_3C June09_2clean.csl
%RAT_NON_GEST_ICF_F083109.CSL  (now 09-11-09)
%dose levels:  0.001, 0.01, 0.1 ug/kg daily for 45 days
%dose levels:  1, 10, 100 ng/kg daily for 45 days

MAXT             = 0.01
CINT             = 0.1
EXP_TIME_ON      = 0.          % delay before begin exposure (HOUR)
EXP_TIME_OFF     = 1080        % TIME EXPOSURE STOP (HOUR)
DAY_CYCLE        = 24
BCK_TIME_ON      = 0.          % DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF     = 0.          % TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT        = 1080        % SIMULATION LIMIT TIME (HOUR)
BW_T0            = 200         % Body weight at the beginning of the
simulation (g)

%EXPOSURE DOSE SCENARIOS (UG/KG)
  %MSTOT          = 0.001        % exposure dose ug/kg
  %MSTOT         = 0.01         % exposure dose ug/kg
  MSTOT         = 0.1          % exposure dose ug/kg
```

C.2.3.2.15. *Li et al. (1997).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% Li et al 1997
% created 1/10/10
% Non-gestational rat model
% dose levels: 3, 10, 30, 100, 300, 1000, 3000, 10000, 30000 nkd one dose via
gavage, sacrificed 24 hrs later

MAXT           =  0.1
CINT           =  0.1
```

```
EXP_TIME_ON     =  0.               %delay before begin exposure (HOUR)
EXP_TIME_OFF    =  24.               %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE       =  24.
BCK_TIME_ON     =  0.                %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF    =  0.                %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT       =  24.               %SIMULATION LIMIT TIME (HOUR)
BW_T0           =  56.5              % Body weight at the beginning of the
simulation (g)

%EXPOSURE DOSE SCENARIOS (UG/KG)
   MSTOT         = 0.003     % ORAL EXPOSURE DOSE (UG/KG)
    %MSTOT         = 0.01      % ORAL EXPOSURE DOSE (UG/KG)
    %MSTOT         = 0.03      % ORAL EXPOSURE DOSE (UG/KG)
    %MSTOT         = 0.1       % ORAL EXPOSURE DOSE (UG/KG)
    %MSTOT         = 0.3       % ORAL EXPOSURE DOSE (UG/KG)
    %MSTOT         = 1.        % ORAL EXPOSURE DOSE (UG/KG)
    %MSTOT         = 3.        % ORAL EXPOSURE DOSE (UG/KG)
    %MSTOT         = 10.       % ORAL EXPOSURE DOSE (UG/KG)
    %MSTOT         = 30.       % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.3.2.16. *Murray et al. (1979).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% Murray et al 1979
%built and check in August 7 2009
%protocol:  dietary exposure for 3 generations (assume 120 day exposure for
each)
%dose levels: 0.001 0.01, 0.1 ug/kg/d
%dose levels: 1, 10, 100  ng/kg/d

MAXT            =  0.01
CINT            =  0.1
EXP_TIME_ON     =  0.               %TIME AT WHICH EXPOSURE BEGINS (HOUR)
EXP_TIME_OFF    =  2880            %TIME AT WHICH EXPOSURE ENDS (HOUR);
CORRESPONDS TO 120 DAYS OF EXPOSURE
DAY_CYCLE       =  24.
BCK_TIME_ON     =  0.               %TIME AT WHICH BACKGROUND EXPOSURE BEGINS
(HOUR)
BCK_TIME_OFF    =  0.               %TIME AT WHICH BACKGROUND EXPOSURE ENDS
(HOUR)
TIMELIMIT       =  2880            %SIMULATION TIME LIMIT (HOUR)
BW_T0           =  4.5              % BODY WEIGHT AT THE BEGINNING OF THE
SIMULATION (G)

%EXPOSURE DOSE SCENARIOS (UG/KG)
  %MSTOT           = 0.001        % ORAL EXPOSURE DOSE IN UG/KG
  %MSTOT         = 0.01         % ORAL EXPOSURE DOSE IN UG/KG
  MSTOT          = 0.1          % ORAL EXPOSURE DOSE IN UG/KG
```

C.2.3.2.17. *NTP (1982) (female) (chronic).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

%NTP 1982
%built and check in August 7 2009
%protocol: twice weekly gavage for 104 weeks + 3 week observation period
%Rat_Dioxin_3C June09_2clean.csl
%RAT_NON_GEST_ICF_F083109.CSL  (now 09-11-09)
%dose levels:  0.005, 0.025, 0.25 ug/kg biweekly for 104 weeks + 3 week
observation period
%dose levels:  5, 25, 250 ng/kg biweekly for 104 weeks + 3 week observation
period
%dose levels equivalent to: 1.43, 7.14, 71.4 ng/kg/day (adj)

MAXT              = 0.01
CINT              = 0.1
EXP_TIME_ON       = 0.            %delay before begin exposure (HOUR)
EXP_TIME_OFF      = 17472         %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE         = 84
BCK_TIME_ON       = 0.            %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF      = 0.            %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT         = 17472         %SIMULATION LIMIT TIME (HOUR)
BW_T0             = 250           % Body weight at the beginning of the
simulation (g)

%EXPOSURE DOSE SCENARIOS (UG/KG)

  %MSTOT          = 0.005        % exposure dose ug/kg
  %MSTOT          = 0.025
  MSTOT          = 0.25
```

C.2.3.2.18. *NTP (1982) (male) (chronic).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

%NTP 1982
%built and check in august 7 2009
%protocol: twice weekly gavage for 104 weeks + 3 week observation period
%Rat_Dioxin_3C June09_2clean.csl
%RAT_NON_GEST_ICF_F083109.CSL  (now 09-11-09)
%dose levels:  0.005, 0.025, 0.25 ug/kg biweekly for 104 weeks + 3 week
observation period
%dose levels:  5, 25, 250 ng/kg biweekly for 104 weeks + 3 week observation
period
%dose levels equivalent to: 1.43, 7.14, 71.4 ng/kg/day (adj)

MAXT              = 0.01
CINT              = 0.1
EXP_TIME_ON       = 0.            %delay before begin exposure (HOUR)
EXP_TIME_OFF      = 17472         %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE         = 84
```

```
BCK_TIME_ON        = 0.            %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF       = 0.            %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT          = 17472         %SIMULATION LIMIT TIME (HOUR)
BW_T0              = 350           % Body weight at the beginning of the
simulation (g)

%EXPOSURE DOSE SCENARIOS (UG/KG)

%MSTOT           = 0.005        % exposure dose ug/kg
%MSTOT           = 0.025
MSTOT           = 0.25
```

C.2.3.2.19. *NTP (2006) 14 weeks.*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% NTP 2006
%built and check in August 7 2009
%protocol:  oral exposure for 14 weeks; SD rats
%Rat_Dioxin_3C June09_2clean.csl
%RAT_NON_GEST_ICF_F083109.CSL  (now 09-11-09)
%dose levels:  0.003, 0.010, 0.022, 0.046 0.1 ug/kg 5 days/weeks for 14 weeks
%dose levels equivalent to: 3, 10, 22, 46 100 ng/kg 5 days/weeks for 14 weeks
%dose levels equivalent to: 2.14, 7.14, 15.7, 32.9 71.4 ng/kg 7 days/weeks for
14 weeks

MAXT             = 0.01
CINT             = 0.1
EXP_TIME_ON      = 0.          %delay before begin exposure (HOUR)
EXP_TIME_OFF     = 2352       %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE        =  24
WEEK_PERIOD      = 168
WEEK_FINISH      = 119
BCK_TIME_ON      = 0.          %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF     = 0.          %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT        = 2352       %SIMULATION LIMIT TIME (HOUR)
BW_T0            = 215         % Body weight at the beginning of the simulation
(g)

%EXPOSURE DOSE SCENARIOS (UG/KG)
     %MSTOT         = 0.003       % exposure dose ug/kg
     %MSTOT       = 0.010              % exposure dose ug/kg
     %MSTOT       = 0.022              % exposure dose ug/kg
     %MSTOT       = 0.046                  % exposure dose ug/kg
     MSTOT       = 0.1             % exposure dose ug/kg
```

C.2.3.2.20. *NTP (2006) 31 weeks.*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% NTP 2006
%built and check in August 7 2009
%protocol:  oral exposure for 31 weeks; SD rats
%Rat_Dioxin_3C June09_2clean.csl
%RAT_NON_GEST_ICF_F083109.CSL  (now 09-11-09)
%dose levels:  0.003, 0.010, 0.022, 0.046 0.1 ug/kg 5 days/weeks for 31 weeks
```

```
%dose levels equivalent to: 3, 10, 22, 46 100 ng/kg 5 days/weeks for 31 weeks
%dose levels equivalent to: 2.14, 7.14, 15.7, 32.9 71.4 ng/kg 7 days/weeks
for 31 weeks

MAXT             = 0.01
CINT             = 0.1
EXP_TIME_ON      = 0.          %delay before begin exposure (HOUR)
EXP_TIME_OFF     = 5208      %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE        =  24
WEEK_PERIOD      = 168
WEEK_FINISH      = 119
BCK_TIME_ON      = 0.          %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF     = 0.          %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT        = 5208      %SIMULATION LIMIT TIME (HOUR)
BW_T0            = 215         % Body weight at the beginning of the
simulation (g)

%EXPOSURE DOSE SCENARIOS (UG/KG)
     %MSTOT        = 0.003      % exposure dose ug/kg
     %MSTOT      = 0.010             % exposure dose ug/kg
     %MSTOT      = 0.022             % exposure dose ug/kg
     %MSTOT      = 0.046                 % exposure dose ug/kg
     MSTOT      = 0.1             % exposure dose ug/kg
```

C.2.3.2.21. *NTP (2006) 53 weeks.*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% NTP 2006
%built and check in August 7 2009
%protocol:  oral exposure for 53 weeks; SD rats
%Rat_Dioxin_3C June09_2clean.csl
%RAT_NON_GEST_ICF_F083109.CSL  (now 09-11-09)
%dose levels:  0.003, 0.010, 0.022, 0.046 0.1 ug/kg 5 days/weeks for 53 weeks
%dose levels equivalent to: 3, 10, 22, 46 100 ng/kg 5 days/weeks for 53 weeks
%dose levels equivalent to: 2.14, 7.14, 15.7, 32.9 71.4 ng/kg 7 days/weeks
for 53 weeks

MAXT             = 0.01
CINT             = 0.1
EXP_TIME_ON      = 0.          %delay before begin exposure (HOUR)
EXP_TIME_OFF     = 8904      %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE        =  24
WEEK_PERIOD      = 168
WEEK_FINISH      = 119
BCK_TIME_ON      = 0.          %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF     = 0.          %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT        = 8904      %SIMULATION LIMIT TIME (HOUR)
BW_T0            = 215         % Body weight at the beginning of the
simulation (g)

%EXPOSURE DOSE SCENARIOS (UG/KG)
     %MSTOT        = 0.003      % exposure dose ug/kg
     %MSTOT      = 0.010             % exposure dose ug/kg
```

```
      %MSTOT      = 0.022              % exposure dose ug/kg
      %MSTOT      = 0.046                  % exposure dose ug/kg
      MSTOT      = 0.1             % exposure dose ug/kg
```

C.2.3.2.22. *NTP (2006) 2 year.*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% NTP 2006
%built and check in August 7 2009
%protocol:  oral exposure for 105 weeks; SD rats
%dose levels:  0.003, 0.010, 0.022, 0.046, 0.1 ug/kg 5 days/week for 105
weeks
%dose levels equivalent to: 3, 10, 22, 46, 100 ng/kg 5 days/week for 105
weeks
%dose levels equivalent to: 2.14, 7.14, 15.7, 32.9, 71.4 ng/kg 7 days/week
for 105 weeks

MAXT             = 0.01
CINT             = 0.1
EXP_TIME_ON      = 0.         %TIME AT WHICH EXPOSURE BEGINS (HOUR)
EXP_TIME_OFF     = 17640      %TIME AT WHICH EXPOSURE ENDS (HOUR)
DAY_CYCLE        =  24
WEEK_PERIOD      = 168
WEEK_FINISH      = 119
BCK_TIME_ON      = 0.         %TIME AT WHICH BACKGROUND EXPOSURE BEGINS
(HOUR)
BCK_TIME_OFF     = 0.         %TIME AT WHICH BACKGROUND EXPOSURE ENDS (HOUR)
TIMELIMIT        = 17640      %SIMULATION TIME LIMIT (HOUR)
BW_T0            = 215        % BODY WEIGHT AT THE BEGINNING OF THE
SIMULATION (G)

%EXPOSURE DOSE SCENARIOS (UG/KG)
     %MSTOT        = 0.003      % EXPOSURE DOSE IN UG/KG
     %MSTOT      = 0.010             % EXPOSURE DOSE IN UG/KG
     %MSTOT      = 0.022             % EXPOSURE DOSE IN UG/KG
     %MSTOT      = 0.046                 % EXPOSURE DOSE IN UG/KG
     MSTOT      = 0.1             % EXPOSURE DOSE IN UG/KG
```

C.2.3.2.23. *Sewall et al. (1995).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG
% Sewall et al. 1995
%Rat_Dioxin_3C June09_2clean.csl
%RAT_NON_GEST_ICF_F083109.CSL  (now 09-11-09)
%protocol: gavage every 2 weeks for 30 weeks
%dose levels: 0.049, 0.1498, 0.49, and 1.75 ug/kg every 2 weeks
%dose levels: 3.5, 10.7, 35, and 125 ng/kg/d or 49, 149.8, 490, and 1750
ng/kg every 2 weeks

MAXT             =  0.01
CINT             =  0.1
```

```
EXP_TIME_ON     =  0.            %delay before begin exposure (HOUR) 5 weeks
after start of experiment (age = 12 weeks)
EXP_TIME_OFF    =  5040            %TIME EXPOSURE STOP (HOUR); 30 doses, 1
every two weeks
DAY_CYCLE       =  336.            % once every two weeks
BCK_TIME_ON     =  0.               %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF    =  0.               %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT       =  5040            %SIMULATION LIMIT TIME (HOUR)
BW_T0           =  250              % Body weight at the beginning of the
simulation (g); corresponds to 12 week old female

%EXPOSURE DOSE SCENARIOS (UG/KG)
  %MSTOT           = 0.049        % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 0.1498        % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT          = 0.49         % ORAL EXPOSURE DOSE (UG/KG)
  MSTOT          = 1.75         % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.3.2.24. *Shi et al. (2007), adult portion.*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% Shi et al 2007
%built and check in August 7 2009
%protocol:  gavage once per week for 322 days
%dose levels: 0.001, 0.005, 0.05 and 0.2 ug TCDD:kg body weight by gavage
once per week
%dose levels: 1, 5, 50 and 200 ng/kg ng TCDD:kg body weight by gavage once
per week
% dose equivalent adjusted 0.143, 0.714, 7.14 and 28.6 ng/kg/d

MAXT            =  0.0001
CINT            =  0.1
EXP_TIME_ON     =  504.             % TIME AT WHICH EXPOSURE BEGINS (HOUR)
EXP_TIME_OFF    =  7728            %TIME AT WHICH EXPOSURE ENDS (HOUR);
CORRESPONDS TO 322 DAYS OF EXPOSURE
DAY_CYCLE       =  168.
BCK_TIME_ON     =  0.               % TIME AT WHICH BACKGROUND EXPOSURE
BEGINS (HOUR)
BCK_TIME_OFF    =  0.               % TIME AT WHICH BACKGROUND EXPOSURE ENDS
(HOUR)
TIMELIMIT       =  7728            %SIMULATION TIME LIMIT (HOUR)
BW_T0           =  4.5              % BODY WEIGHT AT THE BEGINNING OF THE
SIMULATION (G)

%EXPOSURE DOSE SCENARIOS (UG/KG)
   %MSTOT          = 0.001     % ORAL EXPOSURE DOSE IN UG/KG
   %MSTOT         = 0.005     % ORAL EXPOSURE DOSE IN UG/KG
   %MSTOT         = 0.05      % ORAL EXPOSURE DOSE IN UG/KG
   MSTOT         = 0.2       % ORAL EXPOSURE DOSE IN UG/KG
```

C.2.3.2.25. *Van Birgelen et al. (1995).*

```
output @clear
prepare @clear
```

```
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% Van Birgelen et al. (1995)
%protocol:  daily dietary exposure for 13 weeks
%dose levels: 0.0135, 0.0264, 0.0469, 0.320, 1.024 ug/kg every day for 13
weeks
% dose levels = 13.5, 26.4, 46.9, 320, 1024 ng/kg every day for 13 weeks
MAXT            =  0.01
CINT            =  0.1
EXP_TIME_ON     =  0.            %delay before begin exposure (HOUR)
EXP_TIME_OFF    =  2184.           %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE       =  24.            % once every two weeks
BCK_TIME_ON     =  0.              %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF    =  0.              %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT       =  2184.           %SIMULATION LIMIT TIME (HOUR)
BW_T0           =  150.             % Body weight at the beginning of the
simulation (g)

%EXPOSURE DOSE SCENARIOS (UG/KG)
  %MSTOT         = 0.0135        % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.0264         % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.0469          % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.320          % ORAL EXPOSURE DOSE (UG/KG)
  MSTOT         = 1.024          % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.3.2.26. *Vanden Heuvel et al. (1994).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Vanden Heuvel et al. 1994.
%built and check in August 7 2009
%protocol: single gavage
%Rat_Dioxin_3C June09_2clean.csl
%RAT_NON_GEST_ICF_F083109.CSL  (now 09-11-09)
%dose levels:0.00005, 0.0001, 0.001, 0.010, 0.1, 1, 10 ug/kg/d
%dose levels equivalent to: 0.05, 0.1, 1, 10, 100, 1000, 10000 ng/kg/d

MAXT              = 0.001
CINT              = 0.1
EXP_TIME_ON       = 0.            %delay before begin exposure (HOUR)
EXP_TIME_OFF      = 24          %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE         = 24
BCK_TIME_ON       = 0.            %DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
BCK_TIME_OFF      = 0.            %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT         = 24           %SIMULATION LIMIT TIME (HOUR)
BW_T0             = 250           % Body weight at the beginning of the
simulation (g)

%EXPOSURE DOSE SCENARIOS (UG/KG)

  %MSTOT            = 0.00005       % exposure dose ug/kg
  %MSTOT          = 0.0001        % exposure dose ug/kg
  %MSTOT          = 0.001         % exposure dose ug/kg
  %MSTOT          = 0.01          % exposure dose ug/kg
```

```
  %MSTOT           = 0.1           % exposure dose ug/kg
  %MSTOT           = 1             % exposure dose ug/kg
  MSTOT           = 10            % exposure dose ug/kg
```

C.2.4. Rat Gestational Model

C.2.4.1. *Model Code*

PROGRAM: 'Three Compartment PBPK Model for TCDD in Rat (Gestation)'

```
! Parameters were change May 16, 2002
! Come from {8MAI_CHR_PRE-EXP_GD}
! Come from {12_Mouse_GD}file
!*******************************************
!{{IMPORTANT-IMPORTANT-IMPORTANT-IMPORTANT}}
! REDUCTION OF MOTHER AND FETUS COMPARTMENT
! 2M_R_TCDD_JULY2002 ////(JULY 18,2002)////
!TCDD_RED_4Species_2003_4        ////(APR  8 ,2003)////
!TCDD_RED_4Species_2003_9        ////(APR  17 ,2003)////
!TCDD_RED_4Species_2003_12        ////(APR  17 ,2003)////
!*************************************************
!APRIL 18 2003
!TCDD_4C_4SP_2003        ////(APR  18 ,2003)////
! was ''Gest 4 species 1.csl''   but update July 2009

!DevTCDD4Species_ICF_afterKKfix_v3_ratgest.csl
!RAT_GESTATIONAL_ICF_F083109.csl
!RAT_GESTATIONAL_ICF_F100609.csl
!*************************************************

 !Legend/Legend/Legend/Legend/Legend/Legend/Legend/Legend/
 !Legend for this PBPK model
 !Mating: control the tenure of exchange between fetus and
    !Mother and also control imitated tissue growth
    !Control: WTFE, WFO, WPLA0, QPLAF,WT0
    !(for rat, mouse, human, and monkey)
 !Control transfer from mother to fetus or fetus to mother by TRANSTIME_ON
    !SWITCH_trans = 0 NO TRANSFER
    !SWITCH_trans = 1 TRANSFER OCCURS
    !Gest_off = 1
    !Gest_on=   0.0
 ! These switches are also controlled by mating parameters

INITIAL  !

     !SIMULATION PARAMETERS ====
CONSTANT PARA_ZERO          = 1E-30
CONSTANT EXP_TIME_ON     = 0.0       ! TIME AT WHICH EXPOSURE BEGINS (HOURS)
CONSTANT EXP_TIME_OFF    = 530       ! TIME AT WHICH EXPOSURE ENDS (HOURS)
CONSTANT DAY_CYCLE       = 24.0      ! NUMBER OF HOURS BETWEEN DOSES (HOURS)
CONSTANT BCK_TIME_ON     = 0.0       ! TIME AT WHICH BACKGROUND EXPOSURE
BEGINS (HOURS)
CONSTANT BCK_TIME_OFF    = 0.0       ! TIME AT WHICH BACKGROUND EXPOSURE ENDS
(HOURS)
CONSTANT TRANSTIME_ON    = 144.0     !CONTROL TRANSFER FROM MOTHER TO FETUS
AT GESTATIONAL DAY 6
```

```
 !UNIT CONVERSION
CONSTANT MW=322 ! MOLECULAR WEIGHT (NG/NMOL)
CONSTANT SERBLO = 0.55
CONSTANT UNITCORR = 1000


    !INTRAVENOUS SEQUENCE
constant IV_LACK         = 0.0
constant IV_PERIOD       = 0.0

    !PREGNANCY PARAMETER ====
CONSTANT MATTING         = 0.0       !BEGINNING OF MATING (HOUR)
CONSTANT N_FETUS         = 10.0       !NUMBER OF FETUS PRESENT

    !CONSTANT EXPOSURE CONTROL ===========
    !ACUTE, SUBCHRONIC, CHRONIC EXPOSURE =====
    !OR BACKGROUND EXPOSURE (IN THIS CASE 3 TIMES A DAY)===
CONSTANT MSTOTBCKGR      = 0.0       ! ORAL BACKGROUND EXPOSURE DOSE (UG/KG)
CONSTANT MSTOT           = 0.0       ! ORAL EXPOSURE DOSE (UG/KG)

    !ORAL ABSORPTION
  MSTOT_NM = MSTOT/MW              ! CONVERTS THE DOSE TO NMOL/G

    !INTRAVENOUS ABSORPTION
CONSTANT  DOSEIV         = 0.0       ! INJECTED DOSE (UG/KG)
  DOSEIV_NM = DOSEIV/MW            ! CONVERTS THE INJECTED DOSE TO NMOL/G
CONSTANT DOSEIVLATE = 0.0            ! INJECTED DOSE LATE (UG/KG)
  DOSEIVNMlate = DOSEIVLATE/MW     !AMOUNT IN NMOL/G

    !INITIAL GUESS OF THE FREE CONCENTRATION IN THE LIGAND (COMPARTMENT
INDICATED BELOW)====
CONSTANT CFLLI0          = 0.0  !LIVER    (NMOL/ML)
CONSTANT CFLPLA0         = 0.0  !PLACENTA (NMOL/ML)

    !BINDING CAPACITY (AhR) FOR NON LINEAR BINDING (COMPARTMENT INDICATED
BELOW) (NMOL/ML) ===
CONSTANT LIBMAX          = 3.5E-4   ! LIVER  (NMOL/ML), WANG ET AL. 1997
CONSTANT PLABMAX         = 2.0E-4   !TEMPORARY PARAMETER

    ! PROTEIN AFFINITY CONSTANTS (1A2 OR AhR, COMPARTMENT INDICATED BELOW)
(NMOL/ML)===
CONSTANT KDLI            = 1.0E-4   !LIVER (AhR) (NMOL/ML), WANG ET AL. 1997
CONSTANT KDLI2           = 4.0E-2   !LIVER (1A2) (NMOL/ML), EMOND ET AL. 2004
CONSTANT KDPLA           = 1.0E-4   !TEMPORARY PARAMETER; ASSUME IDENTICAL TO
KDLI (AhR)

    !EXCRETION AND ABSORPTION CONSTANT
CONSTANT KST             = 0.36     ! GASTRIC RATE CONSTANT (HR-1), WANG ET
AL. 1997
CONSTANT KABS            = 0.48     !INTESTINAL ABSORPTION CONSTANT (HR-1) ),
WANG ET AL. 1997

    ! ELIMINATION CONSTANTS
CONSTANT CLURI           = 0.01       ! URINARY CLEARANCE (ML/HR), EMOND ET
AL. 2004
```

```
1
2       !INTERSPECIES ELIMINATION VARIABLE
3   CONSTANT kelv            = 0.15     ! INTERSPECIES VARIABLE ELIMINATION
4   CONSTANT (1/HOUR)
5
6       ! CONSTANT TO DIVIDE THE ABSORPTION INTO LYMPHATIC AND PORTAL FRACTIONS
7   CONSTANT A               = 0.7        ! LYMPHATIC FRACTION, WANG ET AL. 1997
8
9       !PARTITION COEFFICIENTS
10  CONSTANT PF              = 100     ! ADIPOSE TISSUE/BLOOD, WANG ET AL. 1997
11  CONSTANT PRE             = 1.5      ! REST OF THE BODY/BLOOD, WANG ET AL.
12  1997
13  CONSTANT PLI             = 6.0       ! LIVER/BLOOD, WANG ET AL. 1997
14  CONSTANT PPLA            = 1.5      ! TEMPORARY PARAMETER NOT CONFIGURED,
15  WANG ET AL. 1997
16
17      !PARAMETER FOR INDUCTION OF CYP 1A2, WANG ET AL. 1997
18  CONSTANT PAS_INDUC       = 1.0        ! INCLUDE INDUCTION? (1 = YES, 0 = NO)
19  CONSTANT CYP1A2_1OUTZ    = 1.6      ! DEGRADATION CONCENTRATION CONSTANT OF
20  1A2 (NMOL/ML)
21  CONSTANT CYP1A2_1A1      = 1.6      ! BASAL CONCENTRATION OF 1A1 (NMOL/ML)
22  CONSTANT CYP1A2_1EC50    = 0.13      ! DISSOCIATION CONSTANT TCDD-CYP1A2
23  (NMOL/ML)
24  CONSTANT CYP1A2_1A2      = 1.6      !BASAL CONCENTRATION OF 1A2 (NMOL/ML)
25  CONSTANT CYP1A2_1KOUT    = 0.1      ! FIRST ORDER RATE OF DEGRADATION (H-1)
26  CONSTANT CYP1A2_1TAU     = 0.25     !HOLDING TIME (H)
27  CONSTANT CYP1A2_1EMAX    = 600      ! MAXIMUM INDUCTION OVER BASAL EFFECT
28  (UNITLESS)
29  CONSTANT HILL            = 0.6      !HILL CONSTANT; COOPERATIVELY LIGAND
30  BINDING EFFECT CONSTANT (UNITLESS)
31
32      !DIFFUSIONAL PERMEABILITY FRACTION
33  CONSTANT PAFF            = 0.0910   !ADIPOSE (UNITLESS), WANG ET AL. 1997
34  CONSTANT PAREF           = 0.0298   !REST OF THE BODY  (UNITLESS), WANG ET
35  AL. 1997
36  CONSTANT PALIF           = 0.3500   !LIVER (UNITLESS), WANG ET AL. 1997
37  CONSTANT PAPLAF          = 0.3      !TEMPORARY PARAMETER NOT CONFIGURED
38
39     !FRACTION OF TISSUE WEIGHT =========
40  CONSTANT WLI0            = 0.0360   !LIVER, WANG ET AL. 1997
41
42     !TISSUE BLOOD FLOW EXPRESSED AS A FRACTION OF CARDIAC OUTPUT
43  CONSTANT QFF             = 0.069    ! ADIPOSE TISSUE BLOOD FLOW FRACTION
44  (UNITLESS), WANG ET AL. 1997
45  CONSTANT QLIF            = 0.183    !LIVER (UNITLESS), WANG ET AL. 1997
46
47     !COMPARTMENT TISSUE BLOOD EXPRESSED AS A FRACTION OF THE TOTAL COMPARTMENT
48  VOLUME
49  CONSTANT WFB0            = 0.050    !ADIPOSE TISSUE, WANG ET AL. 1997
50  CONSTANT WREB0           = 0.030    !REST OF THE BODY, WANG ET AL. 1997
51  CONSTANT WLIB0           = 0.266    !LIVER, WANG ET AL. 1997
52  CONSTANT WPLAB0          = 0.500    !TEMPORARY PARAMETER NOT CONFIGURED
53
54     !EXPOSURE SCENARIO FOR UNIQUE OR REPETITIVE WEEKLY OR MONTHLY EXPOSURE
55     !NUMBER OF EXPOSURES PER WEEK
56  CONSTANT WEEK_LACK       = 0.0       !DELAY BEFORE EXPOSURE ENDS (WEEK)
```

```
CONSTANT WEEK_PERIOD     = 168      ! NUMBER OF HOURS IN THE WEEK (HOURS)
CONSTANT WEEK_FINISH     = 168      ! TIME EXPOSURE ENDS (HOURS)

   !NUMBER OF EXPOSURES PER MONTH
CONSTANT MONTH_LACK      = 0.0       !DELAY BEFORE EXPOSURE BEGINS (MONTHS)

   !CONSTANT FOR BACKGROUND EXPOSURE==========
CONSTANT Day_LACK_BG     = 0.0       !DELAY BEFORE EXPOSURE BEGINS (HOURS)
CONSTANT Day_PERIOD_BG   = 24       !LENGTH OF EXPOSURE (HOURS)

   !NUMBER OF EXPOSURES PER WEEK
CONSTANT WEEK_LACK_BG      = 0.0      !DELAY BEFORE BACKGROUD EXPOSURE BEGINS
(WEEKS)
CONSTANT WEEK_PERIOD_BG    = 168      !NUMBER OF HOURS IN THE WEEK (HOURS)
CONSTANT WEEK_FINISH_BG    = 168      !TIME EXPOSURE ENDS (HOURS)

   !INITIAL BODY WEIGHT
CONSTANT BW_T0             = 250      ! WANG ET AL. 1997
CONSTANT RATIO_RATF_MOUSEF = 1.0        !RATIO OF FETUS MOUSE/RAT AT
GESTATIONAL DAY 22

   ! COMPARTMENT LIPID EXPRESSED AS THE FRACTION OF TOTAL LIPID, POULIN ET AL
2000
CONSTANT F_TOTLIP          = 0.855              ! ADIPOSE TISSUE (UNITLESS)
CONSTANT B_TOTLIP          = 0.0023              ! BLOOD (UNITLESS)
CONSTANT RE_TOTLIP         = 0.019              ! REST OF THE BODY
(UNITLESS)
CONSTANT LI_TOTLIP         = 0.060              ! LIVER (UNITLESS)
CONSTANT PLA_TOTLIP        = 0.019
CONSTANT FETUS_TOTLIP      = 0.019

END     ! END OF THE INITIAL SECTION

DYNAMIC ! DYNAMIC SIMULATION SECTION
ALGORITHM  IALG          =           2        ! GEAR METHOD
CINTERVAL  CINT          =         0.1        ! COMMUNICATION INTERVAL
MAXTERVAL  MAXT          =        1.0e+10     ! MAXIMUM CALCULATION INTERVAL
MINTERVAL  MINT          =        1.0E-10     ! MINIMUM CALCULATION INTERVAL
VARIABLE   T             =         0.0
CONSTANT   TIMELIMIT     =         100        !SIMULATION LIMIT TIME (HOURS)
 CINTXY  = CINT
 PFUNC   = CINT

   !TIME CONVERSION
  DAY         = T/24           ! TIME IN DAYS
  WEEK        = T/168          ! TIME IN WEEKS
  MONTH       = T/730          ! TIME IN MONTHS
  YEAR        = T/8760         ! TIME IN YEARS

DERIVATIVE ! PORTION OF CODE THAT SOLVES DIFFERENTIAL EQUATIONS

   !CHRONIC OR SUBCHRONIC EXPOSURE SCENARIO =======
   !NUMBER OF EXPOSURES PER DAY
 DAY_LACK         = EXP_TIME_ON    ! DELAY BEFORE EXPOSURE BEGINS (HOURS)
 DAY_PERIOD       = DAY_CYCLE      ! EXPOSURE PERIOD (HOURS)
 DAY_FINISH       = CINTXY         ! LENGTH OF EXPOSURE (HOURS)
```

```
 MONTH_PERIOD     = TIMELIMIT      ! EXPOSURE PERIOD (MONTHS)
 MONTH_FINISH     = EXP_TIME_OFF   ! LENGTH OF EXPOSURE (MONTHS)

   !NUMBER OF EXPOSURES PER DAY AND MONTH
 DAY_FINISH_BG    = CINTXY
 MONTH_LACK_BG    = BCK_TIME_ON    !DELAY BEFORE BACKGROUD EXPOSURE BEGINS
(MONTHS)
 MONTH_PERIOD_BG  = TIMELIMIT      !BACKGROUND EXPOSURE (MONTHS)
 MONTH_FINISH_BG  = BCK_TIME_OFF   !LENGTH OF BACKGROUND EXPOSURE (MONTHS)

   !INTRAVENOUS LATE
 IV_FINISH = CINTXY
 B = 1-A ! FRACTION OF DIOXIN ABSORBED IN THE PORTAL FRACTION OF THE LIVER


!FETUS,VOLUME,FETUS,VOLUME,FETUS,VOLUME,FETUS,VOLUME,FETUS,VOLUME,FETUS,VOLUM
E
  ! FROM OFLAHERTY_1992

RTESTGEST= T-MATTING
TESTGEST=DIM(RTESTGEST,0.0)

WTFER_RODENT= (2.3d-3*EXP(1.49d-2*(TESTGEST))+1.3d-2)*Gest_on
WTFER = (WTFER_RODENT*RATIO_RATF_MOUSEF*N_FETUS)
WTFE = DIM(WTFER,0.0)

  !
FAT,VOLUME,FAT,VOLUME,FAT,VOLUME,FAT,VOLUME,FAT,VOLUME,FAT,VOLUME,FAT,VOLUME
  ! FAT GROWTH EXPRESSION LINEAR DURING PREGNANCY
  ! FROM O'FLAHERTY_1992

WF0= (((9.66d-5*(TESTGEST))*gest_on)+0.069)

  ! PLACENTA,VOLUME, PLACENTA,VOLUME, PLACENTA,VOLUME, PLACENTA,VOLUME
  ! WPLA PLACENTA GROWTH  EXPRESSION, SINGLE EXPONENTIAL WITH OFFSET
  ! FROM O'FLAHERTY_1992  ! FOR EACH PUP

WPLA0N_RODENT = (0.6/(1+(5d+3*EXP(-0.0225*(TESTGEST)))))*N_FETUS
WPLA0R = (WPLA0N_RODENT/WT0)*Gest_on
WPLA0 = DIM(WPLA0R,0.0)

  ! PLACENTA,FLOW RATE, PLACENTA,FLOW RATE, PLACENTA,FLOW RATE, PLACENTA,FLOW
RATE
  ! QPLA PLACENTA GROWTH EXPRESSION, DOUBLE EXPONENTIAL WITH OFFSET
  ! FROM O'FLAHERTY_1992

 QPLARF = (1.67d-7 *exp(9.6d-3*(TESTGEST)) &
   +1.6d-3*exp(7.9d-3*(TESTGEST))+0.0)*Gest_on*SWITCH_trans
 QPLAF=DIM(QPLARF,0.0)              !FRACTION OF FLOW RATE IN PLACENTA

  ! GESTATION CONTROL
IF (T.LT.MATTING) THEN
    Gest_off = 1.0
    Gest_on=   0.0
ELSE
    Gest_off = 0.0
```

```
    Gest_on  = 1.0
END IF

  ! MOTHER BODY WEIGHT GROWTH EQUATION========
  ! MODIFICATION TO ADAPT THIS MODEL AT HUMAN MODEL
  ! BECAUSE LINEAR DESCRIPTION IS NOT GOOD ENOUGH FOR MOTHER GROWTH
  ! MOTHER BODY WEIGHT GROWTH

  PARAMETER (BW_RMN = 1.0E-30)
  WT0= BW_T0 *(1+(0.41*T)/(1402.5+T+BW_RMN))

  ! VARIABILITY OF REST OF THE BODY DEPENDS ON OTHER ORGANS
  WRE0 = (0.91 - (WLIB0*WLI0 + WFB0*WF0 +WPLAB0*WPLA0 + WLI0 + WF0 +
WPLA0))/(1+WREB0)  ! REST OF THE BODY FRACTION; UPDATED FOR EPA ASSESSMENT
  QREF = 1-(QFF+QLIF+QPLAF)            !REST OF BODY BLOOD FLOW RATE (ML/HR)
  QTTQF = QFF+QREF+QLIF+QPLAF          ! SUM MUST EQUAL 1

  ! COMPARTMENT VOLUME (ML OR G) =========
 WF  =  WF0  * WT0                    ! ADIPOSE TISSUE
 WRE =  WRE0 * WT0                    ! REST OF THE BODY
 WLI =  WLI0 * WT0                    ! LIVER
 WPLA=  WPLA0* WT0                    ! PLACENTA

   ! COMPARTMENT TISSUE BLOOD (ML OR G) =========
 WFB  =  WFB0  * WF                   ! ADIPOSE TISSUE
 WREB =  WREB0 * WRE                  ! REST OF THE BODY
 WLIB =  WLIB0 * WLI                  ! LIVER
 WPLAB = WPLAB0* WPLA                 ! PLACANTA

   ! CARDIAC OUTPUT FOR THE GIVEN BODY WEIGHT (ML/H) =========
   !QC= QCCAR*60*(WT0/1000.0)**0.75
CONSTANT QCC=18684.0                    ! EQUIVALENT TO 311.4 * 60
QC= QCC*(WT0/UNITCORR)**0.75

   !COMPARTMENT BLOOD FLOW RATE (ML/HR)
QF  = QFF*QC                          !ADIPOSE TISSUE BLOOD FLOW RATE
QLI = QLIF*QC                         !LIVER TISSUE BLOOD FLOW RATE
QRE = QREF*QC                         !REST OF THE BODY BLOOD FLOW RATE
QPLA = QPLAF*QC                       !PLACENTA TISSUE BLOOD FLOW RATE
QTTQ = QF+QRE+QLI+QPLA   !TOTAL FLOW RATE

    !PERMEABILITY ORGAN FLOW (ML/HR)=========
PAF  = PAFF*QF                       ! ADIPOSE TISSUE
PARE = PAREF*QRE                     ! REST OF THE BODY
PALI = PALIF*QLI                     ! LIVER TISSUE
PAPLA = PAPLAF*QPLA                  ! PLACENTA

    !************************************
    ! ABSORPTION SECTION
    ! ORAL
    ! INTRAPERITONEAL
    ! INTRAVENOUS
    !************************************

    !REPETITIVE ORAL BACKGROUND EXPOSURE SCENARIO

```

```
MSTOT_NMBCKGR = MSTOTBCKGR/MW       ! CONVERTS THE BACKGROUND DOSE TO NMOL/G
MSTTBCKGR =MSTOT_NMBCKGR *WT0

DAY_EXPOSURE_BG   = PULSE(DAY_LACK_BG,DAY_PERIOD_BG,DAY_FINISH_BG)
WEEK_EXPOSURE_BG  = PULSE(WEEK_LACK_BG,WEEK_PERIOD_BG,WEEK_FINISH_BG)
MONTH_EXPOSURE_BG = PULSE(MONTH_LACK_BG,MONTH_PERIOD_BG,MONTH_FINISH_BG)

MSTTCH_BG = (DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG)*MSTTBCKGR
MSTTFR_BG = MSTTBCKGR/CINT

CYCLE_BG =DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG

    ! CONDITIONAL ORAL EXPOSURE (BACKGROUND EXPOSURE)

IF (MSTTCH_BG.EQ.MSTTBCKGR) THEN
    ABSMSTT_GB= MSTTFR_BG
ELSE
    ABSMSTT_GB = 0.0
END IF

CYCLETOTBG=INTEG(CYCLE_BG,0.0)

   !REPETITIVE ORAL EXPOSURE SCENARIO

MSTT= MSTOT_NM * WT0                  !AMOUNT IN NMOL

DAY_EXPOSURE   = PULSE(DAY_LACK,DAY_PERIOD,DAY_FINISH)
WEEK_EXPOSURE  = PULSE(WEEK_LACK,WEEK_PERIOD,WEEK_FINISH)
MONTH_EXPOSURE = PULSE(MONTH_LACK,MONTH_PERIOD,MONTH_FINISH)

MSTTCH = (DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE)*MSTT
MSTTFR = MSTT/CINT

CYCLE = DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE
SUMEXPEVENT= INTEG (CYCLE,0.0) !NUMBER OF CYCLE GENERATE DURING SIMULATION

   ! CONDITIONAL ORAL EXPOSURE
IF (MSTTCH.EQ.MSTT) THEN
   ABSMSTT= MSTTFR
ELSE
   ABSMSTT = 0.0
END IF


 CYCLETOT=INTEG(CYCLE,0.0)

   ! MASS CHANGE IN THE LUMEN
 RMSTT= -(KST+KABS)*MST +ABSMSTT +ABSMSTT_GB ! RATE OF CHANGE (NMOL/H)
  MST = INTEG(RMSTT,0.0)                    !AMOUNT REMAINING IN DUODENUM
(NMOL)

   ! ABSORPTION IN LYMPH CIRCULATION
 LYRMLUM = KABS*MST*A
  LYMLUM = INTEG(LYRMLUM,0.0)

   ! ABSORPTION IN PORTAL CIRCULATION
```

```
 LIRMLUM = KABS*MST*B
  LIMLUM = INTEG(LIRMLUM,0.0)


! -----IV EXPOSURE ---------

 IV= DOSEIV_NM * WT0 !AMOUNT IN NMOL
 IVR= IV/PFUNC ! RATE FOR IV INFUSION IN BLOOD
 EXPIV= IVR * (1.0-STEP(PFUNC))
 IVDOSE = integ(EXPIV,0.0)

    !------IV LATE IN THE CYCLE
    ! MODIFICATION ON January 13 2004
 IV_RlateR = DOSEIVNMlate*WT0
 IV_EXPOSURE=PULSE(IV_LACK,IV_PERIOD,IV_FINISH)

 IV_lateT = IV_EXPOSURE *IV_RlateR
 IV_late = IV_lateT/CINT

SUMEXPEVENTIV= integ (IV_EXPOSURE,0.0) !NUMBER OF CYCLE GENERATE DURING
SIMULATION

    !SYSTEMIC CONCENTRATION OF TCDD

     ! MODIFICATION ON OCTOBER 6, 2009
 CB= (QF*CFB+QRE*CREB+QLI*CLIB+EXPIV+LYRMLUM+QPLA*CPLAB+IV_late)/(QC+CLURI) !
  CA = CB ! CONCENTRATION (NMOL/ML)


    !URINARY EXCRETION BY KIDNEY
    ! MODIFICATION ON OCTOBER 6, 2009
RAURI = CLURI *CB
  AURI = INTEG(RAURI,0.0)



  !UNIT CONVERSION POST SIMULATION
 CBSNGKGLIADJ=(CB*MW*UNITCORR*(1.0/B_TOTLIP)*(1.0/SERBLO))![NG of TCDD
Serum/Kg OF LIPIP]
   AUCBS_NGKGLIADJ=integ(CBSNGKGLIADJ,0.0)

  PRCT_B = (CB/(MSTT+1E-30))*100.0 !PERCENT OF ORAL DOSE IN BLOOD
  PRCT_BIV = (CB/(IV_RlateR+1E-30))*100.0 ! PERCENT OF IV DOSE IN BLOOD
  CBNGKG= CB*MW*UNITCORR


   !ADIPOSE COMPARTMENT
   !TISSUE BLOOD COMPARTMENT
RAFB= QF*(CA-CFB)-PAF*(CFB-CF/PF)     !(NMOL/H)
 AFB = INTEG(RAFB,0.0)                 !(NMOL)
  CFB = AFB/WFB                        !(NMOL/ML)
   !TISSUE COMPARTMENT
RAF = PAF*(CFB-CF/PF)                 !(NMOL/H)
 AF = INTEG(RAF,0.0)                   !(NMOL)
  CF  = AF/WF                          !(NM/ML)
```

```
    !UNIT CONVERSION POST SIMULATION
  CFTOTAL= (AF + AFB)/(WF + WFB) ! TOTAL CONCENTRATION IN NMOL/ML
  CFTFREE = CFB + CF !TOTAL FREE CONCENTRATION IN FAT  (NM/ML)
  PRCT_F = (CFTOTAL/(MSTT+1E-30))*100.0 ! PERCENT OF ORAL DOSE IN FAT
  PRCT_FIV = (CFTOTAL/(IV_RlateR+1E-30))*100.0 ! PERCENT OF IV DOSE IN FAT
  CFNGKG=CFTOTAL*MW*UNITCORR ! FAT CONCENTRATION NG/KG
    AUCF_NGKGH=integ(CFNGKG,0.0)

    !REST OF THE BODY COMPARTMENT
RAREB= QRE *(CA-CREB)-PARE*(CREB-CRE/PRE)  !(NMOL/H)
 AREB = INTEG(RAREB,0.0)                    !(NMOL)
  CREB = AREB/WREB                          !(NMOL/H)
   !TISSUE COMPARTMENT
RARE = PARE*(CREB - CRE/PRE)               !(NMOL/H)
 ARE = INTEG(RARE,0.0)                     !(NMOL)
  CRE  = ARE/WRE                            !(NMOL/ML)

    !UNIT CONVERSION POST SIMULATION
  CRETOTAL= (ARE + AREB)/(WRE + WREB)           ! TOTAL CONCENTRATION IN
NMOL/ML
  PRCT_RE = (CRETOTAL/(MSTT+1E-30))*100.0 ! PERCENT OF ORAL DOSE IN REST OF
THE BODY
  PRCT_REIV = (CRETOTAL/(IV_RlateR+1E-30))*100.0   !PERCENT OF IV DOSE IN
REST OF THE BODY
  CRENGKG=CRETOTAL*MW*UNITCORR ! REST OF THE BODY CONCENTRATION IN NG/KG


    !LIVER COMPARTMENT
    !TISSUE BLOOD COMPARTMENT
 RALIB = QLI*(CA-CLIB)-PALI*(CLIB-CFLLIR)+LIRMLUM !
  ALIB = INTEG(RALIB,0.0)                        !(NMOL)
   CLIB = ALIB/WLIB                             !(NMOL/ML)
   !TISSUE COMPARTMENT
 RALI = PALI*(CLIB - CFLLIR)-REXCLI             !  (NMOL/HR)
  ALI = INTEG(RALI,0.0)                           !(NMOL)
   CLI  = ALI/WLI                                !(NMOL/ML)

   !FREE TCDD CONCENTRATION IN LIVER COMPARTMENT
PARAMETER (LIVER_1RMN = 1.0E-30)
 CFLLI= IMPLC(CLI-(CFLLIR*PLI+(LIBMAX*CFLLIR/(KDLI+CFLLIR &
        +LIVER_1RMN))+((CYP1A2_1O3*CFLLIR/(KDLI2 + CFLLIR &
        +LIVER_1RMN)*PAS_INDUC)))-CFLLI,CFLLI0)
     CFLLIR=DIM(CFLLI,0.0) ! FREE CONCENTRATION IN LIVER

  CBNDLI= LIBMAX*CFLLIR/(KDLI+CFLLIR+LIVER_1RMN) !BOUND CONCENTRATION

  !VARIABLE ELIMINATION BASED ON THE CYP1A2
 KBILE_LI_T =((CYP1A2_1OUT-CYP1A2_1A2)/CYP1A2_1A2)*Kelv ! INDUCED BILIARY
EXCRETION RATE CONSTANT IN LIVER
  REXCLI = KBILE_LI_T*CFLLIR*WLI ! DOSE-DEPENDENT BILIARY EXCRETION RATE
    EXCLI = INTEG(REXCLI,0.0)

  !UNIT CONVERSION POST SIMULATION
  CLITOTAL= (ALI + ALIB)/(WLI + WLIB) ! TOTAL CONCENTRATION IN NMOL/ML
  PRCT_LI = (CLITOTAL/(MSTT+1E-30))*100
  PRCT_LIIV = (CLITOTAL/(IV_RlateR+1E-30))*100.0
```

```
  Rec_occ= CFLLIR/(KDLI+CFLLIR)
  CLINGKG=CLITOTAL*MW*UNITCORR ! LIVER CONCENTRATION NG/KG
     AUCLI_NGKGH=INTEG(CLINGKG,0.0)
  CBNDLINGKG = CBNDLI*MW*UNITCORR
     AUCBNDLI_NGKGH =INTEG(CBNDLINGKG,0.0)


   !CHEMICAL IN CYP450 (1A2) COMPARTMENT
CYP1A2_1KINP = CYP1A2_1KOUT* CYP1A2_1OUTZ


    ! MODIFICATION ON OCTOBER 6, 2009
CYP1A2_1OUT =INTEG(CYP1A2_1KINP * (1.0 + CYP1A2_1EMAX *(CBNDLI+1.0e-30)**HILL
&
     /(CYP1A2_1EC50**HILL + (CBNDLI+1.0e-30)**HILL)) &
      - CYP1A2_1KOUT*CYP1A2_1OUT, CYP1A2_1OUTZ)

! EQUATIONS INCORPORATING DELAY OF CYP1A2 PRODUCTION (NOT USED IN
SIMULATIONS)

CYP1A2_1RO2 = (CYP1A2_1OUT - CYP1A2_1O2)/ CYP1A2_1TAU
  CYP1A2_1O2 =INTEG(CYP1A2_1RO2, CYP1A2_1A1)

CYP1A2_1RO3 = (CYP1A2_1O2 - CYP1A2_1O3)/ CYP1A2_1TAU
  CYP1A2_1O3 =INTEG(CYP1A2_1RO3, CYP1A2_1A2)

! TRANSFER OF DIOXIN FROM PLACENTA TO FETUS
! FETAL EXPOSURE ONLY DURING EXPOSURE

IF (T.LT.TRANSTIME_ON) THEN
 SWITCH_trans = 0.0
ELSE
 SWITCH_trans = 1.0
END IF

!TRANSFER OF DIOXIN FROM PLACENTA TO FETUS
! MODIFICATION 26 SEPTEMBER 2003

CONSTANT PFETUS= 4.0 !
CONSTANT CLPLA_FET = 0.17 !

RAMPF = (CLPLA_FET*CPLA) *SWITCH_trans
  AMPF=INTEG(RAMPF,0.0)

!TRANSFER OF DIOXIN FROM FETUS TO PLACENTA
RAFPM = (CLPLA_FET*CFETUS_v)*SWITCH_trans !
  AFPM = INTEG(RAFPM,0.0)

! TCDD IN PLACENTA (MOTHER) COMPARTMENT
RAPLAB= QPLA*(CA - CPLAB)-PAPLA*(CPLAB -CFLPLAR)    ! NMOL/H)
 APLAB = INTEG(RAPLAB,0.0)                           ! (NMOL)
 CPLAB = APLAB/(WPLAB+1E-30)                        ! (NMOL/ML)
RAPLA = PAPLA*(CPLAB-CFLPLAR)-RAMPF + RAFPM         ! (NMOL/H)
 APLA = INTEG(RAPLA,0.0)                             ! (NMOL)
 CPLA  = APLA/(WPLA+1e-30)                          ! (NMOL/ML)
```

```
PARAMETER (PARA_ZERO = 1.0E-30)
CFLPLA= IMPLC(CPLA-(CFLPLAR*PPLA +(PLABMAX*CFLPLAR/(KDPLA&
    +CFLPLAR+PARA_ZERO)))-CFLPLA,CFLPLA0)
CFLPLAR=DIM(CFLPLA,0.0)

   !UNIT CONVERSION POST SIMULATION
  CPLATOTAL= (APLA + APLAB)/((WPLA + WPLAB)+1e-30)! TOTAL CONCENTRATION IN
NMOL/ML
  PRCT_PLA = (CPLATOTAL/(MSTT+1E-30))*100
  PRCT_PLAIV = (CPLATOTAL/(IV_RlateR+1E-30))*100


   !FETUS COMPARTMENT
RAFETUS= RAMPF-RAFPM
 AFETUS=INTEG(RAFETUS,0.0)
CFETUS=AFETUS/(WTFE+1E-30)
CFETOTAL= CFETUS
CFETUS_v = CFETUS/PFETUS

  ! UNIT CONVERSION POST SIMULATION
CFETUSNGKG = CFETUS*MW*UNITCORR                    !(NG/KG)
AUC_FENGKGH = INTEG(CFETUSNGKG,0.0)
PRCT_FE = (CFETOTAL/(MSTT+1E-30))*100
PRCT_FEIV = (CFETOTAL/(IV_RlateR+1E-30))*100


! ------------CONTROL MASS BALANCE ----------
BDOSE= IVDOSE +LYMLUM+LIMLUM
BMASSE = EXCLI+AURI+AFB+AF+AREB+ARE+ALIB+ALI+APLA+APLAB+AFETUS
BDIFF = BDOSE-BMASSE

      !BODY BURDEN (NG)
BODY_BURDEN = AFB+AF+AREB+ARE+ALIB+ALI+APLA+APLAB !
BBFETUSNG     = AFETUS*MW*UNITCORR    ! UNIT (NG)
      ! BODY BURDEN IN TERMS OF CONCENTRATION (NG/KG)
 BBNGKG =(((AFB+AF+AREB+ARE+ALIB+ALI+APLA+APLAB)/WT0)*MW*UNITCORR) !
  AUC_BBNGKGH=INTEG(BBNGKG,0.0)


! ------------COMMAND OF THE END OF SIMULATION ----------
TERMT (T.GE. TimeLimit, 'Time limit has been reached.')
END   ! END OF THE DERIVATIVE SECTION
END   ! END OF THE DYNAMIC SECTION
END   ! END OF THE PROGRAM
```

C.2.4.2. *Input Files*

C.2.4.2.1. *Bell et al. (2007).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH
```

```
%output @nciout=1 T BBFETUSNG %AJS turned off 9/21/09

%Bell et al. 2007 (rat species)
%protocol:  daily dietary dose for 12 weeks followed by a two-week mating
time and 21-day gestation period
%DevTCDD4Species.csl
%RAT_GESTATIONAL_ICF_F083109.csl (now 09-11-09)
%dose levels: 0.0024, 0.008, 0.046 ug/kg/d with 0.00003 ug/kg/d background
%dose levels: 2.4, 8, 46 ng/kg/d with 0.03 ng/kg/day background

   %EXPOSURES SCENARIOS
 MAXT             = 0.01
 CINT             = 0.1  %
 EXP_TIME_ON      = 0            % delay before begin exposure (HOUR)
 EXP_TIME_OFF     = 2856         % TIME EXPOSURE STOP (HOUR) 12 weeks
exposure + 2 weeks for mating + 21 days gestation with exposure
 DAY_CYCLE        = 24
 BCK_TIME_ON      = 0.           % DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
 BCK_TIME_OFF     = 2856.           % TIME OF BACKGROUND EXPOSURE STOP (HOUR)
 IV_LACK          = 505.
 IV_PERIOD        = 505.
 TIMELIMIT        = 2856         % SIMULATION LIMIT TIME (HOUR)
 BW_T0            = 85
 MATTING          = 2352         % BEGINNING MATING (HOUR)
 TRANSTIME_ON     = 2496         % SHOULD BE MATING TIME + 6 DAYS(144 HOURS)
 N_FETUS          = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)
   MSTOT           = 0.00243        % ORAL EXPOSURE DOSE (UG/KG)

   %MSTOT           = 0.008         % ORAL EXPOSURE DOSE (UG/KG)

   %MSTOT = 0.0461          % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.4.2.2. *Haavisto et al. (2006).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH

%Haavisto et al. 2006
%protocol:  single dose on GD 13
%dose levels: 0.04, 0.2, and 1.0 ug/kg on GD 13
%dose levels: 40, 200, and 1,000 ng/kg on GD 13

 MAXT = 0.001
 CINT = 0.1

   %EXPOSURES SCENARIOS
 EXP_TIME_ON         = 312           % TIME AT WHICH EXPOSURE BEGINS (HOUR)
 EXP_TIME_OFF        = 335           % TIME AT WHICH EXPOSURE ENDS (HOUR)
 DAY_CYCLE           = 24
```

```
 BCK_TIME_ON         = 0.             % TIME AT WHICH BACKGROUND EXPOSURE
BEGINS (HOUR)
 BCK_TIME_OFF        = 0.             % TIME AT WHICH BACKGROUND EXPOSURE
ENDS (HOUR)
 IV_LACK             = 505
 IV_PERIOD           = 505
 TIMELIMIT           = 336            % SIMULATION LIMIT TIME (HOUR)
 BW_T0               = 190
 MATTING             = 0.             % BEGINNING MATTING (HOUR)
 TRANSTIME_ON        = 144.           % SHOULD BE MATTING TIME + 6 DAYS(144
HOURS)
 N_FETUS             = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)
  %MSTOT            = 0.04           % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT            = 0.2            % ORAL EXPOSURE DOSE (UG/KG)
  MSTOT            = 1.0            % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.4.2.3. *Hojo et al. (2002).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH
%Hojo et al. 2002
%protocol:  single oral dose at GD8
%DevTCDD4Species.csl
%RAT_GESTATIONAL_ICF_F083109.csl (now 09-11-09)
%RAT_GESTATIONAL_ICF_F092009.csl (now 09-21-09
%dose levels: 0.02 0.06, 0.18 ug/kg  at GD8
%dose levels: 20, 60, 180  ng/kg at GD8
% author provided the body weight for each group at the beginning og
gestation (g)
    %20 ng/kg BW = 271g
    %60 ng/kg BW = 275g
    %180 ng/kg BW = 262g

%EXPOSURES SCENARIOS
 MAXT= 0.001
 CINT =0.1                          %
 EXP_TIME_ON     = 192           % delay before begin exposure (HOUR)
 EXP_TIME_OFF    = 216           % TIME EXPOSURE STOP (HOUR)
 DAY_CYCLE       = 24
 BCK_TIME_ON     = 0.            % DELAY BEFORE BACGROUND EXPOSURE (HOUR)
 BCK_TIME_OFF    = 0.            % TIME OF BACKGROUND EXPOSURE STOP (HOUR)
 IV_LACK         = 505
 IV_PERIOD       = 505
 TIMELIMIT       = 216            % SIMULATION LIMIT TIME (HOUR)
% BW_T0           = 190
 MATTING         = 0.             % BEGINNING MATTING (HOUR)
 TRANSTIME_ON    = 144.           % SHOULD BE MATTING TIME + 6 DAYS(144
HOURS)
 N_FETUS         = 10
```

```
%EXPOSURE DOSE SCENARIOS (UG/KG)

   %MSTOT          = 0.02     % ORAL EXPOSURE DOSE (UG/KG)
   %BW_T0          = 275      % 20 ng/kg BW = 271g

   %MSTOT         = 0.06     % ORAL EXPOSURE DOSE (UG/KG)
   %BW_T0         = 262      %60 ng/kg BW = 275g

   MSTOT         = 0.18     % ORAL EXPOSURE DOSE (UG/KG)
   BW_T0         = 278      %180 ng/kg BW = 262g
```

C.2.4.2.4. *Ikeda et al. (2005).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH

%Ikeda et al. 2005 (rat species)
%protocol:  loading dose of 400 ng/kg followed by weekly maintenance doses of
80 ng/kg for 6 weeks,
%dose levels: 0.4 ug/kg/day followed by weekly 0.08 ug/kg/day
%dose levels: 400 ng/kg/day followed by weekly 80 ng/kg/day

   %EXPOSURES SCENARIOS
 MAXT             =.1
 CINT             = 0.1  %
 EXP_TIME_ON      = 0            % TIME AT WHICH EXPOSURE BEGINS (HOUR)
 EXP_TIME_OFF     = 1008         % TIME AT WHICH EXPOSURE ENDS (HOUR); PRE-
MATING (2 WEEKS) + MATING (1 WEEK) + GESTATION (3 WEEKS)
 DAY_CYCLE        = 168          % WEEKLY CYCLE
 BCK_TIME_ON      = 0.           % TIME AT WHICH BACKGROUND EXPOSURE BEGINS
(HOUR)
 BCK_TIME_OFF     = 167.         % TIME AT WHICH BACKGROUND EXPOSURE ENDS
(HOUR)
 IV_LACK          = 505.
 IV_PERIOD        = 505.
 TIMELIMIT        = 1008         % SIMULATION TIME LIMIT (HOUR)
 BW_T0            = 250
 MATTING          = 504         % BEGINNING OF MATING (HOUR)
 TRANSTIME_ON     = 648         % SHOULD BE MATING TIME + 6 DAYS (144 HOURS)
 N_FETUS          = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)
   MSTOT           = 0.08        % ORAL EXPOSURE DOSE IN UG/KG
   MSTOTBCKGR      = 0.32       % BACKGROUND EXPOSURE IN UG/KG
```

C.2.4.2.5. *Kattainen et al. (2001).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH
```

```
%Kattainen et al. 2001
%protocol:  single gavage at GD15
%DevTCDD4Species.csl
%RAT_GESTATIONAL_ICF_F083109.csl (now 09-11-09)
%dose levels: 0.03 0.1, 0.3, 1 ug/kg  at GD15
%dose levels: 30, 100 300, 1000 ng/kg at GD15

 MAXT=0.001
 CINT =0.1

   %EXPOSURES SCENARIOS
 EXP_TIME_ON         = 336            % delay before begin exposure (HOUR)
 EXP_TIME_OFF        = 360            % TIME EXPOSURE STOP (HOUR)
 DAY_CYCLE           = 24
 BCK_TIME_ON         = 0.             % DELAY BEFORE BACKGROUND EXPOSURE
(HOUR)
 BCK_TIME_OFF        = 0.             % TIME OF BACKGROUND EXPOSURE STOP
(HOUR)
 IV_LACK             = 505
 IV_PERIOD           = 505
 TIMELIMIT           = 360            % SIMULATION LIMIT TIME (HOUR)
 BW_T0               = 190
 MATTING             = 0.             % BEGINNING MATTING (HOUR)
 TRANSTIME_ON        = 144.           % SHOULD BE MATTING TIME + 6 DAYS(144
HOURS)
 N_FETUS             = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)
 %MSTOT            = 0.03           % ORAL EXPOSURE DOSE (UG/KG)
 %MSTOT            = 0.1            % ORAL EXPOSURE DOSE (UG/KG)
 %MSTOT            = 0.3            % ORAL EXPOSURE DOSE (UG/KG)
 MSTOT             = 1              % ORAL EXPOSURE DOSE (UG/KG
```

C.2.4.2.6. *Markowski et al. (2001).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH

%Markowski et al. 2001
%protocol:  single gavage at GD18
%DevTCDD4Species.csl
%RAT_GESTATIONAL_ICF_F083109.csl (now 09-11-09)
%dose levels: 0.02 0.06, 0.18 ug/kg at GD18
%dose levels: 20, 60, 180 ng/kg at GD18

%EXPOSURES SCENARIOS
 MAXT=0.0001
 CINT =0.1                          %
 EXP_TIME_ON     = 408           % delay before begin exposure (HOUR)
 EXP_TIME_OFF    = 432           % TIME EXPOSURE STOP (HOUR)
 DAY_CYCLE       = 24
 BCK_TIME_ON     = 0.            % DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
 BCK_TIME_OFF    = 0.            % TIME OF BACKGROUND EXPOSURE STOP (HOUR)
```

```
 IV_LACK         = 505
 IV_PERIOD       = 505
 TIMELIMIT       = 432            % SIMULATION LIMIT TIME (HOUR)
 BW_T0           = 190
 MATTING         = 0.             % BEGINNING MATING (HOUR)
 TRANSTIME_ON    = 144.           % SHOULD BE MATING TIME + 6 DAYS(144 HOURS)
 N_FETUS         = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)
   %MSTOT         = 0.02  % ORAL EXPOSURE DOSE (UG/KG)
   %MSTOT        = 0.06   % ORAL EXPOSURE DOSE (UG/KG)
   MSTOT        = 0.18   % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.4.2.7. *Miettinen et al. (2006).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH

%Miettinen et al. 2006
%protocol:  single oral dose at GD15
%DevTCDD4Species.csl
%RAT_GESTATIONAL_ICF_F083109.csl (now 09-11-09)
%dose levels: 0.03 0.1, 0.3, 1 ug/kg at GD15
%dose levels: 30, 100, 300, 1000 ng/kg at GD15

 MAXT=0.01
 CINT =0.1                     %

   %EXPOSURES SCENARIOS
 EXP_TIME_ON   = 336          % delay before begin exposure (HOUR)
 EXP_TIME_OFF  = 360          % TIME EXPOSURE STOP (HOUR)
 DAY_CYCLE     = 24
 BCK_TIME_ON   = 0.           % DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
 BCK_TIME_OFF  = 0.           % TIME OF BACKGROUND EXPOSURE STOP (HOUR)
 IV_LACK       = 505
 IV_PERIOD     = 505
 TIMELIMIT     = 360          % SIMULATION LIMIT TIME (HOUR)
 BW_T0         = 180
 MATTING       = 0.           % BEGINNING MATING (HOUR)
 TRANSTIME_ON  = 144.         % SHOULD BE MATING TIME + 6 DAYS(144 HOURS)
 N_FETUS       = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)
   %MSTOT        = 0.03        % ORAL EXPOSURE DOSE (UG/KG)
   %MSTOT      = 0.1         % ORAL EXPOSURE DOSE (UG/KG)
   %MSTOT      = 0.3         % ORAL EXPOSURE DOSE (UG/KG)
   MSTOT      = 1           % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.4.2.8. *Nohara et al. (2000).*

```
%clear variable
output @clear
```

```
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH

%Nohara et al. 2000
%protocol:  single gavage at GD15
%DevTCDD4Species.csl
%RAT_GESTATIONAL_ICF_F083109.csl (now 09-11-09)
%dose levels: 0.0125, 0.050, 0.2, or 0.8 ug TCDD:kg body weight by gavage on
GD15.
%dose levels: 12.5, 50, 200, or 800 ng TCDD:kg body weight by gavage on GD15.

 MAXT=0.01
 CINT =0.1                      %

   %EXPOSURES SCENARIOS
 EXP_TIME_ON   = 336          % delay before begin exposure (HOUR)
 EXP_TIME_OFF  = 360          % TIME EXPOSURE STOP (HOUR)
 DAY_CYCLE     = 24
 BCK_TIME_ON   = 0.           % DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
 BCK_TIME_OFF  = 0.           % TIME OF BACKGROUND EXPOSURE STOP (HOUR)
 IV_LACK       = 505
 IV_PERIOD     = 505
 TIMELIMIT     = 360           % SIMULATION LIMIT TIME (HOUR)
 BW_T0         = 180
 MATTING       = 0.             % BEGINNING MATTING (HOUR)
 TRANSTIME_ON  = 144.     % SHOULD BE MATTING TIME + 6 DAYS(144 HOURS)
 N_FETUS       = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)
  %MSTOT         = 0.0125     % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.050   % ORAL EXPOSURE DOSE (UG/KG)
  %MSTOT         = 0.2     % ORAL EXPOSURE DOSE (UG/KG)
  MSTOT         = 0.8     % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.4.2.9. *Ohsako et al. (2001).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH

%Ohsako et al. 2001
%protocol:  single oral dose at GD15
%DevTCDD4Species.csl
%RAT_GESTATIONAL_ICF_F083109.csl (now 09-11-09)
%RAT_GESTATIONAL_ICF_F092009.csl (now 09-21-09)
%dose levels: 0.0125, 0.05, 0.2, 0.8 ug/kg at GD15
%dose levels: 12.5, 50, 200, 800 ng/kg at GD15

%EXPOSURES SCENARIOS
 MAXT=0.01
 CINT =0.1                        %
 EXP_TIME_ON     = 360          % delay before begin exposure (HOUR)
 EXP_TIME_OFF    = 384          % TIME EXPOSURE STOP (HOUR)
```

```
 DAY_CYCLE       = 24
 BCK_TIME_ON     = 0.            % DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
 BCK_TIME_OFF    = 0.            % TIME OF BACKGROUND EXPOSURE STOP (HOUR)
 IV_LACK         = 505
 IV_PERIOD       = 505
 TIMELIMIT       = 384            % SIMULATION LIMIT TIME (HOUR)
 BW_T0           = 200
 MATTING         = 0.             % BEGINNING MATTING (HOUR)
 TRANSTIME_ON    = 144.           % SHOULD BE MATTING TIME + 6 DAYS(144
HOURS)
 N_FETUS         = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)

 %MSTOT        = 0.0125    % ORAL EXPOSURE DOSE (UG/KG)
 %MSTOT        = 0.05      % ORAL EXPOSURE DOSE (UG/KG)
 %MSTOT        = 0.20      % ORAL EXPOSURE DOSE (UG/KG)
 MSTOT          = 0.80      % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.4.2.10. *Schantz et al. (1996) and Amin et al. (2000).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH

%Amin et al. 2000 (rat species) and Schantz et al. 1996
%protocol:  daily doses on GDs 10 to 16
%DevTCDD4Species.csl
%RAT_GESTATIONAL_ICF_F083109.csl (now 09-11-09)
%dose levels: 25 and 100 ng/kg/day
%dose levels: 0.025 and 0.100 ug/kg/day

   %EXPOSURES SCENARIOS
 MAXT             = 0.001
 CINT             = 0.1  %
 EXP_TIME_ON      = 240.           % TIME AT WHICH EXPOSURE BEGINS (HOUR)
 EXP_TIME_OFF     = 384.         % TIME AT WHICH EXPOSURE ENDS (HOUR) GD 10
to 16
 DAY_CYCLE        = 24          % weekly cycle
 BCK_TIME_ON      = 1000.          % DELAY BEFORE BACKGROUND EXPOSURE (HOUR)
 BCK_TIME_OFF     = 1000.         % TIME OF BACKGROUND EXPOSURE STOP (HOUR)
 IV_LACK          = 505.
 IV_PERIOD        = 505.
 TIMELIMIT        = 384.         % SIMULATION LIMIT TIME (HOUR)
 BW_T0            = 250.
 MATTING          = 0         % BEGINNING MATTING (HOUR)
 TRANSTIME_ON     = 144.         % SHOULD BE MATTING TIME + 6 DAYS(144 HOURS)
 N_FETUS          = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)
   %MSTOT           = .025        % ORAL EXPOSURE DOSE (UG/KG)
   MSTOT           = .100
   MSTOTBCKGR      = 0    % Background Exposure (UG/KG)
```

C.2.4.2.11. *Seo et al. (1995).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH

%Seo et al. 1995
%protocol:  daily doses on GDs 10-16
%DevTCDD4Species.csl
%RAT_GESTATIONAL_ICF_F083109.csl (now 09-11-09)
%dose levels: 0.025 and 0.1 ug/kg on GDs 10-16
%dose levels: 25 and 100 ng/kg on GDs 10-16

 MAXT = 0.01
 CINT = 0.1

   %EXPOSURES SCENARIOS
 EXP_TIME_ON         = 240            % TIME AT WHICH EXPOSURE BEGINS (HOUR)
 EXP_TIME_OFF        = 384            % TIME AT WHICH EXPOSURE ENDS (HOUR)
 DAY_CYCLE           = 24
 BCK_TIME_ON         = 0.             % TIME AT WHICH BACKGROUND EXPOSURE
BEGINS (HOUR)
 BCK_TIME_OFF        = 0.             % TIME AT WHICH BACKGROUND EXPOSURE
ENDS (HOUR)
 IV_LACK             = 505
 IV_PERIOD           = 505
 TIMELIMIT           = 384            % SIMULATION LIMIT TIME (HOUR)
 BW_T0               = 190
 MATTING             = 0.             % BEGINNING MATTING (HOUR)
 TRANSTIME_ON        = 144.           % SHOULD BE MATTING TIME + 6 DAYS(144
HOURS)
 N_FETUS             = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)
 %MSTOT            = 0.025            % ORAL EXPOSURE DOSE (UG/KG)
 MSTOT            = 0.1             % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.5. Mouse Standard Model

C.2.5.1. *Model Code*

PROGRAM: 'Three Compartment PBPK Model for TCDD in Mice: Standard Model (Non-Gestation)'

```
!Mice_Dioxin_3C_June09_1_icf_afterKKfix_v3_mousenongest.csl
!MICE_NON_GESTAT_ICF_F083109.csl
!MICE_NON_GESTAT_ICF_F093009.csl
!MICE_NON_GESTAT_ICF_F100609.csl
!*************************************************

INITIAL  ! INITIALIZATION OF PARAMETERS

    !SIMULATION PARAMETERS ====
```

```
CONSTANT PARA_ZERO       =    1D-30
CONSTANT EXP_TIME_ON     =     0.0        ! TIME AT WHICH EXPOSURE BEGINS
(HOURS)
CONSTANT EXP_TIME_OFF    =    2832        ! TIME AT WHICH EXPOSURE ENDS
(HOURS)
CONSTANT DAY_CYCLE       =     24         ! NUMBER OF HOURS BETWEEN DOSES
(HOURS)
CONSTANT BCK_TIME_ON     =      0.0       ! TIME AT WHICH BACKGROUND EXPOSURE
BEGINS (HOURS)
CONSTANT BCK_TIME_OFF    =      0.0       ! TIME AT WHICH BACKGROUND EXPOSURE
ENDS (HOURS)

CONSTANT MW=322 ! MOLECULAR WEIGHT (NG/NMOL)
CONSTANT SERBLO = 0.55
CONSTANT UNITCORR = 1000

    !CONSTANT EXPOSURE CONTROL ===========
    !ACUTE, SUBCHRONIC, CHRONIC EXPOSURE =====
    !OR BACKGROUND EXPOSURE (IN THIS CASE 3 TIMES A DAY)===
CONSTANT MSTOTBCKGR     =       0.0       !ORAL BACKGROUND EXPOSURE DOSE
(UG/KG)
CONSTANT MSTOT          =       0.15     !ORAL EXPOSURE DOSE (UG/KG)
CONSTANT MSTOTsc        =       0.0       ! SUBCUTANEOUS EXPOSURE DOSE
(UG/KG)

    !ORAL ABSORPTION
  MSTOT_NM              =   MSTOT/MW    !AMOUNT IN NMOL/G

    ! INTRAVENOUS ABSORPTION
CONSTANT  DOSEIV   = 0.0                  !INJECTED DOSE (UG/KG)
  DOSEIV_NM = DOSEIV/MW   ! CONVERTS THE INJECTED DOSE TO NMOL/G

  !INITIAL GUESS OF THE FREE CONCENTRATION IN THE LIGAND (COMPARTMENT
INDICATED BELOW)====
CONSTANT CFLLI0          =       0.0       !LIVER (NMOL/ML)

  !BINDING CAPACITY (AhR) FOR NON LINEAR BINDING (COMPARTMENT INDICATED
BELOW) (NMOL/ML)
CONSTANT LIBMAX          =     3.5e-4     ! LIVER  (NMOL/ML), WANG ET AL.
1997

! PROTEIN AFFINITY CONSTANTS (1A2 OR AhR, COMPARTMENT INDICATED BELOW)
(NMOL/ML)===
CONSTANT KDLI            =     1.0e-4     !LIVER (AhR)(NMOL/ML), WANG ET AL.
1997
CONSTANT KDLI2           =     2.0e-2     !LIVER (1A2)(NMOL/ML), EMOND ET AL.
2004

!===EXCRETION AND ABSORPTION CONSTANT (OPTIMIZED)
CONSTANT KST             =    0.3   ! GASTRIC RATE CONSTANT (HR-1),
CONSTANT KABS            =    0.48  !INTESTINAL ABSORPTION CONSTANT (HR-1) ),
WANG ET AL. 1997

! ELIMINATION CONSTANTS
CONSTANT CLURI           =      0.09 ! URINARY CLEARANCE (ML/HR)

```

```
! ==test elimination variable
constant kelv            =     0.4        ! INTERSPECIES VARIABLE ELIMINATION
CONSTANT (1/HOUR)

! CONSTANT TO DIVIDE THE ABSORPTION INTO LYMPHATIC AND PORTAL FRACTIONS
CONSTANT A               =      0.7       ! LYMPHATIC FRACTION, WANG ET AL.
1997

!PARTITION COEFFICIENTS OPTIMIZED
CONSTANT PF              =   400          ! ADIPOSE TISSUE/BLOOD
CONSTANT PRE             =    3           ! REST OF THE BODY/BLOOD, WANG ET
AL. 2000
CONSTANT PLI             =    6           ! LIVER/BLOOD, WANG ET AL. 1997

!===PARAMETER FOR INDUCTION OF CYP 1A2
CONSTANT PAS_INDUC=     1.0      ! INCLUDE INDUCTION? (1 = YES, 0 = NO)
CONSTANT CYP1A2_1OUTZ = 1.6   ! DEGRADATION CONCENTRATION CONSTANT OF 1A2
(NMOL/ML)
CONSTANT CYP1A2_1A1 =   1.5   ! BASAL CONCENTRATION OF 1A1 (NMOL/ML)
CONSTANT CYP1A2_1EC50 = 0.13   ! DISSOCIATION CONSTANT TCDD-CYP1A2 (NMOL/ML)
CONSTANT CYP1A2_1A2 =   1.5   ! BASAL CONCENTRATION OF 1A2 (NMOL/ML)
CONSTANT CYP1A2_1KOUT = 0.1   ! FIRST ORDER RATE OF DEGRADATION (H-1)
CONSTANT CYP1A2_1TAU  = 1.5  ! HOLDING TIME (H)
CONSTANT CYP1A2_1EMAX = 600   ! MAXIMUM INDUCTION OVER BASAL EFFECT
(UNITLESS)
CONSTANT HILL         = 0.6      !HILL CONSTANT; COOPERATIVELY LIGAND BINDING
EFFECT CONSTANT (UNITLESS)
    !DIFFUSIONAL PERMEABILITY FRACTION
CONSTANT PAFF     = 0.12      ! ADIPOSE (UNITLESS), WANG ET AL. 2000
CONSTANT PAREF    = 0.03      ! REST OF THE BODY (UNITLESS)
CONSTANT PALIF    = 0.35      ! LIVER (UNITLESS)

    !COMPARTMENT TISSUE BLOOD VOLUME  =========
CONSTANT WLI0     =  0.0549   ! LIVER, ILSI 1994
CONSTANT WF0      =  0.069    ! ADIPOSE

    !TISSUE BLOOD FLOW EXPRESSED AS A FRACTION OF CARDIAC OUTPUT
CONSTANT QFF  = 0.070         ! ADIPOSE TISSUE BLOOD FLOW FRACTION
(UNITLESS), LEUNG ET AL. 1990
CONSTANT QLIF = 0.161         ! LIVER (UNITLESS)  ILSI ET AL. 1994

    !COMPARTMENT TISSUE BLOOD EXPRESSED AS A FRACTION OF THE TOTAL
COMPARTMENT VOLUME
CONSTANT WFB0 =    0.050      ! ADIPOSE TISSUE, WANG ET AL. 1997
CONSTANT WREB0 =   0.030      ! REST OF THE BODY, WANG ET AL. 1997
CONSTANT WLIB0 =   0.266      ! LIVER, WANG ET AL. 1997

    ! EXPOSURE SCENARIO FOR UNIQUE OR REPETITIVE WEEKLY OR MONTHLY EXPOSURE
    ! NUMBER OF EXPOSURES PER WEEK
CONSTANT WEEK_LACK   = 0.0     ! DELAY BEFORE EXPOSURE ENDS (WEEK)
CONSTANT WEEK_PERIOD = 168    ! NUMBER OF HOURS IN THE WEEK (HOURS)
CONSTANT WEEK_FINISH = 120    ! TIME EXPOSURE ENDS (HOURS)

    ! NUMBER OF EXPOSURES PER MONTH
CONSTANT MONTH_LACK   = 0.0    ! DELAY BEFORE EXPOSURE (MONTH)
```

```
    !SET FOR BACKGROUND EXPOSURE===========
    !CONSTANT FOR BACKGROUND EXPOSURE===========
CONSTANT Day_LACK_BG = 0.0     ! DELAY BEFORE EXPOSURE BEGINS (HOURS)
CONSTANT Day_PERIOD_BG = 24    ! LENGTH OF EXPOSURE (HOURS)

    ! NUMBER OF EXPOSURES PER WEEK
CONSTANT WEEK_LACK_BG   = 0.0 ! DELAY BEFORE BACKGROUD EXPOSURE (WEEK)
CONSTANT WEEK_PERIOD_BG = 168 !NUMBER OF HOURS IN THE WEEK (HOURS)
CONSTANT WEEK_FINISH_BG = 168 ! TIME EXPOSURE ENDS (HOURS)

    !GROWTH CONSTANT FOR RAT AND MOUSE
    !CONSTANT FOR MOTHER BODY WEIGHT GROWTH ======
CONSTANT BW_T0 = 20          !CHANGED FOR SIMULATION

    !CONSTANT USED IN CARDIAC OUTPUT EQUATION, HADDAD 2001
CONSTANT QCCAR =275         !CONSTANT (ML/MIN/KG)

    ! COMPARTMENT LIPID EXPRESSED AS THE FRACTION OF TOTAL LIPID
CONSTANT F_TOTLIP =  0.855    !ADIPOSE TISSUE (UNITLESS)
CONSTANT B_TOTLIP =  0.0033   !BLOOD (UNITLESS)
CONSTANT RE_TOTLIP = 0.019    !REST OF THE BODY (UNITLESS)
CONSTANT LI_TOTLIP = 0.06     !LIVER (UNITLESS)

END  ! END OF THE INITIAL SECTION

DYNAMIC ! DYNAMIC SIMULATION SECTION

ALGORITHM  IALG          =           2        !GEAR METHOD
CINTERVAL  CINT          =         1.0        !COMMUNICATION INTERVAL
MAXTERVAL  MAXT          =       1.0e+10      !MAXIMUM CALCULATION INTERVAL
MINTERVAL  MINT          =       1.0E-10      !MINIMUM CALCULATION INTERVAL
VARIABLE   T             =         0.0        !HOUR
CONSTANT   TIMELIMIT     =         2904.0       !SIMULATION TIME LIMIT
(HOURS)
 CINTXY  = CINT
 PFUNC   = CINT

    !TIME CONVERSION
  DAY         = T/24.0          ! TIME IN DAYS
  WEEK        = T/168.0         ! TIME IN WEEKS
  MONTH       = T/730.0         ! TIME IN MONTHS
  YEAR        = T/8760.0        ! TIME IN YEARS

    !NMAX =MAX(T,CTFNGKG)
nmax =max(T,CFNGKG)

DERIVATIVE ! PORTION OF CODE THAT SOLVES DIFFERENTIAL EQUATIONS

    !CHRONIC OR SUBCHRONIC EXPOSURE SCENARIO =======
    !NUMBER OF EXPOSURES PER DAY
 DAY_LACK     = EXP_TIME_ON       ! DELAY BEFORE EXPOSURE BEGINS (HOURS)
 DAY_PERIOD   = DAY_CYCLE         ! EXPOSURE PERIOD (HOURS)
 DAY_FINISH   = CINTXY            ! LENGTH OF EXPOSURE (HOURS)
 MONTH_PERIOD = TIMELIMIT         ! EXPOSURE PERIOD (MONTHS)
 MONTH_FINISH = EXP_TIME_OFF      ! LENGTH OF EXPOSURE (MONTHS)

```

```
      !NUMBER OF EXPOSURES PER DAY AND MONTH
 DAY_FINISH_BG   = CINTXY
 MONTH_LACK_BG   = BCK_TIME_ON    ! DELAY BEFORE BACKGROUD EXPOSURE BEGINS
(MONTHS)
 MONTH_PERIOD_BG = TIMELIMIT      ! BACKGROUND EXPOSURE PERIOD (MONTHS)
 MONTH_FINISH_BG = BCK_TIME_OFF   ! LENGTH OF BACKGROUND EXPOSURE (MONTHS)

      ! FRACTION OF DIOXIN ABSORBED IN THE PORTAL FRACTION OF THE LIVER
 B = 1.0-A


      !GROWTH UP EQUATION (G)

PARAMETER (BW_RMN = 1.0E-30)
WT0= (BW_T0 *(1.0+(0.41*T)/(1402.5+T+BW_RMN)))

      ! VARIABILITY OF REST OF THE BODY DEPENDS ON OTHER ORGANS
      !REST OF THE BODY FRACTION; UPDATED FOR EPA ASSESSMENT
 WRE0 = (0.91 - (WLIB0*WLI0 + WFB0*WF0 + WLI0 + WF0))/(1+WREB0)

      ! REST OF THE BODY BLOOD FLOW FRACTION
 QREF = 1.0-(QFF+QLIF)            !REST OF BODY BLOOD FLOW (ML/HR)
      !SUMMATION OF BLOOD FLOW FRACTION (SHOULD BE EQUAL TO 1)
 QTTQF = QFF+QREF+QLIF          ! SUM MUST EQUAL 1

      !COMPARTMENT VOLUME (G)
 WF  =  WF0  * WT0              ! ADIPOSE
 WRE =  WRE0 * WT0              ! REST OF THE BODY
 WLI =  WLI0 * WT0              ! LIVER

      !COMPARTMENT TISSUE BLOOD (G)
 WFB  =  WFB0  * WF             ! ADIPOSE
 WREB =  WREB0 * WRE            ! REST OF THE BODY
 WLIB =  WLIB0 * WLI            ! LIVER

      !CARDIAC OUTPUT FOR THE GIVEN BODY WEIGHT
  QC= QCCAR*60*(WT0/1000.0)**0.75

QF  = QFF*QC         ! ADIPOSE TISSUE BLOOD FLOW RATE (ML/HR)
QLI = QLIF*QC        ! LIVER TISSUE BLOOD FLOW RATE (ML/HR)
QRE = QREF*QC        ! REST OF THE BODY BLOOD FLOW RATE (ML/HR)

QTTQ = QF+QRE+QLI   !TOTAL FLOW RATE (ML/HR)

      !PERMEABILITY ORGAN FLOW (ML/HR) =======
PAF  = PAFF*QF       ! ADIPOSE TISSUE
PARE = PAREF*QRE     ! REST OF THE BODY
PALI = PALIF*QLI     ! LIVER TISSUE

      !ABSORPTION SECTION
      !ORAL
      !BACKGROUND EXPOSURE
      !EXPOSURE FOR STEADY STATE CONSIDERATION
      !REPETITIVE EXPOSURE SCENARIO

MSTOT_NMBCKGR = MSTOTBCKGR/322 !AMOUNT IN NMOL/G
```

```
MSTTBCKGR =MSTOT_NMBCKGR *WT0

     !REPETITIVE ORAL BACKGROUND EXPOSURE SCENARIOS
DAY_EXPOSURE_BG   = PULSE(DAY_LACK_BG,DAY_PERIOD_BG,DAY_FINISH_BG)
WEEK_EXPOSURE_BG  = PULSE(WEEK_LACK_BG,WEEK_PERIOD_BG,WEEK_FINISH_BG)
MONTH_EXPOSURE_BG = PULSE(MONTH_LACK_BG,MONTH_PERIOD_BG,MONTH_FINISH_BG)

MSTTCH_BG = (DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG)*MSTTBCKGR
MSTTFR_BG = MSTTBCKGR/CINT

totalBG= integ (MSTTCH_BG,0.0)
CYCLE_BG =DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG


     !CONDITIONAL ORAL EXPOSURE (BACKGROUND EXPOSURE)
IF (MSTTCH_BG.EQ.MSTTBCKGR) THEN
    ABSMSTT_GB= MSTTFR_BG
ELSE
    ABSMSTT_GB = 0.0
END IF

     !EXPOSURE + !REPETITIVE EXPOSURE SCENARIO
IV= DOSEIV_NM * WT0  !AMOUNT IN NMOL
MSTT= MSTOT_NM * WT0 !AMOUNT IN NMOL

DAY_EXPOSURE   = PULSE(DAY_LACK,DAY_PERIOD,DAY_FINISH)
WEEK_EXPOSURE  = PULSE(WEEK_LACK,WEEK_PERIOD,WEEK_FINISH)
MONTH_EXPOSURE = PULSE(MONTH_LACK,MONTH_PERIOD,MONTH_FINISH)

MSTTCH = (DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE)*MSTT
CYCLE = DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE

SUMEXPEVENT= integ (CYCLE,0.0)*cint !NUMBER OF CYCLE GENERATE DURING
SIMULATION

MSTTFR = MSTT/CINT

    ! CONDITIONAL ORAL EXPOSURE
IF (MSTTCH.EQ.MSTT) THEN
   ABSMSTT= MSTTFR
ELSE
   ABSMSTT = 0.0
END IF

 CYCLETOT=INTEG(CYCLE,0.0)


    !MASS CHANGE IN THE LUMEN
RMSTT= -(KST+KABS)*MST+ABSMSTT +ABSMSTT_GB ! RATE OF CHANGE (NMOL/H)
 MST = INTEG(RMSTT,0.0)  !AMOUNT OF STAY IN DUODENUM (NMOL)

    !ABSORPTION IN LYMPH CIRCULATION
LYRMLUM = KABS*MST*A
 LYMLUM = INTEG(LYRMLUM,0.0)

    !ABSORPTION IN PORTAL CIRCULATION
```

```
LIRMLUM = KABS*MST*B
  LIMLUM = INTEG(LIRMLUM,0.0)

    !PERCENT OF DOSE REMAINING IN THE GI TRACT
PRCT_remain_GIT  = (MST/(MSTT+1E-30))*100

RFECES = KST*MST + REXCLI
  FECES = INTEG(RFECES,0.0)
prctFECES = (FECES/(BDOSE_TOTAL+1E-30))*100


    !ABSORPTION OF DIOXIN BY IV ROUTE---------
 IVR= IV/PFUNC ! RATE FOR IV INFUSION IN BLOOD
 EXPIV= IVR * (1.0-STEP(PFUNC))
 IVDOSE = integ(EXPIV,0.0)

    !SYSTEMIC BLOOD CONCENTRATION (NMOL/ML)
    ! MODIFICATION ON OCTOBER 6, 2009
CB=(QF*CFB+QRE*CREB+QLI*CLIB+EXPIV+LYRMLUM)/(QC+CLURI) !
 CA = CB

    !URINARY EXCRETION BY KIDNEY
    ! MODIFICATION ON OCTOBER 6, 2009
RAURI = CLURI *CB
  AURI = INTEG(RAURI,0.0)

prctAURI = (AURI/(BDOSE_TOTAL+1E-30))*100


    !UNIT CONVERSION POST SIMULATION
PRCT_B = (CB/(MSTT+1E-30))*100  ! PERCENT OF DOSE/G TISSUE
CBNGKG=CB*MW*UNITCORR
CBSNGKGLIADJ= (CB*MW*UNITCORR*(1.0/B_TOTLIP)*(1.0/SERBLO))![NG of TCDD
Serum/Kg OF LIPIP]
CBPMOL_KG= CB*UNITCORR*UNITCORR       !CONCENTRATION IN PMOL/KG
CBNGG = CB*MW
    !ADIPOSE TISSUE COMPARTMENT
    !TISSUE BLOOD SUBCOMPARTMENT
RAFB = QF*(CA-CFB)-PAF*(CFB-CF/PF)       !(NMOL/HR)
 AFB = INTEG(RAFB,0.0)                    !(NMOL)
 CFB = AFB/WFB                           !(NMOL/ML)
    !TISSUE SUBCOMPARTMENT
RAF  = PAF*(CFB-CF/PF)                   !(NMOL/HR)
 AF  = INTEG(RAF,0.0)                     !(NMOL)
 CF  = AF/WF                             !(NMOL/ML)

    !POST SIMULATION UNIT CONVERSION
CFTOTAL    = (AF + AFB)/(WF + WFB) ! TOTAL CONCENTRATION IN FAT(NM/ML)
PRCT_F  = (CFTOTAL/(MSTT+1E-30))*100 ! PERCENT OF DOSE IN FAT
CFNGKG  = CFTOTAL*MW*UNITCORR
CFUGG=(CFTOTAL*MW)/UNITCORR
CFPMOL_KG= CFTOTAL*UNITCORR*UNITCORR       !CONCENTRATION IN PMOL/KG
CFNGG = CFTOTAL*MW

    !REST OF THE BODY COMPARTMENT
    !TISSUE BLOOD SUBCOMPARTMENT
```

```
RAREB= QRE*(CA-CREB)-PARE*(CREB-CRE/PRE)          !(NMOL/HR)
 AREB = INTEG(RAREB,0.0)                           !(NMOL)
 CREB = AREB/WREB                                 !(NMOL/ML)
    !TISSUE SUBCOMPARTMENT
RARE = PARE*(CREB - CRE/PRE)                      !(NMOL/HR)
 ARE = INTEG(RARE,0.0)                            !(NMOL)
 CRE  = ARE/WRE                                    !(NMOL/ML)

    !POST SIMULATION UNIT CONVERSION
CRETOTAL= (ARE + AREB)/(WRE + WREB)               ! CONCENTRATION AT STEADY
STATE
PRCT_RE = (CRETOTAL/(MSTT+1E-30))*100


    !LIVER COMPARTMENT
    !TISSUE BLOOD SUBCOMPARTMENT
RALIB = QLI*(CA-CLIB)-PALI*(CLIB-CFLLIR)+LIRMLUM   !(NMOL/HR)
  ALIB = INTeg(RALIB,0.0)                           !(NMOL)
 CLIB = ALIB/WLIB
    !TISSUE SUBCOMPARTMENT
 RALI =  PALI*(CLIB-CFLLIR)-REXCLI                 !(NMOL/HR)
  ALI = integ(RALI,0.0)                                    !(NMOL)
  CLI  = ALI/WLI                                    !(NMOL/ML)

    !FREE TCCD CONCENTRATION IN LIVER (NMOL/ML)
PARAMETER (LIVER_1RMN = 1.0E-30)
 CFLLI= IMPLC(CLI-(CFLLIR*PLI+(LIBMAX*CFLLIR/(KDLI+CFLLI &
       +LIVER_1RMN))+((CYP1A2_1O3*CFLLIR/(KDLI2+CFLLIR &
       +LIVER_1RMN)*PAS_INDUC)))-CFLLI,CFLLI0)
     CFLLIR=DIM(CFLLI,0.0) ! FREE CONCENTRATION IN LIVER

CBNDLI= LIBMAX*CFLLIR/(KDLI+CFLLIR+LIVER_1RMN) !BOUND CONCENTRATION

   !POST SIMULATION UNIT CONVERSION
CLITOTAL= (ALI + ALIB)/(WLI + WLIB)!
PRCT_LI = (CLITOTAL/(MSTT+1E-30))*100 ! PERCENT OF DOSE IN LIVER
rec_occ_AHR= (CFLLIR/(KDLI+CFLLIR+1E-30))*100.0  ! PERCENT OF AhR OCCUPANCY
PROT_occ_1A2= (CFLLIR/(KDLI2+CFLLIR))*100.0  ! PERCENT OF 1A2 OCCUPANCY
CLINGKG =(CLITOTAL*MW*UNITCORR)
CBNDLINGKG = CBNDLI*MW*UNITCORR
CLIUGG=(CLITOTAL*MW)/UNITCORR
CLIPMOL_KG= CLITOTAL*UNITCORR*UNITCORR       !CONCENTRATION IN PMOL/KG
CLINGG = CLITOTAL*MW

   !Fraction increase of induction of CYP1A2
fold_ind=(CYP1A2_1OUT/CYP1A2_1A2)
VARIATIONOfAC =(CYP1A2_1OUT-CYP1A2_1A2)/CYP1A2_1A2

   !VARIABLE ELIMINATION BASED ON THE CYP1A2
KBILE_LI_T =((CYP1A2_1OUT-CYP1A2_1A2)/CYP1A2_1A2)*Kelv !INDUCED BILIARY
EXCRETION RATE CONSTANT

REXCLI= (KBILE_LI_T*CFLLIR*WLI) !DOSE-DEPENDENT EXCRETION RATE
 EXCLI = INTEG(REXCLI,0.0)

   !CHEMICAL IN CYP450 (1A2) COMPARTMENT
```

```
   !EQUATION FOR INDUCTION OF CYP1A2

CYP1A2_1KINP = CYP1A2_1KOUT* CYP1A2_1OUTZ

   ! MODIFICATION ON OCTOBER 6, 2009
CYP1A2_1OUT =INTEG(CYP1A2_1KINP * (1.0 + CYP1A2_1EMAX *(CBNDLI+1.0e-30)**HILL
&
     /(CYP1A2_1EC50**HILL + (CBNDLI+1.0e-30)**HILL)) &
      - CYP1A2_1KOUT*CYP1A2_1OUT, CYP1A2_1OUTZ)
! EQUATIONS INCORPORATING DELAY OF CYP1A2 PRODUCTION (NOT USED IN
SIMULATIONS)

CYP1A2_1RO2 = (CYP1A2_1OUT - CYP1A2_1O2)/ CYP1A2_1TAU
  CYP1A2_1O2 =INTEG(CYP1A2_1RO2, CYP1A2_1A1)
CYP1A2_1RO3 = (CYP1A2_1O2 - CYP1A2_1O3)/ CYP1A2_1TAU
  CYP1A2_1O3 =INTEG(CYP1A2_1RO3, CYP1A2_1A2)

      ! MASS BALANCE CONTROL
 BDOSE= LYMLUM+LIMLUM+IVDOSE
 BMASSE = EXCLI+AURI+AFB+AF+AREB+ARE+ALIB+ALI
 BDIFF = BDOSE-BMASSE
      ! AMOUNT TOTAL PRESENT IN THE GI TRACT
BDOSE_TOTAL =LYMLUM+LIMLUM+FECES

      !BODY BURDEN IN NG
 Body_burden =(AFB+AF+AREB+ARE+ALIB+ALI)*MW

      !BODY BURDEN CONCENTRATION (NG/KG)
 BBNGKG =(((AFB+AF+AREB+ARE+ALIB+ALI)*MW)/(WT0/UNITCORR)) !

      !COMMAND FOR END OF SIMULATION
TERMT (T.GE. TimeLimit, 'Time limit has been reached.')

END   ! END OF THE DERIVATIVE SECTION
END   ! END OF THE DYNAMIC SECTION
END   ! END OF PROGRAM
```

C.2.5.2. *Input Files*

C.2.5.2.1. *Della Porta (1987) (female)*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Della Porta 1987 for female mice.
%dose levels:  2.5 and 5 ug/kg/week for 52 weeks
%dose levels:  2500 and 5000 ng/kg/week for 52 weeks
%dose levels equivalent to: 357 and 714 ng/kg/d

MAXT =  0.01
CINT  = 0.1
EXP_TIME_ON       = 0.       %delay before begin exposure (HOUR)
EXP_TIME_OFF      = 8736     %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE         = 168
BCK_TIME_ON       = 0.       %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
```

```
BCK_TIME_OFF      = 0.       %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT         = 8736     %SIMULATION LIMIT TIME (HOUR)
BW_T0             = 20       % Body weight at the beginning of the simulation
(g); corresponds to 6 weeks of age and taken from Figure 3


%EXPOSURE DOSE SCENARIOS (UG/KG)
    %MSTOT          = 2.5      % exposure dose ug/kg
    MSTOT         = 5.0      % exposure dose ug/kg
```

C.2.5.2.2. *Della Porta (1987) (male)*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Della Porta 1987 for male mice.
%dose levels:  2.5 and 5 ug/kg/week for 52 weeks
%dose levels:  2500 and 5000 ng/kg/week for 52 weeks
%dose levels equivalent to: 357 and 714 ng/kg/d

MAXT =  0.01
CINT  = 0.1
EXP_TIME_ON       = 0.       %delay before begin exposure (HOUR)
EXP_TIME_OFF      = 8736     %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE         = 168
BCK_TIME_ON       = 0.       %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF      = 0.       %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT         = 8736     %SIMULATION LIMIT TIME (HOUR)
BW_T0             = 26       % Body weight at the beginning of the simulation
(g); corresponds to 6 weeks of age and taken from Figure 3


%EXPOSURE DOSE SCENARIOS (UG/KG)
    %MSTOT          = 2.5      % exposure dose ug/kg
    MSTOT         = 5.0      % exposure dose ug/kg
```

C.2.5.2.3. *NTP (1982) (female) (chronic)*

```
%RAT2.m
%clear variable
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG
%output @nciout=168 T SUMEXPEVENT

% NTP 1982.
%built and check in September 20, 2009
%protocol: twice weekly gavage for 104 weeks
%Rat_Dioxin_3C June09_2clean_2.csl
%MICE_NON_GESTAT_ICF_F083109.csl
%MICE_NON_GESTAT_ICF_F092009.csl (now 09-20-09)
%dose levels:  0.02, 0.1, 1 ug/kg/biweekly, ug/kg for 104 weeks
%dose levels:  20, 100, 1000 ng/kg/biweekly,ng/kg for 104 weeks
%dose levels equivalent to: 5.71, 28.57, 285.1 ng/kg/d
```

```
MAXT =  0.01
CINT  = 0.1
EXP_TIME_ON       = 0.       %delay before begin exposure (HOUR)
EXP_TIME_OFF      = 17472     %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE         = 84
BCK_TIME_ON       = 0.       %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF      = 0.       %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT         = 17472     %SIMULATION LIMIT TIME (HOUR)
BW_T0             = 23       % Body weight at the beginning of the simulation
(g)


%EXPOSURE DOSE SCENARIOS (UG/KG)
    %MSTOT          = 0.02      % exposure dose ug/kg
    %MSTOT         = 0.1       % exposure dose ug/kg
    MSTOT         = 1.0         % exposure dose ug/kg
```

C.2.5.2.4. *NTP (1982) (male) (chronic).*

```
%RAT2.m
%clear variable
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG
%output @nciout=168 T SUMEXPEVENT

% NTP 1982.
%built and check in September 20, 2009
%protocol: twice weekly gavage for 104 weeks
%Rat_Dioxin_3C June09_2clean_2.csl
%MICE_NON_GESTAT_ICF_F083109.csl
%MICE_NON_GESTAT_ICF_F092009.csl (now 09-20-09)
%dose levels:  0.005, 0.025, 0.25 ug/kg/biweekly, ug/kg for 104 weeks
%dose levels:  5, 25, 250 ng/kg/biweekly,ng/kg for 104 weeks
%dose levels equivalent to: 1.4, 7.1, 71 ng/kg/d

MAXT =  0.01
CINT  = 0.1
EXP_TIME_ON       = 0.       %delay before begin exposure (HOUR)
EXP_TIME_OFF      = 17472     %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE         = 84
BCK_TIME_ON       = 0.       %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF      = 0.       %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
TIMELIMIT         = 17472     %SIMULATION LIMIT TIME (HOUR)
BW_T0             = 25       % Body weight at the beginning of the simulation
(g)


%EXPOSURE DOSE SCENARIOS (UG/KG)
    %MSTOT          = 0.005      % exposure dose ug/kg
    %MSTOT         = 0.025       % exposure dose ug/kg
    MSTOT         = 0.25         % exposure dose ug/kg
```

C.2.5.2.5. *Smialowicz et al. (2008).*

```
output @clear
```

```
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Smialowicz et al. 2008.
%built and check in August 7 2009
%protocol:  oral gavage 5 days/week for 13 weeks
%Mice_Dioxin_3C_June09_1.csl
%MICE_NON_GESTAT_ICF_F083109.csl (now 09-11-09)
%dose levels:  0, 0.0015, 0.015, 0.15, 0.45 ug/kg
%dose levels:  0, 1.5, 15, 150, 450 nkd (0, 1.07, 10.7, 107, 321 nkd adj)

MAXT          = 0.01
CINT          = 0.1
TIMELIMIT     = 2184          %SIMULATION LIMIT TIME (HOUR)
EXP_TIME_ON   = 0.            %delay before begin exposure (HOUR)
EXP_TIME_OFF  = 2184          %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE     =  24
WEEK_PERIOD   = 168
WEEK_FINISH   = 119
BCK_TIME_ON   = 0.           %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF  = 0.           %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
BW_T0         = 28           % Body weight at the beginning of the simulation
(g)

%EXPOSURE DOSE SCENARIOS (UG/KG)
   %MSTOT      = 0.0015       % exposure dose (ug/kg)
   %MSTOT   = 0.015        % exposure dose (ug/kg)
   %MSTOT   = 0.150        % exposure dose (ug/kg)
   MSTOT   = 0.450        % exposure dose (ug/kg)
```

C.2.5.2.6. *Toth et al. (1979) (1 year).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG CBNGKG

% Toth et al. 1979
%built and check in August 7 2009
%protocol:  weekly gavage for 1 year
%Mice_Dioxin_3C_June09_1.csl
%MICE_NON_GESTAT_ICF_F083109.csl (now 09-11-09)
%dose levels:  7, 700, 7000 ng/kg 1/week for 52 weeks (1 year)
%dose levels:  0.007, 0.7, 7 ug/kg 1/week for 52 weeks (1 year)
%dose equivalent: 1, 100, 1000 ng/kg/day

MAXT          = 0.01
CINT          = 0.1
TIMELIMIT     = 8760
EXP_TIME_ON   = 0.          %delay before begin exposure (HOUR)
EXP_TIME_OFF  = 8760        %2208 %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE     =  168
WEEK_PERIOD   = 8760
WEEK_FINISH   = 8760
BCK_TIME_ON   = 0.          %DELAY BEFORE BACGROUND EXPOSURE (HOUR)
BCK_TIME_OFF  = 0.          %TIME OF BACKGROUND EXPOSURE STOP (HOUR)
```

```
BW_T0        = 27          % Body weight at the beginning of the simulation
(g)


%EXPOSURE DOSE SCENARIOS (UG/KG)
    %MSTOT    = 0.007       % exposure dose (ug/kg)
    %MSTOT = 0.7          % exposure dose (ug/kg)
    MSTOT = 7           % exposure dose (ug/kg)
```

C.2.5.2.7. *White et al. (1986).*

```
output @clear
prepare @clear
prepare T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CBNDLINGKG

% White et al 1986
%built and check in August 7 2009

%protocol:  oral exposure single dose
%dose levels:  0.714, 3.57, 7.14, 35.71, 71.43, 142.86 ng /kg/d ug/kg 1/day
for 14 consecutive days
%dose have been modified following Jeff email on Friday August 21 2009
%dose levels: 10, 50, 100, 500, 1000, 2000 ng /kg/d ug/kg 1/day for 14
consecutive days
%dose levels: 0.010, 0.050, 0.100, 0.500, 1.0, 2.0 ug /kg/d ug/kg 1/day for
14 consecutive days

MAXT         = 0.01
CINT         = 0.1
TIMELIMIT    = 336
EXP_TIME_ON  = 0.        %TIME AT WHICH EXPOSURE BEGINS (HOUR)
EXP_TIME_OFF = 336       %TIME AT WHICH EXPOSURE ENDS (HOUR)
DAY_CYCLE    =  24
WEEK_PERIOD  = 336
WEEK_FINISH  = 336
BCK_TIME_ON  = 0.        %TIME AT WHICH BACKGROUND EXPOSURE BEGINS (HOUR)
BCK_TIME_OFF = 0.        %TIME AT WHICH BACKGROUND EXPOSURE ENDS (HOUR)
BW_T0        = 23        % BODY WEIGHT AT THE BEGINNING OF THE SIMULATION (G)

%EXPOSURE DOSE SCENARIOS (UG/KG)
   %MSTOT = 0.010         % EXPOSURE DOSE IN UG/KG
   %MSTOT = 0.050       % EXPOSURE DOSE IN UG/KG
   %MSTOT = 0.100       % EXPOSURE DOSE IN UG/KG
   %MSTOT = 0.500       % EXPOSURE DOSE IN UG/KG
   %MSTOT =  1          % EXPOSURE DOSE IN UG/KG
   MSTOT =  2          % EXPOSURE DOSE IN UG/KG
```

C.2.6. Mouse Gestational Model

C.2.6.1. *Model Code*

PROGRAM: 'Three Compartment PBPK Model for TCDD in Mice (Gestation)'

```
! Parameters were change may 16, 2002
! Come from {8MAI_CHR_PRE-EXP_GD}
```

```
! Come from {12_Mouse_GD}file
!*****************************************
!{{IMPORTANT-IMPORTANT-IMPORTANT-IMPORTANT}}
! REDUCTION OF MOTHER AND FETUS COMPARTMENT
! 2M_R_TCDD_JULY2002 ////(JULY 18,2002)////
!TCDD_RED_4Species_2003_4        ////(APR  8 ,2003)////
!TCDD_RED_4Species_2003_9        ////(APR  17 ,2003)////
!TCDD_RED_4Species_2003_12        ////(APR  17 ,2003)////
!*************************************************
!APRIL 18 2003
!TCDD_4C_4SP_2003        ////(APR  18 ,2003)////
! was ''Gest 4 species 1.csl''   but update July 2009

!DevTCDD4Species_ICF_afterKKfix_v3_ratgest.csl
!MICE_GESTATIONAL_ICF_F092309.csl
!MICE_GESTATIONAL_ICF_F100609.csl
!*************************************************

 !Legend/Legend/Legend/Legend/Legend/Legend/Legend/Legend/
 !Legend for this PBPK model
 !Mating: control the tenure of exchange between fetus and
    !Mother and also control imitated tissue growth
    !Ctrl: WTFE, WFO, WPLA0, QPLAF,WT0
    !(for rat, mouse, human, and monkey)
 !Control transfer from mother to fetus and fetus to mother by TRANSTIME_ON
    !SWITCH_trans = 0 NO TRANSFER
    !SWITCH_trans = 1 TRANSFER OCCURS
    !Gest_off = 1
    !Gest_on=   0.
 ! These switches are also controlled by mating parameters

INITIAL  !

     !SIMULATION PARAMETERS ====
CONSTANT PARA_ZERO          = 1E-30
CONSTANT EXP_TIME_ON     = 288.       ! TIME AT WHICH EXPOSURE BEGINS (HOURS)
CONSTANT EXP_TIME_OFF    = 504      ! TIME AT WHICH EXPOSURE ENDS (HOURS)
CONSTANT DAY_CYCLE       =  504.     ! NUMBER OF HOURS BETWEEN DOSES (HOURS)
CONSTANT BCK_TIME_ON     = 0.0       ! TIME AT WHICH BACKGROUND EXPOSURE
BEGINS (HOURS)
CONSTANT BCK_TIME_OFF    = 0.0       ! TIME AT WHICH BACKGROUND EXPOSURE ENDS
(HOURS)
CONSTANT TRANSTIME_ON    = 144       !CONTROL TRANSFER FROM MOTHER TO FETUS
AT GESTATIONAL DAY 6

    !UNIT CONVERSION
CONSTANT MW=322 ! MOLECULAR WEIGHT (NG/NMOL)
CONSTANT SERBLO = 0.55
CONSTANT UNITCORR = 1000

    !INTRAVENOUS SEQUENCY
constant IV_LACK         = 0.0
constant IV_PERIOD       = 0.0

    !PREGNANCY PARAMETER ====
CONSTANT MATTING         = 0.0       !BEGINNING OF MATING (HOUR)
```

```
CONSTANT N_FETUS         = 10       !NUMBER OF FETUS PRESENT

    !CONSTANT EXPOSURE CONTROL ===========
    !ACUTE, SUBCHRONIC, CHRONIC EXPOSURE =====
    !OR BACKGROUND EXPOSURE (IN THIS CASE 3 TIMES A DAY)===
CONSTANT MSTOTBCKGR      = 0.0       ! ORAL BACKGROUND EXPOSURE DOSE (UG/KG)
CONSTANT MSTOT           = 0.0       ! ORAL EXPOSURE DOSE (UG/KG)

    !ORAL ABSORPTION
  MSTOT_NM = MSTOT/MW              !CONVERTS THE DOSE TO NMOL/G

    ! INTRAVENOUS ABSORPTION
CONSTANT  DOSEIV         = 0.0       ! INJECTED DOSE (UG/KG)
  DOSEIV_NM = DOSEIV/MW            ! CONVERTS THE INJECTED DOSE TO NMOL/G
CONSTANT DOSEIVLATE = 0.0            ! INJECTED DOSE LATE (UG/KG)
  DOSEIVNMlate = DOSEIVLATE/MW     !AMOUNT IN NMOL/G

    !INITIAL GUESS OF THE FREE CONCENTRATION IN THE LIGAND (COMPARTMENT
INDICATED BELOW)====
CONSTANT CFLLI0          = 0.0  !LIVER    (NMOL/ML)
CONSTANT CFLPLA0         = 0.0  !PLACENTA (NMOL/ML)

    !BINDING CAPACITY (AhR) FOR NON LINEAR BINDING (COMPARTMENT INDICATED
BELOW) (NMOL/ML) ===
CONSTANT LIBMAX          = 3.5E-4   ! LIVER  (NMOL/ML), WANG ET AL. 1997
CONSTANT PLABMAX         = 2.0E-4   !TEMPORARY PARAMETER

    ! PROTEIN AFFINITY CONSTANTS (1A2 OR AhR, COMPARTMENT INDICATED BELOW)
(NMOL/ML)===
CONSTANT KDLI            = 1.0E-4   !LIVER (AhR) (NMOL/ML), WANG ET AL. 1997
CONSTANT KDLI2           = 4.0E-2   !LIVER (1A2) (NMOL/ML), EMOND ET AL. 2004
CONSTANT KDPLA           = 1.0E-4   !TEMPORARY PARAMETER (AhR)

    !EXCRETION AND ABSORPTION CONSTANT
CONSTANT KST             =    0.3   ! GASTRIC RATE CONSTANT (HR-1)
CONSTANT KABS            =    0.48  !INTESTINAL ABSORPTION CONSTANT (HR-1) ),
WANG ET AL. 1997

! ELIMINATION CONSTANTS
CONSTANT CLURI           =      0.09 ! URINARY CLEARANCE (ML/HR)

   !TEST ELIMINATION VARIABLE
constant kelv            =     0.4        ! INTERSPECIES VARIABLE ELIMINATION
CONSTANT (1/HOUR)

    ! CONSTANT TO DIVIDE THE ABSORPTION INTO LYMPHATIC AND PORTAL FRACTIONS
CONSTANT A               = 0.7        ! LYMPHATIC FRACTION, WANG ET AL. 1997

    !PARTITION COEFFICIENTS
CONSTANT PF              = 400    ! ADIPOSE TISSUE/BLOOD
CONSTANT PRE             = 3      ! REST OF THE BODY/BLOOD, WANG ET AL. 2000
CONSTANT PLI             = 6     ! LIVER/BLOOD, WANG ET AL. 1997
CONSTANT PPLA            = 3      ! TEMPORARY PARAMETER NOT CONFIGURED

    !PARAMETER FOR INDUCTION OF CYP 1A2, WANG ET AL. 1997 OR OPTIMIZED
CONSTANT PAS_INDUC       = 1        ! INCLUDE INDUCTION? (1 = YES, 0 = NO)
```

```
CONSTANT CYP1A2_1OUTZ    = 1.6      ! DEGRADATION CONCENTRATION CONSTANT OF
1A2 (NMOL/ML) (OPTIMIZED)
CONSTANT CYP1A2_1A1      = 1.5      ! BASAL CONCENTRATION OF 1A1 (NMOL/ML),
WANG ET AL . (2000)
CONSTANT CYP1A2_1EC50    = 0.13      ! DISSOCIATION CONSTANT TCDD-CYP1A2
(NMOL/ML)
CONSTANT CYP1A2_1A2      = 1.5      !BASAL CONCENTRATION OF 1A2
(NMOL/ML),WANG ET AL. (2000)
CONSTANT CYP1A2_1KOUT    = 0.1      ! FIRST ORDER RATE OF DEGRADATION (H-1)
CONSTANT CYP1A2_1TAU     = 1.5      !HOLDING TIME (H) (OPTIMIZED), WANG ET AL
. (2000)
CONSTANT CYP1A2_1EMAX    = 600      ! MAXIMUM INDUCTION OVER BASAL EFFECT
(UNITLESS)
CONSTANT HILL            = 0.6      !HILL CONSTANT; COOPERATIVELY LIGAND
BINDING EFFECT CONSTANT (UNITLESS)

    !DIFFUSIONAL PERMEABILITY FRACTION, WANG ET AL. 1997
CONSTANT PAFF            = 0.12   !ADIPOSE (UNITLESS) OPTIMIZED, WANG ET AL.
2000
CONSTANT PAREF           = 0.03   !REST OF THE BODY (UNITLESS)
CONSTANT PALIF           = 0.35   !LIVER (UNITLESS)
CONSTANT PAPLAF          = 0.03   !TEMPORARY PARAMETER NOT CONFIGURED

   !FRACTION OF TISSUE WEIGHT =========
CONSTANT WLI0            = 0.0549   !LIVER  ILSI (1994)

   !TISSUE BLOOD FLOW EXPRESSED AS A FRACTION OF CARDIAC OUTPUT CONSTANT QFF
= 0.070    ! ADIPOSE TISSUE BLOOD FLOW FRACTION (UNITLESS), LEUNG ET AL. 1990
CONSTANT QLIF            = 0.161    !LIVER (UNITLESS), ILSI 1994

   !COMPARTMENT TISSUE BLOOD EXPRESSED AS A FRACTION OF THE TOTAL COMPARTMENT
VOLUME
CONSTANT WFB0            = 0.050    !ADIPOSE TISSUE, WANG ET AL. 1997
CONSTANT WREB0           = 0.030    !REST OF THE BODY, WANG ET AL. 1997
CONSTANT WLIB0           = 0.266    !LIVER, WANG ET AL. 1997
CONSTANT WPLAB0          = 0.500    !TEMPORARY PARAMETER NOT CONFIGURED

   !EXPOSURE SCENARIO FOR UNIQUE OR REPETITIVE WEEKLY OR MONTHLY EXPOSURE
   !NUMBER OF EXPOSURES PER WEEK
CONSTANT WEEK_LACK       = 0.0       !DELAY BEFORE EXPOSURE ENDS (WEEK)
CONSTANT WEEK_PERIOD     = 168      ! NUMBER OF HOURS IN THE WEEK (HOURS)
CONSTANT WEEK_FINISH     = 168      ! TIME EXPOSURE ENDS (HOURS)

   !NUMBER OF EXPOSURES PER MONTH
CONSTANT MONTH_LACK      = 0.0       !DELAY BEFORE EXPOSURE BEGINS (MONTH)

   !CONSTANT FOR BACKGROUND EXPOSURE===========
CONSTANT Day_LACK_BG     = 0.0       ! DELAY BEFORE EXPOSURE BEGINS (HOUR)
CONSTANT Day_PERIOD_BG   = 24       !LENGTH OF EXPOSURE (HOUR)

   !NUMBER OF EXPOSURES PER WEEK
CONSTANT WEEK_LACK_BG      = 0.0      !DELAY BEFORE BACKGROUD EXPOSURE (WEEK)
CONSTANT WEEK_PERIOD_BG    = 168      ! NUMBER OF HOURS IN THE WEEK (HOURS)
CONSTANT WEEK_FINISH_BG    = 168      !TIME EXPOSURE ENDS (HOURS)

   !INITIAL BODY WEIGHT
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```
CONSTANT BW_T0              = 30      ! WANG ET AL. 1997
CONSTANT RATIO_RATF_MOUSEF = 0.2        !RATIO OF FETUS MOUSE/RAT AT
GESTATIONAL DAY 22
                                        ! FOR RAT (1) AND FOR MOUSE (0.2)

   !COMPARTMENT LIPID EXPRESSED AS THE FRACTION OF TOTAL LIPID, POULIN ET AL.
2000
CONSTANT F_TOTLIP          = 0.855              ! ADIPOSE TISSUE (UNITLESS)
CONSTANT B_TOTLIP          = 0.0033              ! BLOOD (UNITLESS)
CONSTANT RE_TOTLIP         = 0.019              ! REST OF THE BODY
(UNITLESS)
CONSTANT LI_TOTLIP         = 0.060              ! LIVER (UNITLESS)
CONSTANT PLA_TOTLIP        = 0.019              ! PLACENTA (UNITLESS)
CONSTANT FETUS_TOTLIP      = 0.019            ! FETUS (UNITLESS)

END     ! END OF THE INITIAL SECTION

DYNAMIC ! DYNAMIC SIMULATION SECTION
ALGORITHM  IALG          =           2        ! GEAR METHOD
CINTERVAL  CINT          =         0.1        ! COMMUNICATION INTERVAL
MAXTERVAL  MAXT          =       1.0e+10      ! MAXIMUM CALCULATION INTERVAL
MINTERVAL  MINT          =       1.0E-10      ! MINIMUM CALCULATION INTERVAL
VARIABLE   T             =         0.0
CONSTANT   TIMELIMIT     =         313        !SIMULATION LIMIT TIME (HOUR)
 CINTXY  = CINT
 PFUNC   = CINT

   !TIME CONVERSION
  DAY         = T/24           ! TIME IN DAYS
  WEEK        = T/168          ! TIME IN WEEKS
  MONTH       = T/730          ! TIME IN MONTHS
  YEAR        = T/8760         ! TIME IN YEARS

DERIVATIVE ! PORTION OF CODE THAT SOLVES DIFFERENTIAL EQUATIONS

   !CHRONIC OR SUBCHRONIC EXPOSURE SCENARIO =======
   !NUMBER OF EXPOSURES PER DAY
 DAY_LACK         = EXP_TIME_ON    ! DELAY BEFORE EXPOSURE BEGINS (HOURS)
 DAY_PERIOD       = DAY_CYCLE      ! EXPOSURE PERIOD (HOURS)
 DAY_FINISH       = CINTXY         ! LENGTH OF EXPOSURE (HOURS)
 MONTH_PERIOD     = TIMELIMIT      ! EXPOSURE PERIOD (MONTHS)
 MONTH_FINISH     = EXP_TIME_OFF   ! LENGTH OF EXPOSURE (MONTHS)

   !NUMBER OF EXPOSURES PER DAY AND MONTH
 DAY_FINISH_BG    = CINTXY
 MONTH_LACK_BG    = BCK_TIME_ON    !DELAY BEFORE BACKGROUD EXPOSURE BEGINS
(MONTHS)
 MONTH_PERIOD_BG  = TIMELIMIT      !BACKGROUND EXPOSURE PERIOD (MONTHS)
 MONTH_FINISH_BG  = BCK_TIME_OFF   !LENGTH OF BACKGROUND EXPOSURE (MONTHS)

   !INTRAVENOUS LATE
 IV_FINISH = CINTXY
 B = 1-A ! FRACTION OF DIOXIN ABSORBED IN THE PORTAL FRACTION OF THE LIVER
```

```
1
2   !FETUS,VOLUME,FETUS,VOLUME,FETUS,VOLUME,FETUS,VOLUME,FETUS,VOLUME,FETUS,VOLUM
3   E
4     ! FROM OFLAHERTY_1992
5
6   RTESTGEST= T-MATTING
7   TESTGEST=DIM(RTESTGEST,0.0)
8
9   WTFER_RODENT= (2.3d-3*EXP(1.49d-2*(TESTGEST))+1.3d-2)*Gest_on
10  WTFER = (WTFER_RODENT*RATIO_RATF_MOUSEF*N_FETUS)
11  WTFE = DIM(WTFER,0.0)
12
13    !
14  FAT,VOLUME,FAT,VOLUME,FAT,VOLUME,FAT,VOLUME,FAT,VOLUME,FAT,VOLUME,FAT,VOLUME
15    ! FAT GROWTH EXPRESSION LINEAR DURING PREGNANCY
16    ! FROM O'FLAHERTY_1992
17
18  WF0= (((9.66d-5*(TESTGEST))*gest_on)+0.069)
19
20    ! PLACENTA,VOLUME, PLACENTA,VOLUME, PLACENTA,VOLUME, PLACENTA,VOLUME
21    ! WPLA PLACENTA GROWTH EXPRESSION, SINGLE EXPONENTIAL WITH OFFSET
22    ! FROM O'FLAHERTY_1992  ! FOR EACH PUP
23
24  WPLA0N_RODENT = (0.6/(1+(5d+3*EXP(-0.0225*(TESTGEST)))))*N_FETUS
25  WPLA0R = (WPLA0N_RODENT/WT0)*Gest_on
26  WPLA0 = DIM(WPLA0R,0.0)
27
28    ! PLACENTA,FLOW RATE, PLACENTA,FLOW RATE, PLACENTA,FLOW RATE, PLACENTA,FLOW
29  RATE
30    ! QPLA PLACENTA GROWTH EXPRESSION, DOUBLE EXPONENTIAL WITH OFFSET
31    ! FROM O'FLAHERTY_1992
32
33   QPLARF = (1.67d-7 *exp(9.6d-3*(TESTGEST)) &
34     +1.6d-3*exp(7.9d-3*(TESTGEST))+0.0)*Gest_on*SWITCH_trans
35   QPLAF=DIM(QPLARF,0.0)               !FRACTION OF FLOW RATE IN PLACENTA
36
37    ! GESTATION CONTROL
38  IF (T.LT.MATTING) THEN
39      Gest_off = 1
40      Gest_on=   0.0
41  ELSE
42      Gest_off = 0.0
43      Gest_on  = 1
44  END IF
45
46    ! MOTHER BODY WEIGHT GROWTH EQUATION========
47    ! MODIFICATION TO ADAPT THIS MODEL AT HUMAN MODEL
48    ! BECAUSE LINEAR DESCRIPTION IS NOT GOOD ENOUGH FOR MOTHER GROWTH
49    ! MOTHER BODY WEIGHT GROWTH
50
51    PARAMETER (BW_RMN = 1.0E-30)
52    WT0= BW_T0 *(1.0+(0.41*T)/(1402.5+T+BW_RMN))
53
54    ! VARIABILITY OF REST OF THE BODY DEPENDS ON OTHER ORGANS
55    WRE0 = (0.91 - (WLIB0*WLI0 + WFB0*WF0 +WPLAB0*WPLA0 + WLI0 + WF0 +
56  WPLA0))/(1.0+WREB0)  ! REST OF THE BODY FRACTION; UPDATED FOR EPA ASSESSMENT
```

```
  QREF = 1.0-(QFF+QLIF+QPLAF)            !REST OF BODY BLOOD FLOW RATE
(ML/HR)
  QTTQF = QFF+QREF+QLIF+QPLAF          ! SUM MUST EQUAL 1

  ! COMPARTMENT VOLUME (ML OR G) =========
 WF  =  WF0  * WT0                    ! ADIPOSE TISSUE
 WRE =  WRE0 * WT0                    ! REST OF THE BODY
 WLI =  WLI0 * WT0                    ! LIVER
 WPLA=  WPLA0* WT0                    ! PLACENTA

   ! COMPARTMENT TISSUE BLOOD (ML OR G) =========
 WFB  =  WFB0  * WF                   ! ADIPOSE TISSUE
 WREB =  WREB0 * WRE                  ! REST OF THE BODY
 WLIB =  WLIB0 * WLI                  ! LIVER
 WPLAB = WPLAB0* WPLA                 ! PLACANTA

   ! CARDIAC OUTPUT FOR THE GIVEN BODY WEIGHT
   !QC= QCCAR*60*(WT0/1000.0)**0.75
CONSTANT QCC=16500                    ! EQUIVALENT TO 275 * 60
QC= QCC*(WT0/UNITCORR)**0.75

   !COMPARTMENT BLOOD FLOW RATE (ML/HR)
QF  = QFF*QC                          !ADIPOSE TISSUE BLOOD FLOW RATE
QLI = QLIF*QC                         !LIVER TISSUE BLOOD FLOW RATE
QRE = QREF*QC                         !REST OF THE BODY BLOOD FLOW RATE
QPLA = QPLAF*QC                       !PLACENTA TISSUE BLOOD FLOW RATE
QTTQ = QF+QRE+QLI+QPLA   !TOTAL FLOW RATE

    !PERMEABILITY ORGAN FLOW (ML/HR)=========
PAF  = PAFF*QF                       ! ADIPOSE TISSUE
PARE = PAREF*QRE                     ! REST OF THE BODY
PALI = PALIF*QLI                     ! LIVER TISSUE
PAPLA = PAPLAF*QPLA                  ! PLACENTA

    !************************************
    ! ABSORPTION SECTION
    ! ORAL,
    ! INTRAPERITONEAL,
    ! INTRAVENOUS
    !************************************

    !REPETITIVE ORAL BACKGROUND EXPOSURE SCENARIO

MSTOT_NMBCKGR = MSTOTBCKGR/322       !AMOUNT IN NMOL/G
MSTTBCKGR =MSTOT_NMBCKGR *WT0

DAY_EXPOSURE_BG   = PULSE(DAY_LACK_BG,DAY_PERIOD_BG,DAY_FINISH_BG)
WEEK_EXPOSURE_BG  = PULSE(WEEK_LACK_BG,WEEK_PERIOD_BG,WEEK_FINISH_BG)
MONTH_EXPOSURE_BG = PULSE(MONTH_LACK_BG,MONTH_PERIOD_BG,MONTH_FINISH_BG)

MSTTCH_BG = (DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG)*MSTTBCKGR
MSTTFR_BG = MSTTBCKGR/CINT

CYCLE_BG =DAY_EXPOSURE_BG*WEEK_EXPOSURE_BG*MONTH_EXPOSURE_BG

    ! CONDITIONAL ORAL EXPOSURE (BACKGROUND EXPOSURE)
```

```
1
2   IF (MSTTCH_BG.EQ.MSTTBCKGR) THEN
3       ABSMSTT_GB= MSTTFR_BG
4   ELSE
5       ABSMSTT_GB = 0.0
6   END IF
7
8   CYCLETOTBG=INTEG(CYCLE_BG,0.0)
9
10     !REPETITIVE ORAL EXPOSURE SCENARIO
11
12  MSTT= MSTOT_NM * WT0                    !AMOUNT IN NMOL
13
14  DAY_EXPOSURE   = PULSE(DAY_LACK,DAY_PERIOD,DAY_FINISH)
15  WEEK_EXPOSURE  = PULSE(WEEK_LACK,WEEK_PERIOD,WEEK_FINISH)
16  MONTH_EXPOSURE = PULSE(MONTH_LACK,MONTH_PERIOD,MONTH_FINISH)
17
18  MSTTCH = (DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE)*MSTT
19  MSTTFR = MSTT/CINT
20
21  CYCLE = DAY_EXPOSURE*WEEK_EXPOSURE*MONTH_EXPOSURE
22  SUMEXPEVENT= INTEG (CYCLE,0.0)/cint !NUMBER OF CYCLES GENERATED DURING
23  SIMULATION
24
25     ! CONDITIONAL ORAL EXPOSURE
26  IF (MSTTCH.EQ.MSTT) THEN
27     ABSMSTT= MSTTFR
28  ELSE
29     ABSMSTT = 0.0
30  END IF
31
32
33   CYCLETOT=INTEG(CYCLE,0.0)
34
35     ! MASS CHANGE IN THE LUMEN
36   RMSTT= -(KST+KABS)*MST +ABSMSTT +ABSMSTT_GB ! RATE OF CHANGE (NMOL/H)
37    MST = INTEG(RMSTT,0.0)                      !AMOUNT REMAINING IN DUODENUM
38  (NMOL)
39
40     ! ABSORPTION IN LYMPH CIRCULATION
41   LYRMLUM = KABS*MST*A
42    LYMLUM = INTEG(LYRMLUM,0.0)
43
44     ! ABSORPTION IN PORTAL CIRCULATION
45   LIRMLUM = KABS*MST*B
46    LIMLUM = INTEG(LIRMLUM,0.0)
47
48
49  ! -----IV EXPOSURE ---------
50
51   IV= DOSEIV_NM * WT0 !AMOUNT IN NMOL
52   IVR= IV/PFUNC ! RATE FOR IV INFUSION IN BLOOD
53   EXPIV= IVR * (1.0-STEP(PFUNC))
54   IVDOSE = integ(EXPIV,0.0)
55
56      !------IV late in the cycle
```

```
    ! MODIFICATION ON January 13 2004
 IV_RlateR = DOSEIVNMlate*WT0
 IV_EXPOSURE=PULSE(IV_LACK,IV_PERIOD,IV_FINISH)

 IV_lateT = IV_EXPOSURE *IV_RlateR
 IV_late = IV_lateT/CINT

SUMEXPEVENTIV= integ (IV_EXPOSURE,0.0) !NUMBER OF CYCLE GENERATE DURING
SIMULATION

    !SYSTEMIC CONCENTRATION OF TCDD
    ! MODIFICATION ON OCTOBER 6, 2009
 CB=(QF*CFB+QRE*CREB+QLI*CLIB+EXPIV+LYRMLUM+QPLA*CPLAB+IV_late)/(QC+CLURI) !
 CA = CB ! CONCENTRATION (NMOL/ML)

    !URINARY EXCRETION BY KIDNEY
    !MODIFICATION ON OCTOBER 6, 2009
RAURI = CLURI *CB
  AURI = INTEG(RAURI,0.0)

  !UNIT CONVERSION POST SIMULATION
 CBSNGKGLIADJ=(CB*MW*UNITCORR*(1/B_TOTLIP)*(1/SERBLO))![NG of TCDD Serum/Kg
OF LIPIP]
   AUCBS_NGKGLIADJ=integ(CBSNGKGLIADJ,0.0)

  PRCT_B = (CB/(MSTT+1E-30))*100 ! PERCENT OF ORAL DOSE IN BLOOD
  PRCT_BIV = (CB/(IV_RlateR+1E-30))*100 ! PERCENT OF IV DOSE IN BLOOD
  CBNGKG= CB*MW*UNITCORR
  CBNGG = CB*MW

   !ADIPOSE COMPARTMENT
   !TISSUE BLOOD COMPARTMENT
RAFB= QF*(CA-CFB)-PAF*(CFB-CF/PF)     !(NMOL/H)
 AFB = INTEG(RAFB,0.0)                 !(NMOL)
  CFB = AFB/WFB                        !(NMOL/ML)
   !TISSUE COMPARTMENT
RAF = PAF*(CFB-CF/PF)                 !(NMOL/H)
 AF = INTEG(RAF,0.0)                  !(NMOL)
  CF  = AF/WF                          !(NMOL/ML)

   !UNIT CONVERSION POST SIMULATION
  CFTOTAL= (AF + AFB)/(WF + WFB) ! TOTAL CONCENTRATION IN NMOL/ML
  CFTFREE = CFB + CF !TOTAL FREE CONCENTRATION IN FAT  (NM/ML)
  PRCT_F = (CFTOTAL/(MSTT+1E-30))*100 ! PERCENT OF ORAL DOSE IN FAT
  PRCT_FIV = (CFTOTAL/(IV_RlateR+1E-30))*100 ! PERCENT OF IV DOSE IN FAT
  CFNGKG=CFTOTAL*MW*UNITCORR ! FAT CONCENTRATION IN NG/KG
    AUCF_NGKGH=integ(CFNGKG,0.0)
  CFNGG = CFTOTAL*MW

   !REST OF THE BODY COMPARTMENT
RAREB= QRE *(CA-CREB)-PARE*(CREB-CRE/PRE)  !(NMOL/H)
 AREB = INTEG(RAREB,0.0)                    !(NMOL)
  CREB = AREB/WREB                          !(NMOL/H)
   !TISSUE COMPARTMENT
RARE = PARE*(CREB - CRE/PRE)               !(NMOL/H)
 ARE = INTEG(RARE,0.0)                     !(NMOL)
```

```
  CRE  = ARE/WRE                                !(NMOL/ML)

   !UNIT CONVERSION POST SIMULATION
  CRETOTAL= (ARE + AREB)/(WRE + WREB)           ! TOTAL CONCENTRATION IN
NMOL/ML
  PRCT_RE = (CRETOTAL/(MSTT+1E-30))*100 ! PERCENT OF ORAL DOSE IN REST OF
BODY
  PRCT_REIV = (CRETOTAL/(IV_RlateR+1E-30))*100   ![ PERCENT OF IV DOSE IN
REST OF THE BODY ]
  CRENGKG=CRETOTAL*MW*UNITCORR ! REST OF THE BODY CONCENTRATION IN NG/KG


   !LIVER COMPARTMENT
   !TISSUE BLOOD COMPARTMENT
 RALIB = QLI*(CA-CLIB)-PALI*(CLIB-CFLLIR)+LIRMLUM !
  ALIB = INTEG(RALIB,0.0)                        !(NMOL)
   CLIB = ALIB/WLIB                             !(NMOL/ML)
   !TISSUE COMPARTMENT
 RALI = PALI*(CLIB - CFLLIR)-REXCLI             !  (NMOL/HR)
  ALI = INTEG(RALI,0.0)                           !(NMOL)
   CLI  = ALI/WLI                                !(NMOL/ML)

   !FREE TCDD IN LIVER COMPARTMENT
PARAMETER (LIVER_1RMN = 1.0E-30)
 CFLLI= IMPLC(CLI-(CFLLIR*PLI+(LIBMAX*CFLLIR/(KDLI+CFLLIR &
        +LIVER_1RMN))+((CYP1A2_1O3*CFLLIR/(KDLI2 + CFLLIR &
        +LIVER_1RMN)*PAS_INDUC)))-CFLLI,CFLLI0)
     CFLLIR=DIM(CFLLI,0.0) ! FREE CONCENTRATION IN LIVER

  CBNDLI= LIBMAX*CFLLIR/(KDLI+CFLLIR+LIVER_1RMN) !BOUND CONCENTRATION

  !VARIABLE ELIMINATION BASED ON THE CYP1A2
 KBILE_LI_T =((CYP1A2_1OUT-CYP1A2_1A2)/CYP1A2_1A2)*Kelv ! INDUCED BILIARY
EXCRETION RATE CONSTANT
  REXCLI = KBILE_LI_T*CFLLIR*WLI ! DOSE-DEPENDENT EXCRETION RATE
    EXCLI = INTEG(REXCLI,0.0)

  !UNIT CONVERSION POST SIMULATION
  CLITOTAL= (ALI + ALIB)/(WLI + WLIB) ! TOTAL CONCENTRATION IN NMOL/ML
  PRCT_LI = (CLITOTAL/(MSTT+1E-30))*100 ! PERCENT ORAL DOSE IN LIVER
  PRCT_LIIV = (CLITOTAL/(IV_RlateR+1E-30))*100 ! PERCENT IV DOSE IN LIVER
  Rec_occ= CFLLIR/(KDLI+CFLLIR)
  CLINGKG=CLITOTAL*MW*UNITCORR ! LIVER CONCENTRATION IN NG/KG
     AUCLI_NGKGH=INTEG(CLINGKG,0.0)
  CBNDLINGKG = CBNDLI*MW*UNITCORR
     AUCBNDLI_NGKGH =INTEG(CBNDLINGKG,0.0)
  CLINGG = CLITOTAL*MW

   !CHEMICAL IN CYP450 (1A2) COMPARTMENT
CYP1A2_1KINP = CYP1A2_1KOUT* CYP1A2_1OUTZ ! BASAL RATE OF CYP1A2 PRODUCTION
SET EQUAL TO BASAL RATE OF DEGREDATION

   ! MODIFICATION ON OCTOBER 6, 2009
CYP1A2_1OUT =INTEG(CYP1A2_1KINP * (1.0 + CYP1A2_1EMAX *(CBNDLI+1.0e-30)**HILL
&
     /(CYP1A2_1EC50**HILL + (CBNDLI+1.0e-30)**HILL)) &
```

```
      - CYP1A2_1KOUT*CYP1A2_1OUT, CYP1A2_1OUTZ)

! EQUATIONS INCORPORATING DELAY OF CYP1A2 PRODUCTION (NOT USED IN
SIMULATIONS)

      CYP1A2_1RO2 = (CYP1A2_1OUT - CYP1A2_1O2)/ CYP1A2_1TAU
  CYP1A2_1O2 =INTEG(CYP1A2_1RO2, CYP1A2_1A1)

CYP1A2_1RO3 = (CYP1A2_1O2 - CYP1A2_1O3)/ CYP1A2_1TAU
  CYP1A2_1O3 =INTEG(CYP1A2_1RO3, CYP1A2_1A2)

! TRANSFER OF DIOXIN FROM PLACENTA TO FETUS
! FETAL EXPOSURE ONLY DURING EXPOSURE

IF (T.LT.TRANSTIME_ON) THEN
 SWITCH_trans = 0.0
ELSE
 SWITCH_trans = 1
END IF

!TRANSFER OF DIOXIN FROM PLACENTA TO FETUS
! MODIFICATION 26 SEPTEMBER 2003

CONSTANT PFETUS= 4 !
CONSTANT CLPLA_FET = 0.17 !

RAMPF = (CLPLA_FET*CPLA) *SWITCH_trans
  AMPF=INTEG(RAMPF,0.0)

!TRANSFER OF DIOXIN FROM FETUS TO PLACENTA
RAFPM = (CLPLA_FET*CFETUS_v)*SWITCH_trans !
  AFPM = INTEG(RAFPM,0.0)

! TCDD IN PLACENTA MOTHER COMPARTMENT
RAPLAB= QPLA*(CA - CPLAB)-PAPLA*(CPLAB -CFLPLAR)    ! NMOL/H)
 APLAB = INTEG(RAPLAB,0.0)                           ! (NMOL)
 CPLAB = APLAB/(WPLAB+1E-30)                        ! (NMOL/ML)
RAPLA = PAPLA*(CPLAB-CFLPLAR)-RAMPF + RAFPM         ! (NMOL/H)
 APLA = INTEG(RAPLA,0.0)                             ! (NMOL)
 CPLA  = APLA/(WPLA+1e-30)                          ! (NMOL/ML)

PARAMETER (PARA_ZERO = 1.0E-30)
CFLPLA= IMPLC(CPLA-(CFLPLAR*PPLA +(PLABMAX*CFLPLAR/(KDPLA&
    +CFLPLAR+PARA_ZERO)))-CFLPLA,CFLPLA0)
CFLPLAR=DIM(CFLPLA,0.0)

   !UNIT CONVERSION POST SIMULATION
  CPLATOTAL= (APLA + APLAB)/((WPLA + WPLAB)+1e-30)! TOTAL CONCENTRATION IN
NMOL/ML
  PRCT_PLA = (CPLATOTAL/(MSTT+1E-30))*100
  PRCT_PLAIV = (CPLATOTAL/(IV_RlateR+1E-30))*100
  CPLANGG = CPLATOTAL*MW

   !FETUS COMPARTMENT
RAFETUS= RAMPF-RAFPM
 AFETUS=INTEG(RAFETUS,0.0)
```

```
CFETUS=AFETUS/(WTFE+1E-30)
CFETOTAL= CFETUS
CFETUS_v = CFETUS/PFETUS

  ! UNIT CONVERSION POST SIMULATION
CFETUSNGKG = CFETUS*MW*UNITCORR                    !(NG/KG)
AUC_FENGKGH = INTEG(CFETUSNGKG,0.0)
PRCT_FE = (CFETOTAL/(MSTT+1E-30))*100
PRCT_FEIV = (CFETOTAL/(IV_RlateR+1E-30))*100
CFETUSNGG = CFETOTAL*MW

! ------------CONTROL MASS BALANCE ----------
BDOSE= IVDOSE +LYMLUM+LIMLUM
BMASSE = EXCLI+AURI+AFB+AF+AREB+ARE+ALIB+ALI+APLA+APLAB+AFETUS
BDIFF = BDOSE-BMASSE

      !BODY BURDEN (NG)
BODY_BURDEN = AFB+AF+AREB+ARE+ALIB+ALI+APLA+APLAB !
BBFETUSNG     = AFETUS*MW*UNITCORR    ! NG
      ! BODY BURDEN IN TERMS OF CONCENTRATION (NG/KG)
 BBNGKG =(((AFB+AF+AREB+ARE+ALIB+ALI+APLA+APLAB)/WT0)*MW*UNITCORR) !
  AUC_BBNGKGH=INTEG(BBNGKG,0.0)


! ------------COMMAND OF THE END OF SIMULATION ----------
TERMT (T.GE. TimeLimit, 'Time limit has been reached.')
END   ! END OF THE DERIVATIVE SECTION
END   ! END OF THE DYNAMIC SECTION
END   ! END OF THE PROGRAM
```

C.2.6.2. *Input Files*

C.2.6.2.1. *Keller et al. (2007).*

```
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH

%output @nciout=10 T SUMEXPEVENT wt0

%Keller et al. 2007
%protocol:  single oral dose at GD13
%DevTCDD4Species.csl
%MICE_GESTATIONAL_ICF_F092309.csl
%dose levels: 0.01, 0.100 1 ug/kg at GD13
%dose levels:   10, 100 1000 ng/kg at GD13

%EXPOSURES SCENARIOS
 MAXT=0.01
 CINT =0.1
 EXP_TIME_ON     = 312.          % delay before begin exposure (HOUR)
 EXP_TIME_OFF    = 336           % TIME EXPOSURE STOP (HOUR)
 DAY_CYCLE       = 24
 BCK_TIME_ON     = 0.            % DELAY BEFORE BACGROUND EXPOSURE (HOUR)
```

```
 BCK_TIME_OFF    = 0.            % TIME OF BACKGROUND EXPOSURE STOP (HOUR)
 IV_LACK         = 505
 IV_PERIOD       = 505
 TIMELIMIT       = 336            % SIMULATION LIMIT TIME (HOUR)
 BW_T0           = 24
 MATTING         = 0.             % BEGINNING MATTING (HOUR)
 TRANSTIME_ON    = 144.           % SHOULD BE MATTING TIME + 6 DAYS(144
HOURS)
 N_FETUS         = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)

   %MSTOT         = 0.01           % ORAL EXPOSURE DOSE (UG/KG)
   %MSTOT        = 0.1           % ORAL EXPOSURE DOSE (UG/KG)
   MSTOT        = 1           % ORAL EXPOSURE DOSE (UG/KG)
```

C.2.6.2.2. *Li et al. (2006).*

```
%TO BE USED AFTER THE
%clear variable
output @clear
prepare @clear T CLINGKG CFNGKG CBSNGKGLIADJ BBNGKG CFETUSNGKG AUCLI_NGKGH
AUCF_NGKGH AUCBS_NGKGLIADJ AUC_BBNGKGH AUC_FENGKGH CBNDLINGKG AUCBNDLI_NGKGH
CBNGKG AUC_CBNGKGH
%output @nciout=10 T SUMEXPEVENT
%Li et al.2006
%protocol:  daily oral dose from GD1 to GD3
%DevTCDD4Species.csl
%MICE_GESTATIONAL_ICF_F092309.csl
%dose levels: 0.002, 0.050, 0.10 ug/kg/day at GD1 to GD3
%dose levels: 2, 50, 100 ng/kg/day from GD1 to GD3

%EXPOSURES SCENARIOS
 MAXT=0.01
 CINT =0.1
 EXP_TIME_ON     = 0.            % delay before begin exposure (HOUR)
 EXP_TIME_OFF    = 72           % TIME EXPOSURE STOP (HOUR) 2 HOURS LESS THAN
GD3  put 70 to be sure 3 doses will be administrate
                                 % BECAUSE i STARTED TIME 0 FOR GD1
 DAY_CYCLE       = 24
 BCK_TIME_ON     = 0.            % DELAY BEFORE BACGROUND EXPOSURE (HOUR)
 BCK_TIME_OFF    = 0.            % TIME OF BACKGROUND EXPOSURE STOP (HOUR)
 IV_LACK         = 505
 IV_PERIOD       = 505
 TIMELIMIT       = 72.            % SIMULATION LIMIT TIME (HOUR) Run for 3
days
 BW_T0           = 27
 MATTING         = 0.             % BEGINNING MATTING (HOUR)
 TRANSTIME_ON    = 144.           % SHOULD BE MATTING TIME + 6 DAYS(144
HOURS)
 N_FETUS         = 10

%EXPOSURE DOSE SCENARIOS (UG/KG)

   %MSTOT         = 0.002           % ORAL EXPOSURE DOSE (UG/KG)
   %MSTOT        = 0.05           % ORAL EXPOSURE DOSE (UG/KG)
```

```
MSTOT          = 0.10              % ORAL EXPOSURE DOSE (UG/KG)
```

C.3. TOXICOKINETIC MODELING RESULTS FOR KEY ANIMAL BIOASSAY STUDIES

The simulated TCDD serum-adjusted lipid concentrations reported in this appendix for the rodent bioassays were converted to TCDD concentrations in rodent whole blood. Initially, EPA multiplied the serum-adjusted lipid concentrations by 0.0033, the ratio of lipid content to total serum volume, then by 0.55, the value of the hematocrit. This product yields the TCDD concentration in whole rodent blood as predicted by the PBPK model. EPA assumed that the same whole blood TCDD concentration would result in the same effects in humans and rodents.

This conversion accomplishes the following:

1. Allows the human equivalent dose (HED) to be based on equivalent blood concentration (that represents serum plus erythrocyte TCDD), which is proportional to tissue exposure;
2. Avoids criticism that the total blood concentration is normalized to serum lipid alone in an unbalanced way (thus EPA does not contradict Centers for Disease Control and Prevention (CDC) data or methods);
3. Factors out any impact of the lipid content used in the PBPK model; and
4. TCDD concentration in whole blood is encouraged for use in the assessments by the NAS (NAS, 2006, p. 43); see additional information in Section 3.3.

C.3.1. Nongestational Studies

C.3.1.1. *Cantoni et al. (1981)*

Type:	Rat	**Dose:**	10, 100, 1000 ng/kg/week
Strain:	CD-COBS rats	**Route:**	Oral gavage exposure
Body weight:	BW set to 125g	**Regime:**	1 dose/week for 45 weeks
Sex:	Female	**Simulation time:**	7,560 hours (45 weeks)

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.43	Emond	1.85	3.70 (@ 7,392 hours)	1.82
	CADM	-	-	-
14.29	Emond	8.84	26.6 (@ 7,392 hours)	7.97

	CADM	-	-	-
142.86	Emond	50.0	227 (@ 7,392 hours)	41.9
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.43	Emond	247	328 (@ 7,398 hours)	242
	CADM	374	431	431
14.29	Emond	2,176	2,860 (@ 7,231 hours)	1,928
	CADM	3,884	4,330	4,330
142.86	Emond	20,500	26,978 (@ 7,399 hours)	17,255
	CADM	39,067	43,329	43,329
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.43	Emond	175	200 (@ 7,431 hours)	181
	CADM	250	280	244
14.29	Emond	837	937 (@ 7,427 hours)	807
	CADM	1,209	1,352	1,167
142.86	Emond	4,741	5,374 (@ 7,424 hours)	4,349
	CADM	10,050	11,224	9,734
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.43	Emond	26.1	31.7 (@ 7,398 hours)	26.3
	CADM	32.0	35.0	35.0
14.29	Emond	170	210 (@ 7,230 hours)	156
	CADM	225	243	243
142.86	Emond	1,337	1,695 (@ 7,398 hours)	1,151
	CADM	2,106	2,266	2,266

BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.43	Emond	6.04	7.76 (@ 7,396 hours)	6.01
	CADM	-	-	-
14.29	Emond	23.7	29.1 (@ 7,228 hours)	22.2
	CADM	-	-	-
142.86	Emond	66.8	80.0 (@ 1 hours)	63.4
	CADM	-	-	-

C.3.1.2. *Chu et al. (2007)*

Type:	Rat	**Dose:**	2.5, 25, 250, and 1,000 ng/kg-day
Strain:	Sprague-Dawley	**Route:**	Oral exposure
Body weight:	200 g	**Regime:**	1 dose per day for 28 days
Sex:	Female	**Simulation time:**	672 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.5	Emond	1.26	2.35 (@ 648 hours)	1.88
	CADM	-	-	-
25	Emond	7.66	15.3 (@ 648 hours)	10.4
	CADM	-	-	-
250	Emond	48.8	113 (@ 648 hours)	63.7
	CADM	-	-	-
1,000	Emond	169	418 (@ 648 hours)	222
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.5	Emond	148	268 (@ 652 hours)	255
	CADM	-	-	-
25	Emond	1,777	2,953 (@ 653 hours)	2,806
	CADM	-	-	-

250	Emond	19,232	30,262 (@ 653 hours)	28,668
	CADM	-	-	-
1,000	Emond	77,819	120,400 (@ 653 hours)	113,890
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.5	Emond	108	180 (@ 668 hours)	180
	CADM	-	-	-
25	Emond	660	1,020 (@ 659 hours)	1,015
	CADM	-	-	-
250	Emond	4,210	6,433 (@ 655 hours)	6,354
	CADM	-	-	-
1,000	Emond	14,576	22,610 (@ 655 hours)	22,280
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.5	Emond	16.1	27.5 (@ 652 hours)	26.9
	CADM	-	-	-
25	Emond	138	222 (@ 652 hours)	214
	CADM	-	-	-
250	Emond	1,239	1,935 (@ 652 hours)	1,842
	CADM	-	-	-
1,000	Emond	4,801	7,444 (@ 652 hours)	7,067
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.5	Emond	4.15	6.51 (@ 652 hours)	6.21
	CADM	-	-	-
25	Emond	20.5	28.5 (@ 652 hours)	27.4
	CADM	-	-	-
250	Emond	63.3	76.0 (@ 652 hours)	74.7
	CADM	-	-	-

1,000	Emond	90.2	99.0 (@ 653 hours)	98.3
	CADM	-	-	-

C.3.1.3. *Crofton et al. (2005)*

Type:	Rats	**Dose:**	0, 0.1, 3, 10, 30, 100, 300, 1000, 3000, and 10,000 ng/kg-day
Strain:	Long Evans	**Route:**	Oral exposure
Body weight:	4 weeks old BW set to 190 g	**Regime:**	One dose per day for four days
Sex:	Female	**Simulation time:**	96 hours

The CADM model was not run because the dosing duration is lower than the resolution of the model (1 week)

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.1	Emond	0.0202	0.041 (@ 72 hours)	0.0244
	CADM	-	-	-
3	Emond	0.488	1.10 (@ 72 hours)	0.582
	CADM	-	-	-
10	Emond	1.38	3.40 (@ 72 hours)	1.62
	CADM	-	-	-
30	Emond	3.46	9.44 (@ 72 hours)	3.93
	CADM	-	-	-
100	Emond	9.26	29.0 (@ 72 hours)	10.2
	CADM	-	-	-
300	Emond	23.1	81.8 (@ 72 hours)	24.5
	CADM	-	-	-
1000	Emond	65.7	260 (@ 72 hours)	68.2
	CADM	-	-	-
3000	Emond	181	764 (@ 72 hours)	187
	CADM	-	-	-
10,000	Emond	583	2,527 (@ 72 hours)	607
	CADM	-	-	-

LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.1	Emond	0.919	1.55 (@ 75 hours)	1.18
	CADM	-	-	-
3	Emond	37.4	62.6 (@ 76 hours)	53.3
	CADM	-	-	-
10	Emond	145	242 (@ 77 hours)	214
	CADM	-	-	-
30	Emond	494	818 (@ 78 hours)	742
	CADM	-	-	-
100	Emond	1,839	3,025 (@ 78 hours)	2,793
	CADM	-	-	-
300	Emond	5,925	9,692 (@ 78 hours)	9,028
	CADM	-	-	-
1000	Emond	20,717	33,738 (@ 79 hours)	31,564
	CADM	-	-	-
3000	Emond	63,511	103,140 (@ 79 hours)	96,545
	CADM	-	-	-
10,000	Emond	212,890	344,910 (@ 79 hours)	321,960
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.1	Emond	1.00	1.93 (@ 96 hours)	1.93
	CADM	-	-	-
3	Emond	24.6	45.9 (@ 96 hours)	45.9
	CADM	-	-	-
10	Emond	70.3	129 (@ 96 hours)	129
	CADM	-	-	-
30	Emond	177	317 (@ 96 hours)	317
	CADM	-	-	-
100	Emond	480	838 (@ 96 hours)	838
	CADM	-	-	-

300	Emond	1,206	2,065 (@ 96 hours)	2,065
	CADM	-	-	-
1000	Emond	3,452	5,836 (@ 96 hours)	5,836
	CADM	-	-	-
3000	Emond	9,522	16,050 (@ 96 hours)	16,050
	CADM	-	-	-
10,000	Emond	30,657	51,918 (@ 96 hours)	51,918
	CADM	-	-	-

BODY BURDEN (ng/kg)

Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.1	Emond	0.138	0.224 (@ 79 hours)	0.223
	CADM	-	-	-
3	Emond	4.04	6.56 (@ 78 hours)	6.44
	CADM	-	-	-
10	Emond	13.3	21.5 (@ 78 hours)	21.0
	CADM	-	-	-
30	Emond	39.3	63.5 (@ 78 hours)	61.5
	CADM	-	-	-
100	Emond	129	208 (@ 78 hours)	200
	CADM	-	-	-
300	Emond	384	618 (@ 77 hours)	590
	CADM	-	-	-
1000	Emond	1,270	2,041 (@ 77 hours)	1,942
	CADM	-	-	-
3000	Emond	3,793	6,094 (@ 77 hours)	5,784
	CADM	-	-	-
10,000	Emond	12,595	20,226 (@ 77 hours)	19,154
	CADM	-	-	-

BOUND LIVER (ng/kg)

Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.1	Emond	0	0.115 (@ 75 hours)	0
	CADM	-	-	-

3	Emond	2	2.47 (@ 76 hours)	2
	CADM	-	-	-
10	Emond	4	6.42 (@ 76 hours)	5
	CADM	-	-	-
30	Emond	10	14.1 (@ 76 hours)	12
	CADM	-	-	-
100	Emond	22	29.9 (@ 76 hours)	27
	CADM	-	-	-
300	Emond	41	51.9 (@ 77 hours)	49
	CADM	-	-	-
1000	Emond	68	80.2 (@ 1 hours)	77
	CADM	-	-	-
3000	Emond	90	98.6 (@ 1 hours)	96
	CADM	-	-	-
10,000	Emond	104	108 (@ 1 hours)	107
	CADM	-	-	-

C.3.1.4. *Della Porta et al. (2001) (female)*

Type:	Mouse	**Dose:**	2,500 and 5,000 ng/kg-week (equivalent to 357 and 714 ng/kg-day)
Strain:	B6C3	**Route:**	Gavage
Body weight:	6 weeks old (BW 20g)	**Regime:**	Once a week for 52 weeks
Sex:	Female	**Simulation time:**	8,736 hours

The CADM model was not run because the study duration is longer than the allowed model duration

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
357	Emond	67.0	741 (@ 8,568 hours)	46.8
	CADM	-	-	-
714	Emond	37.6	374 (@ 8,568 hours)	27.2
	CADM	-	-	-

LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
357	Emond	50,269	70,070 (@ 8,577 hours)	37,389
	CADM	-	-	-
714	Emond	25,422	35,352 (@ 8,577 hours)	19,105
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
357	Emond	25,235	28,559 (@ 8,589 hours)	22,498
	CADM	-	-	-
714	Emond	14,162	15,914 (@ 8,590 hours)	12,810
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
357	Emond	5,473	7,247 (@ 8,574 hours)	4,335
	CADM	-	-	-
714	Emond	2,878	3,774 (@ 8,574 hours)	2,318
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
357	Emond	71.5	99.1 (@ 2 hours)	65.4
	CADM	-	-	-
714	Emond	56.4	88.6 (@ 2 hours)	50.4
	CADM	-	-	-

C.3.1.5. *Della Porta et al. (2001) (male)*

Type:	Mouse	**Dose:**	2,500 and 5,000 ng/kg-week (equivalent to 357 and 714 ng/kg-day)
Strain:	B6C3	**Route:**	Gavage
Body weight:	6 weeks old (BW 26g)	**Regime:**	Once a week for 52 weeks
Sex:	Male	**Simulation time:**	8,736 hours

The CADM model was not run because the study duration is longer than the allowed model duration

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
357	Emond	67.8	787 (@ 8,568 hours)	47.0
	CADM	-	-	-
714	Emond	38.0	398 (@ 8,568 hours)	27.3
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
357	Emond	50,397	70,052 (@ 8,577 hours)	37,483
	CADM	-	-	-
714	Emond	25,493	35,347 (@ 8,577 hours)	19,155
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
357	Emond	25,516	28,851 (@ 8,589 hours)	22,861
	CADM	-	-	-
714	Emond	14,306	16,061 (@ 8,590 hours)	12,999
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
357	Emond	5,504	7,282 (@ 8,574 hours)	4,368
	CADM	-	-	-

714	Emond	2,894	3,791 (@ 8,574 hours)	2,335
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
357	Emond	71.6	99.2 (@ 2 hours)	65.4
	CADM	-	-	-
714	Emond	56.4	88.6 (@ 2 hours)	50.4
	CADM	-	-	-

C.3.1.6. *Fattore et al. (2000)*

Type:	Rat	**Dose:**	20, 200, 2,000 ng/kg-day
Strain:	Sprague Dawley	**Route:**	Oral in the diet
Body weight:	7 weeks old (BW 150g)	**Regime:**	Every day for 13 weeks
Sex:	Female and male	**Simulation time:**	2,184 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
20	Emond	9.59	15.0 (@ 2,160 hours)	11.1
	CADM	-	-	-
200	Emond	57.6	102 (@ 2,160 hours)	63.9
	CADM	-	-	-
2,000	Emond	476	903 (@ 2,160 hours)	522
	CADM	-	-	-

LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
20	Emond	2,448	3,228 (@ 2,164 hours)	3,078
	CADM	4,471	5,639	5,639
200	Emond	24,136	30,245 (@ 2,164 hours)	28,709
	CADM	45,337	56,499	56,499
2,000	Emond	234,170	288,020 (@ 2,164 hours)	272,590
	CADM	454,031	565,103	565,103
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
20	Emond	890	1,113 (@ 2,166 hours)	1,101
	CADM	1,545	1,796	1,756
200	Emond	5,355	6,542 (@ 2,165 hours)	6,430
	CADM	13,351	15,604	15,292
2,000	Emond	44,176	54,246 (@ 2,165 hours)	53,140
	CADM	131,259	153,534	150,516
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
20	Emond	187	242 (@ 2,164 hours)	233
	CADM	261	324	324
200	Emond	1,556	1,940 (@ 2,164 hours)	1,850
	CADM	2,496	3,084	3,084
2,000	Emond	14,432	17,797 (@ 2,164 hours)	16,891
	CADM	24,836	30,674	30,674
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
20	Emond	24.9	29.8 (@ 2,164 hours)	28.8
	CADM	-	-	-
200	Emond	69.4	76.0 (@ 2,164 hours)	74.7
	CADM	-	-	-

2,000	Emond	104	106 (@ 2,164 hours)	106
	CADM	-	-	-

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3 **C.3.1.7.** ***Franc et al. (2001) Sprague Dawley Rats***

Type:	Rats	**Dose:**	140, 420, and 1400 ng/kg every two weeks (equivalent to 10, 30, and 100 ng/kg-day)
Strain:	Sprague Dawley,	**Route:**	Oral gavage
Body weight:	200 g (10 weeks old)	**Regime:**	Once every two weeks for 22 weeks
Sex:	Female	**Simulation time:**	3,696 hours

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WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	6.59	34.6 (@ 3,360 hours)	5.52
	CADM	-	-	-
30	Emond	14.5	98.1 (@ 3,360 hours)	11.3
	CADM	-	-	-
WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
100	Emond	36.4	315 (@ 3,360 hours)	26.4
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	1,447	2,458 (@ 3,368 hours)	1,150
	CADM	2,616	3,620	2,174
30	Emond	4,228	7,161 (@ 3,368 hours)	3,120
	CADM	7,936	10,899	6,510
100	Emond	13,821	23,417 (@ 3,368 hours)	9,658
	CADM	26,564	36,361	21,703

FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	619	787 (@ 3,417 hours)	560
	CADM	966	1,230	759
30	Emond	1,362	1,741 (@ 3,415 hours)	1,161
	CADM	2,448	3,203	1,849
100	Emond	3,430	4,464 (@ 3,412 hours)	2,755
	CADM	7,573	10,052	5,606
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	119	177 (@ 3,366 hours)	99.5
	CADM	159	212	133
30	Emond	308	472 (@ 3,366 hours)	240
	CADM	450	603	367
100	Emond	921	1,445 (@ 3,366 hours)	671
	CADM	1,462	1,969	1,181
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	18.6	32.9 (@ 1 hours)	16.4
	CADM	-	-	-
30	Emond	33.7	59.2 (@ 1 hours)	29.0
	CADM	-	-	-
100	Emond	57.5	86.9 (@ 1 hours)	50.4
	CADM	-	-	-

C.3.1.8. *Franc et al. (2001) Long-Evans Rats*

Type:	Rats	**Dose:**	140, 420, and 1400 ng/kg every two weeks (equivalent to 10, 30, and 100 ng/kg-day)
Strain:	Long-Evans	**Route:**	Oral gavage
Body weight:	190 g (10 weeks old)	**Regime:**	Once every two weeks for 22 weeks
Sex:	Female	**Simulation time:**	3,696 hours

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WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	6.58	34.2 (@ 3,360 hours)	5.52
	CADM	-	-	-
30	Emond	14.5	97.0 (@ 3,360 hours)	11.3
	CADM	-	-	-
100	Emond	36.4	312 (@ 3,360 hours)	26.4
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	1,447	2,458 (@ 3,368 hours)	1,150
	CADM	2,616	3,620	2,174
30	Emond	4,228	7,161 (@ 3,368 hours)	3,121
	CADM	7,936	10,899	6,510
100	Emond	13,821	23,421 (@ 3,368 hours)	9,659
	CADM	26,564	36,361	21,703
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	619	788 (@ 3,417 hours)	560
	CADM	966	1,230	759
30	Emond	1,362	1,742 (@ 3,414 hours)	1,160
	CADM	2,448	3,203	1,849
100	Emond	3,429	4,466 (@ 3,412 hours)	2,752
	CADM	7,573	10,052	5,606
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	119	177 (@ 3,366 hours)	99.5
	CADM	159	212	133
30	Emond	308	472 (@ 3,366 hours)	240
	CADM	450	603	367
100	Emond	921	1,445 (@ 3,366 hours)	671
	CADM	1,462	1,969	1,181

BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	18.6	32.9 (@ 1 hours)	16.4
	CADM	-	-	-
30	Emond	33.7	59.2 (@ 1 hours)	29.0
	CADM	-	-	-
100	Emond	57.5	86.9 (@ 1 hours)	50.4
	CADM	-	-	-

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C.3.1.9. *Franc et al. (2001) Hans Wistar Rats*

Type:	Rats	**Dose:**	140, 420, and 1400 ng/kg every two weeks (equivalent to 10, 30, and 100 ng/kg-day)
Strain:	Hans Wistar	**Route:**	Oral gavage
Body weight:	205 g (10 weeks old)	**Regime:**	Once every two weeks for 22 weeks
Sex:	Female	**Simulation time:**	3,696 hours

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WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	6.59	34.7 (@ 3,360 hours)	5.52
	CADM	-	-	-
30	Emond	14.5	98.7 (@ 3,360 hours)	11.3
	CADM	-	-	-
100	Emond	36.4	317 (@ 3,360 hours)	26.4
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	1,447	2,458 (@ 3,368 hours)	1,150
	CADM	2,616	3,620	2,174
30	Emond	4,228	7,160 (@ 3,368 hours)	3,120
	CADM	7,936	10,899	6,510

100	Emond	13,821	23,416 (@ 3,368 hours)	9,658
	CADM	26,564	36,361	21,703
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	619	787 (@ 3,418 hours)	560
	CADM	966	1,230	759
30	Emond	1,363	1,741 (@ 3,415 hours)	1,162
	CADM	2,448	3,203	1,849
100	Emond	3,431	4,463 (@ 3,412 hours)	2,757
	CADM	7,573	10,052	5,606
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	119	177 (@ 3,366 hours)	99.5
	CADM	159	212	133
30	Emond	308	472 (@ 3,366 hours)	240
	CADM	450	603	367
100	Emond	921	1,446 (@ 3,366 hours)	671
	CADM	1,462	1,969	1,181
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	18.6	32.9 (@ 1 hours)	16.4
	CADM	-	-	-
30	Emond	33.7	59.2 (@ 1 hours)	29.0
	CADM	-	-	-
100	Emond	57.5	86.9 (@ 1 hours)	50.4
	CADM	-	-	-

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C.3.1.10. *Hassoun et al. (2000)*

Type:	Rat	**Dose:**	0, 3, 10, 22, 46, 100 ng/kg/day (2.14, 7.14, 15.7, 32.9, and 71.4 ng/kg/day adjusted doses)
Strain:	Sprague Dawley	**Route:**	Oral gavage
Body weight:	8 weeks old (BW=215g)	**Regime:**	5 days/week for 13 weeks
Sex:	Female	**Simulation time:**	2184 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	1.94	3.12 (@ 2,112 hours)	1,303.17
	CADM	-	-	-
7.14	Emond	4.6136	7.71 (@ 2,112 hours)	2,901.26
	CADM	-	-	-
15.7	Emond	8.147	14.2 (@ 2,112 hours)	4,947.3
	CADM	-	-	-
32.9	Emond	14.009	25.8 (@ 2,112 hours)	8,277
	CADM	-	-	-
71.4	Emond	25.34	49.7 (@ 2,112 hours)	14,637
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	266.8	399 (@ 2,116 hours)	349
	CADM	-	-	-
7.14	Emond	888	1,259 (@ 2,117 hours)	1,079
	CADM	-	-	-
15.7	Emond	1,948.499	2,689 (@ 2,117 hours)	2,278.182
	CADM	-	-	-
32.9	Emond	4,055.031	5,484 (@ 2,117 hours)	4,607.265
	CADM	-	-	-
71.4	Emond	8,774.97	11,692 (@ 2,117 hours)	9,754.31
	CADM	-	-	-

FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	179.2	243 (@ 2,126 hours)	234.9
	CADM	-	-	-
7.14	Emond	427	553 (@ 2,124 hours)	528
	CADM	-	-	-
15.7	Emond	755	958 (@ 2,123 hours)	908
	CADM	-	-	-
32.9	Emond	1,299	1,627 (@ 2,122 hours)	1,529
	CADM	-	-	-
71.4	Emond	2,349.892	2,928 (@ 2,121 hours)	2,727.240
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	27.425	38.9 (@ 2,116 hours)	35.720
	CADM	-	-	-
7.14	Emond	76.87	105 (@ 2,116 hours)	93.67
	CADM	-	-	-
15.7	Emond	153.1	205 (@ 2,116 hours)	180.2
	CADM	-	-	-
32.9	Emond	295	390 (@ 2,116 hours)	339
	CADM	-	-	-
71.4	Emond	600	785 (@ 2,116 hours)	674
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	6	8.48 (@ 2,116 hours)	8
	CADM	-	-	-
7.14	Emond	13.7242	17.5 (@ 2,116 hours)	15.7348
	CADM	-	-	-
15.7	Emond	21.9703	27.1 (@ 2,116 hours)	24.4047
	CADM	-	-	-
32.9	Emond	32.817	39.2 (@ 2,116 hours)	35.608
	CADM	-	-	-

71.4	Emond	47.54	55.0 (@ 2,116 hours)	50.63
	CADM	-	-	-

C.3.1.11. *Hutt et al. (2008)*

Type:	Rat	**Dose:**	50 ng/kg-week
Strain:	Sprague-Dawley	**Route:**	Oral gavage
Body weight:	4.5 g	**Regime:**	1/week for 13 weeks
Sex:	Female	**Simulation time:**	2,184 hours (weekly exposure)

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
7.14	Emond	4.49	8.86 (@ 2,016 hours)	4.71
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
7.14	Emond	867.4	1,363 (@ 2,021 hours)	928.1
	CADM	1,678	2,007	2,007
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
7.14	Emond	423.6	555 (@ 2,040 hours)	459.9
	CADM	730	787.1	769
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
7.14	Emond	76	108 (@ 2,022 hours)	81
	CADM	108	126	126

BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
7.14	Emond	14	19.4 (@ 2,020 hours)	14
	CADM	-	-	-

C.3.1.12. *Kitchin and Woods (1979)*

Type:	Rats	**Dose:**	0, 0.6, 2, 4, 20, 60, 200, 600, 2000, 5000, 20,000 ng/kg/day
Strain:	Sprague-Dawley	**Route:**	Oral exposure
Body weight:	200 to 250 g (BW set to 225 g)	**Regime:**	Single dose
Sex:	Female	**Simulation time:**	24 hours*

* 1 week is the minimum that can be simulated with the CADM model, so the CADM model was not used.

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day)	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.6	Emond	0.0645	0.126 (@ 0 hours)	0.0441
	CADM	-	-	-
2	Emond	0.202	0.421 (@ 0 hours)	0.137
	CADM	-	-	-
4	Emond	0.384	0.841 (@ 0 hours)	0.258
	CADM	-	-	-
20	Emond	1.61	4.21 (@ 0 hours)	1.04
	CADM	-	-	-
60	Emond	4.15	12.6 (@ 0 hours)	2.55
	CADM	-	-	-
200	Emond	11.6	42.1 (@ 0 hours)	6.61
	CADM	-	-	-
600	Emond	30.3	126 (@ 0 hours)	15.8
	CADM	-	-	-
2000	Emond	90.9	422 (@ 0 hours)	42.8
	CADM	-	-	-

5000	Emond	218	1,056 (@ 0 hours)	96.9
	CADM	-	-	-
20000	Emond	863	4,233 (@ 0 hours)	365
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day)	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.6	Emond	2.95	3.81 (@ 4 hours)	2.31
	CADM	-	-	-
2	Emond	10.5	12.9 (@ 4 hours)	8.69
	CADM	-	-	-
4	Emond	22.2	26.3 (@ 4 hours)	18.9
	CADM	-	-	-
20	Emond	128	143 (@ 6 hours)	118
	CADM	-	-	-
60	Emond	420	463 (@ 8 hours)	406
	CADM	-	-	-
200	Emond	1,523	1,666 (@ 9 hours)	1,526
	CADM	-	-	-
600	Emond	4,821	5,258 (@ 10 hours)	4,932
	CADM	-	-	-
2000	Emond	16,603	18,080 (@ 11 hours)	17,226
	CADM	-	-	-
5000	Emond	41,971	45,674 (@ 11 hours)	43,803
	CADM	-	-	-
20000	Emond	167,820	182,580 (@ 11 hours)	175,890
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day)	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.6	Emond	1.60	2.47 (@ 24 hours)	2.47
	CADM	-	-	-
2	Emond	5.07	7.71 (@ 24 hours)	7.71
	CADM	-	-	-

4	Emond	9.68	14.6 (@ 24 hours)	14.6
	CADM	-	-	-
20	Emond	41.7	60.7 (@ 24 hours)	60.7
	CADM	-	-	-
60	Emond	110	155 (@ 24 hours)	155
	CADM	-	-	-
200	Emond	317	427 (@ 24 hours)	427
	CADM	-	-	-
600	Emond	851	1,102 (@ 24 hours)	1,102
	CADM	-	-	-
2000	Emond	2,620	3,276 (@ 24 hours)	3,276
	CADM	-	-	-
5000	Emond	6,361	7,816 (@ 24 hours)	7,816
	CADM	-	-	-
20000	Emond	25,401	30,827 (@ 24 hours)	30,827
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day)	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.6	Emond	0.322	0.341 (@ 9 hours)	0.338
	CADM	-	-	-
2	Emond	1.07	1.14 (@ 8 hours)	1.12
	CADM	-	-	-
4	Emond	2.14	2.27 (@ 8 hours)	2.23
	CADM	-	-	-
20	Emond	10.6	11.3 (@ 8 hours)	11.0
	CADM	-	-	-
60	Emond	31.7	33.8 (@ 7 hours)	32.8
	CADM	-	-	-
200	Emond	105	112 (@ 7 hours)	108
	CADM	-	-	-
600	Emond	315	337 (@ 7 hours)	324
	CADM	-	-	-
2000	Emond	1,049	1,123 (@ 7 hours)	1,074
	CADM	-	-	-

5000	Emond	2,621	2,806 (@ 7 hours)	2,680
	CADM	-	-	-
20000	Emond	10,468	11,215 (@ 7 hours)	10,693
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day)	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.6	Emond	0.216	0.309 (@ 3 hours)	0.159
	CADM	-	-	-
2	Emond	0.668	0.975 (@ 3 hours)	0.494
	CADM	-	-	-
4	Emond	1.25	1.86 (@ 3 hours)	0.927
	CADM	-	-	-
20	Emond	4.87	7.67 (@ 2 hours)	3.66
	CADM	-	-	-
60	Emond	11.2	18.3 (@ 2 hours)	8.55
	CADM	-	-	-
200	Emond	25.1	40.8 (@ 1 hours)	19.7
	CADM	-	-	-
600	Emond	45.8	68.2 (@ 1 hours)	37.6
	CADM	-	-	-
2000	Emond	73.3	93.1 (@ 1 hours)	64.7
	CADM	-	-	-
5000	Emond	90.9	104 (@ 1 hours)	84.7
	CADM	-	-	-
20000	Emond	106	110 (@ 1 hours)	104
	CADM	-	-	-

C.3.1.13. *Kociba et al. (1976)*

Type:	Rats	**Dose:**	1, 10, 100, 1000 ng/kg-day
Strain:	Sprague-Dawley (Spartan)	**Route:**	Diet exposure
Body weight:	170–190 g (bw=180g)	**Regime:**	5 days/week for 13 weeks
Sex:	Female	**Simulation time:**	2,184 hours (13wk exposed)

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WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.714	Emond	0.859	1.38 (@ 2,112 hours)	1.13
	CADM	-	-	-
7.143	Emond	4.61	7.62 (@ 2,112 hours)	5.27
	CADM	-	-	-
WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
71.43	Emond	25.3	48.8 (@ 2,112 hours)	26.6
	CADM	-	-	-
714.3	Emond	181	403 (@ 2,112 hours)	184
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.714	Emond	88.3	140 (@ 2,116 hours)	126
	CADM	89.0	192	12.1
7.143	Emond	888	1,259 (@ 2,117 hours)	1,079
	CADM	970	2,007	29.0
71.43	Emond	8,776	11,693 (@ 2,117 hours)	9,756
	CADM	9,841	20,170	88.0
714.3	Emond	86,329	112,580 (@ 2,117 hours)	92,835
	CADM	98,617	201,814	455
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.714	Emond	79.4	114 (@ 2,129 hours)	111
	CADM	120	190	43.0
7.143	Emond	427	553 (@ 2,124 hours)	528
	CADM	456	787	67.0
71.43	Emond	2,348	2,925 (@ 2,121 hours)	2,720
	CADM	3,036	5,748	117

714.3	Emond	16,815	21,126 (@ 2,120 hours)	19,233
	CADM	28,382	55,013	274
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.714	Emond	10.8	16.1 (@ 2,116 hours)	15.1
	CADM	11.5	20.0	3.75
7.143	Emond	76.9	105 (@ 2,116 hours)	93.6
	CADM	65.3	126	6.22
71.43	Emond	600	785 (@ 2,116 hours)	673
	CADM	553	1,113	12.0
714.3	Emond	5,366	6,960 (@ 2,116 hours)	5,842
	CADM	5,401	10,967	37.0
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.714	Emond	2.89	4.17 (@ 2,116 hours)	3.81
	CADM	-	-	-
7.143	Emond	13.7	17.5 (@ 2,116 hours)	15.7
	CADM	-	-	-
71.43	Emond	47.5	55.0 (@ 2,116 hours)	50.6
	CADM	-	-	-
714.3	Emond	93.4	98.2 (@ 2,117 hours)	95.7
	CADM	-	-	-

C.3.1.14. *Kociba et al. (1978) Female*

Type:	Rats	**Dose:**	0, 1, 10, 100 ng/kg-day
Strain:	Sprague-Dawley (Spartan)	**Route:**	Diet exposure
Body weight:	170–190 g (bw=180)	**Regime:**	7 days/week for 104 weeks
Sex:	Female	**Simulation time:**	17,472 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	1.55	1.92 (@ 17,448 hours)	1.69
	CADM	-	-	-
10	Emond	7.15	9.25 (@ 17,448 hours)	7.16
	CADM	-	-	-
100	Emond	38.6	57.5 (@ 17,448 hours)	37.1
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	192	226 (@ 17,452 hours)	218
	CADM	292	333	333
10	Emond	1,618	1,742 (@ 17,452 hours)	1,665
	CADM	2,981	3,342	3,342
100	Emond	14,892	15,673 (@ 17,452 hours)	14,907
	CADM	29,917	33,432	33,432
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	147	165 (@ 17,457 hours)	164
	CADM	196	229	181
10	Emond	680	713 (@ 17,454 hours)	706
	CADM	861	1,015	789
100	Emond	3,663	3,788 (@ 17,454 hours)	3,731
	CADM	6,756	7,939	6,203
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	21.2	24.3 (@ 17,452 hours)	23.8
	CADM	26.0	27.0	27.0
10	Emond	131	140 (@ 17,452 hours)	136
	CADM	169	176	176

100	Emond	989	1,039 (@ 17,452 hours)	994
	CADM	1,546	1,601	1,601
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	5.11	5.77 (@ 17,452 hours)	5.59
	CADM	-	-	-
10	Emond	20.0	21.1 (@ 17,452 hours)	20.4
	CADM	-	-	-
100	Emond	59.9	61.5 (@ 17,452 hours)	60.1
	CADM	-	-	-

C.3.1.15. *Kociba et al. (1978) Male*

Type:	Rats	**Dose:**	0, 1, 10, 100 ng/kg-day
Strain:	Sprague-Dawley (Spartan)	**Route:**	Diet exposure
Body weight:	Body weight approximated to be 250 g	**Regime:**	7 days/week for 104 weeks
Sex:	Male	**Simulation time:**	17,472 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	1.56	1.96 (@ 17,448 hours)	1.70
	CADM	-	-	-
10	Emond	7.16	9.35 (@ 17,448 hours)	7.11
	CADM	-	-	-
100	Emond	38.7	59.3 (@ 17,448 hours)	37.1
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	194	229 (@ 17,452 hours)	221
	CADM	-	-	-

10	Emond	1,616	1,723 (@ 17,452 hours)	1,649
	CADM	-	-	-
100	Emond	14,898	15,671 (@ 17,452 hours)	14,912
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	148	167 (@ 17,456 hours)	166
	CADM	-	-	-
10	Emond	680	709 (@ 17,454 hours)	703
	CADM	-	-	-
100	Emond	3,677	3,803 (@ 17,453 hours)	3,747
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	21.4	24.6 (@ 17,452 hours)	24.1
	CADM	-	-	-
10	Emond	131	139 (@ 17,452 hours)	134
	CADM	-	-	-
100	Emond	991	1,041 (@ 17,452 hours)	995
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	5.15	5.83 (@ 17,452 hours)	5.64
	CADM	-	-	-
10	Emond	20.0	21.0 (@ 17,452 hours)	20.3
	CADM	-	-	-
100	Emond	60.0	61.5 (@ 17,452 hours)	60.1
	CADM	-	-	-

C.3.1.16. *Latchoumycandane and Mathur (2002)*

Type:	Rat	**Dose:**	0, 1, 10, 100 ng/kg-day
Strain:	Wistar	**Route:**	Oral gavage
Body weight:	45 days old (BW set to 200g)	**Regime:**	1/day for 45 days
Sex:	Male	**Simulation time:**	1,080 hours (daily exposure)

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	0.785	1.37 (@ 1,056 hours)	1.18
	CADM	-	-	-
10	Emond	4.65	8.18 (@ 1,056 hours)	6.18
	CADM	-	-	-
100	Emond	27.3	53.9 (@ 1,056 hours)	33.8
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	78.5	138 (@ 1,060 hours)	133
	CADM	116	217	217
10	Emond	902	1,423 (@ 1,060 hours)	1,358
	CADM	1,669	2,550	2,550
100	Emond	9,579	14,015 (@ 1,061 hours)	13,306
	CADM	17,681	25,915	25,915
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	69.8	113 (@ 1,072 hours)	113
	CADM	150	220	220
10	Emond	416	608 (@ 1,065 hours)	604
	CADM	744	1,009	1,009
100	Emond	2,448	3,425 (@ 1,062 hours)	3,380
	CADM	5,719	7,866	7,866

BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	9.56	15.9 (@ 1,060 hours)	15.6
	CADM	14.0	22.2	22.2
10	Emond	76.7	117 (@ 1,060 hours)	113
	CADM	106	157	157
100	Emond	646	933 (@ 1,060 hours)	891
	CADM	988	1,439	1,439
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	2.64	4.12 (@ 1,060 hours)	3.96
	CADM	-	-	-
10	Emond	13.7	18.8 (@ 1,060 hours)	18.1
	CADM	-	-	-
100	Emond	48.6	59.0 (@ 1,060 hours)	57.5
	CADM	-	-	-

C.3.1.17. *Li et al. (1997)*

Type:	Rats	**Dose:**	0, 3, 10, 30, 100, 300, 1000, 3000, 10000, 30000 ng/kg/day
Strain:	Sprague-Dawley	**Route:**	Gastric intubation
Body weight:	22 day old, 55 to 58 g (BW set to 56.5 g)	**Regime:**	One dose for one day
Sex:	Female	**Simulation time:**	24 hours

The CADM model was not run because the dosing duration is lower than the resolution of the model (1 week)

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day)	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
3	Emond	0.266	0.470 (@ 1 hours)	0.180
	CADM	-	-	-
10	Emond	0.799	1.57 (@ 1 hours)	0.535
	CADM	-	-	-

30	Emond	2.10	4.68 (@ 1 hours)	1.37
	CADM	-	-	-
100	Emond	5.87	15.6 (@ 1 hours)	3.68
	CADM	-	-	-
300	Emond	15.0	46.8 (@ 0 hours)	8.83
	CADM	-	-	-
1,000	Emond	43.3	156 (@ 0 hours)	23.4
	CADM	-	-	-
3,000	Emond	120	469 (@ 0 hours)	59.9
	CADM	-	-	-
10,000	Emond	386	1,570 (@ 0 hours)	182
	CADM	-	-	-
30,000	Emond	1,172	4,762 (@ 0 hours)	535
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day)	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
3	Emond	14.7	18.6 (@ 4 hours)	11.9
	CADM	-	-	-
10	Emond	55.0	65.2 (@ 5 hours)	47.6
	CADM	-	-	-
30	Emond	185	210 (@ 6 hours)	170
	CADM	-	-	-
100	Emond	690	768 (@ 7 hours)	666
	CADM	-	-	-
300	Emond	2,248	2,473 (@ 8 hours)	2,240
	CADM	-	-	-
1,000	Emond	7,938	8,671 (@ 9 hours)	8,094
	CADM	-	-	-
3,000	Emond	24,474	26,639 (@ 9 hours)	25,267
	CADM	-	-	-
10,000	Emond	82,349	89,464 (@ 9 hours)	85,597
	CADM	-	-	-
30,000	Emond	245,610	265,670 (@ 10 hours)	255,390
	CADM	-	-	-

FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day)	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
3	Emond	8.75	12.7 (@ 24 hours)	12.7
	CADM	-	-	-
10	Emond	26.6	38.0 (@ 24 hours)	38.0
	CADM	-	-	-
30	Emond	70.8	98.9 (@ 24 hours)	98.9
	CADM	-	-	-
100	Emond	202	273 (@ 24 hours)	273
	CADM	-	-	-
300	Emond	530	689 (@ 24 hours)	689
	CADM	-	-	-
1,000	Emond	1,573	1,958 (@ 24 hours)	1,958
	CADM	-	-	-
3,000	Emond	4,433	5,358 (@ 24 hours)	5,358
	CADM	-	-	-
10,000	Emond	14,428	17,119 (@ 24 hours)	17,119
	CADM	-	-	-
30,000	Emond	44,361	51,948 (@ 22 hours)	51,898
	CADM	-	-	-

BODY BURDEN (ng/kg)				
Dose (ng/kg-day)	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
3	Emond	1.60	1.70 (@ 8 hours)	1.68
	CADM	-	-	-
10	Emond	5.33	5.66 (@ 8 hours)	5.56
	CADM	-	-	-
30	Emond	15.9	16.9 (@ 8 hours)	16.5
	CADM	-	-	-
100	Emond	52.8	56.2 (@ 7 hours)	54.5
	CADM	-	-	-
300	Emond	158	169 (@ 7 hours)	163
	CADM	-	-	-

1,000	Emond	525	561 (@ 7 hours)	539
	CADM	-	-	-
3,000	Emond	1,574	1,684 (@ 7 hours)	1,611
	CADM	-	-	-
10,000	Emond	5,240	5,610 (@ 7 hours)	5,360
	CADM	-	-	-
30,000	Emond	15,758	16,815 (@ 7 hours)	16,041
	CADM	-	-	-

BOUND LIVER (ng/kg)

Dose (ng/kg-day)	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
3	Emond	0.89	1.37 (@ 3 hours)	0.64
	CADM	-	-	-
10	Emond	2.58	4.10 (@ 2 hours)	1.88
	CADM	-	-	-
30	Emond	6.37	10.5 (@ 2 hours)	4.71
	CADM	-	-	-
100	Emond	15.54	25.9 (@ 2 hours)	11.77
	CADM	-	-	-
300	Emond	31.25	50.1 (@ 1 hours)	24.57
	CADM	-	-	-
1,000	Emond	56.75	79.8 (@ 1 hours)	47.62
	CADM	-	-	-
3,000	Emond	81.28	98.4 (@ 1 hours)	73.32
	CADM	-	-	-
10,000	Emond	99.77	108 (@ 1 hours)	95.68
	CADM	-	-	-
30,000	Emond	107.69	111 (@ 1 hours)	106.24
	CADM	-	-	-

C.3.1.18. *Murray et al. (1979) Adult Portion*

Type:	Rat	**Dose:**	1, 10, and 100 ng/kg-day
Strain:	Sprague Dawley	**Route:**	Diet oral dose
Body weight:	BW set to 4.5 g	**Regime:**	Once per day for 120 days
Sex:	Female	**Simulation time:**	2880 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	1.12	1.51 (@ 2,856 hours)	1.42
	CADM	-	-	-
10	Emond	5.88	7.59 (@ 2,856 hours)	6.75
	CADM	-	-	-
100	Emond	32.7	44.3 (@ 2,856 hours)	36.0
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	128	180 (@ 2,859 hours)	173
	CADM	-	-	-
10	Emond	1,273	1,618 (@ 2,860 hours)	1,540
	CADM	-	-	-
100	Emond	12,601	15,281 (@ 2,860 hours)	14,460
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	106	139 (@ 2,865 hours)	138
	CADM	-	-	-
10	Emond	556	665 (@ 2,864 hours)	657
	CADM	-	-	-
100	Emond	3,095	3,604 (@ 2,862 hours)	3,534
	CADM	-	-	-

BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	14.8	20.0 (@ 2,860 hours)	19.6
	CADM	-	-	-
10	Emond	105	130 (@ 2,860 hours)	126
	CADM	-	-	-
100	Emond	837	1,003 (@ 2,860 hours)	957
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	3.77	4.95 (@ 2,859 hours)	4.77
	CADM	-	-	-
10	Emond	17.1	20.3 (@ 2,859 hours)	19.5
	CADM	-	-	-
100	Emond	55.3	60.9 (@ 2,860 hours)	59.4
	CADM	-	-	-

C.3.1.19. *NTP (1982)—Female Rats, Chronic*

Type:	Rat	**Dose:**	10, 50 and 500 ng/kg/wk, two doses per week
Strain:	Osborne-Mendel	**Route:**	Oral exposure
Body weight	6 weeks old (BW set to 250g)	**Regime:**	Biweekly (Simulation has been perform using female BW
Sex:	**Female**	**Simulation time**	17,472 hours (104 weeks of exposure)

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	1.96	3.11 (@ 17,220 hours)	1.94
	CADM	-	-	-
7.1	Emond	5.69	11.0 (@ 17,388 hours)	5.40
	CADM	-	-	-

71	Emond	29.8	82.2 (@ 17,388 hours)	26.9
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	265	308 (@ 17,226 hours)	265
	CADM	15,318	20,170	7,102
7.1	Emond	1,175	1,338 (@ 17,394 hours)	1,117
	CADM	30,700	40,353	14,200
71	Emond	10,734	12,182 (@ 17,395 hours)	9,882
	CADM	30,700	40,353	14,200
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	186	200 (@ 17,328 hours)	193
	CADM	4,655	5,748	2,107
7.1	Emond	541	569 (@ 17,409 hours)	544
	CADM	9,064	11,224	3,964
71	Emond	2,826	2,973 (@ 17,404 hours)	2,769
	CADM	17,879	22,172	7,671
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	27.9	31.1 (@ 17,225 hours)	28.4
	CADM	855	1,113	403
7.1	Emond	99.4	110 (@ 17,393 hours)	96.7
	CADM	1,695	2,208	787
71	Emond	729	814 (@ 17,393 hours)	683
	CADM	3,375	4,395	1,556
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	6.37	7.26 (@ 17,224 hours)	6.38
	CADM	-	-	-

7.1	Emond	16.6	18.5 (@ 17,392 hours)	16.1
	CADM	-	-	-
71	Emond	52.7	56.4 (@ 17,393 hours)	50.9
	CADM	-	-	-

C.3.1.20. *NTP (1982)—Male Rats, Chronic*

Type:	Rat	**Dose:**	10, 50 and 500 ng/kg/wk, two doses per week
Strain:	Osborne-Mendel	**Route:**	Oral exposure
Body weight	6 weeks old (BW set to 350g)	**Regime:**	Biweekly (Simulation has been perform using female BW
Sex:	**Male**	**Simulation time**	17,472 hours (104 weeks of exposure)

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	1.96	3.18 (@ 17,388 hours)	1.93
	CADM	-	-	-
7.1	Emond	5.70	11.4 (@ 17,388 hours)	5.39
	CADM	-	-	-
71	Emond	29.9	87.0 (@ 17,388 hours)	26.9
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	265	306 (@ 17,394 hours)	263
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
7.1	Emond	1,174	1,334 (@ 17,394 hours)	1,114
	CADM	-	-	-
71	Emond	10,736	12,170 (@ 17,395 hours)	9,881
	CADM	-	-	-

FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	186	199 (@ 17,412 hours)	193
	CADM	-	-	-
7.1	Emond	541	569 (@ 17,409 hours)	544
	CADM	-	-	-
71	Emond	2,836	2,983 (@ 17,404 hours)	2,784
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	27.8	30.9 (@ 17,393 hours)	28.2
	CADM	-	-	-
7.1	Emond	99.5	110 (@ 17,393 hours)	96.6
	CADM	-	-	-
71	Emond	730	816 (@ 17,393 hours)	684
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	6.36	7.22 (@ 17,392 hours)	6.35
	CADM	-	-	-
7.1	Emond	16.6	18.4 (@ 17,392 hours)	16.0
	CADM	-	-	-
71	Emond	52.7	56.3 (@ 17,393 hours)	50.9
	CADM	-	-	-

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C.3.1.21. *NTP (1982)—Female Mice, Chronic*

Type:	Mice	**Dose:**	40, 200 and 2000 ng/kg/wk, two doses during the week
Strain:	B6C3F1	**Route:**	Oral exposure
Body weight	6 weeks old (BW set to 23g)	**Regime:**	Biweekly (Simulation has been perform using female BW)
Sex:	Female	**Simulation time**	17,472 hours (104 weeks of exposure)

* The mice chronic exposure could not be simulated with the CADM model because this model simulates for only 123 days.

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
5.7	Emond	1.95	4.86 (@ 16,800 hours)	1.82
	CADM	-	-	-
28.6	Emond	5.84	19.8 (@ 17,388 hours)	5.17
	CADM	-	-	-
286	Emond	32.1	171 (@ 16,884 hours)	26.0
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
5.7	Emond	490	582 (@ 16,807 hours)	463
	CADM	-	-	-
28.6	Emond	2,236	2,629 (@ 17,395 hours)	2,025
	CADM	-	-	-
286	Emond	20,841	24,353 (@ 17,396 hours)	18,182
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
5.7	Emond	737	785 (@ 17,408 hours)	757
	CADM	-	-	-
28.6	Emond	2,213	2,337 (@ 17,404 hours)	2,216
	CADM	-	-	-
286	Emond	12,138	12,861 (@ 17,400 hours)	11,775
	CADM	-	-	-

BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
5.7	Emond	91.9	103 (@ 17,393 hours)	91.2
	CADM	-	-	-
28.6	Emond	329	370 (@ 17,393 hours)	313
	CADM	-	-	-
286	Emond	2,400	2,740 (@ 17,393 hours)	2,176
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
5.7	Emond	6.18	7.29 (@ 16,805 hours)	5.93
	CADM	-	-	-
28.6	Emond	16.3	18.9 (@ 17,393 hours)	15.3
	CADM	-	-	-
286	Emond	52.3	67.8 (@ 2 hours)	49.3
	CADM	-	-	-

C.3.1.22. *NTP (1982)—Male Mice, Chronic*

Type:	Mice	**Dose:**	10, 50 and 500ng/kg/wk, two doses during the week
Strain:	B6C3F1	**Route:**	Oral exposure
Body weight	6 weeks old (BW set to 25g)	**Regime:**	Biweekly
Sex:	Male	**Simulation time**	17,472 hours (104 weeks of exposure)

* The mice chronic exposure could not be simulated with the CADM model because this model simulates for only 123 days.

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	0.767	1.53 (@ 17,304 hours)	0.749
	CADM	-	-	-
7.1	Emond	2.27	5.99 (@ 17,052 hours)	2.11
	CADM	-	-	-

71	Emond	11.2	46.7 (@ 17,388 hours)	9.59
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	138	165 (@ 17,310 hours)	136
	CADM	-	-	-
7.1	Emond	606	722 (@ 17,059 hours)	571
	CADM	-	-	-
71	Emond	5,409	6,328 (@ 17,395 hours)	4,805
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	290	314 (@ 17,411 hours)	306
	CADM	-	-	-
7.1	Emond	860	918 (@ 17,155 hours)	883
	CADM	-	-	-
71	Emond	4,257	4,490 (@ 17,402 hours)	4,204
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	32.3	36.2 (@ 17,309 hours)	33.3
	CADM	-	-	-
7.1	Emond	110	123 (@ 17,057 hours)	108
	CADM	-	-	-
71	Emond	710	802 (@ 17,393 hours)	660
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.4	Emond	2.56	3.03 (@ 17,309 hours)	2.53
	CADM	-	-	-

7.1	Emond	7.12	8.40 (@ 17,057 hours)	6.82
	CADM	-	-	-
71	Emond	27.1	32.4 (@ 2 hours)	25.3
	CADM	-	-	-

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3 **C.3.1.23.** ***NTP (2006) 14 Weeks***

Type:	Rat	**Dose:**	0, 3, 10, 22, 46, 100 ng/kg-day
Strain:	Sprague Dawley	**Route:**	Oral gavage
Body weight:	8 weeks old (BW=215g)	**Regime:**	5 days/weeks for 14 weeks
Sex:	Female and male	**Simulation time:**	2,352 hours (14 weeks)

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WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	1.98	3.15 (@ 2,280 hours)	2.39
	CADM	-	-	-
7.14	Emond	4.69	7.75 (@ 2,280 hours)	5.30
	CADM	-	-	-
15.7	Emond	8.27	14.3 (@ 2,280 hours)	9.02
	CADM	-	-	-
32.9	Emond	14.2	25.9 (@ 2,280 hours)	15.1
	CADM	-	-	-
71.4	Emond	25.7	49.8 (@ 2,280 hours)	26.6
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	275	404 (@ 2,284 hours)	354
	CADM	-	-	-
7.14	Emond	909	1,270 (@ 2,285 hours)	1,089
	CADM	-	-	-
15.7	Emond	1,988	2,703 (@ 2,285 hours)	2,291
	CADM	-	-	-

32.9	Emond	4,129	5,508 (@ 2,285 hours)	4,628
	CADM	-	-	-
71.4	Emond	8,921	11,734 (@ 2,285 hours)	9,792
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	184	246 (@ 2,294 hours)	237
	CADM	-	-	-
7.14	Emond	436	557 (@ 2,292 hours)	532
	CADM	-	-	-
15.7	Emond	768	962 (@ 2,291 hours)	912
	CADM	-	-	-
32.9	Emond	1,319	1,633 (@ 2,289 hours)	1,535
	CADM	-	-	-
71.4	Emond	2,385	2,938 (@ 2,289 hours)	2,736
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	28.2	39.4 (@ 2,284 hours)	36.1
	CADM	-	-	-
7.14	Emond	78.5	106 (@ 2,284 hours)	94.4
	CADM	-	-	-
15.7	Emond	156	206 (@ 2,284 hours)	181
	CADM	-	-	-
32.9	Emond	300	391 (@ 2,284 hours)	340
	CADM	-	-	-
71.4	Emond	610	788 (@ 2,284 hours)	676
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	6.41	8.55 (@ 2,284 hours)	7.74
	CADM	-	-	-

7.14	Emond	13.9	17.6 (@ 2,284 hours)	15.8
	CADM	-	-	-
15.7	Emond	22.2	27.2 (@ 2,284 hours)	24.5
	CADM	-	-	-
32.9	Emond	33.2	39.3 (@ 2,284 hours)	35.7
	CADM	-	-	-
71.4	Emond	47.9	55.1 (@ 2,284 hours)	50.7
	CADM	-	-	-

C.3.1.24. *NTP (2006) 31 Weeks*

Type:	Rat	**Dose:**	0, 3, 10, 22, 46, 100 ng/kg-day
Strain:	Sprague Dawley	**Route:**	Oral gavage
Body weight:	8 weeks old (BW=215g)	**Regime:**	5 days/weeks for 31 weeks
Sex:	Female and male	**Simulation time:**	5,208 hours (31 weeks)

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	2.33	3.25 (@ 3,960 hours)	2.48
	CADM	-	-	-
7.14	Emond	5.32	7.89 (@ 3,960 hours)	5.40
	CADM	-	-	-
15.7	Emond	9.21	14.5 (@ 3,960 hours)	9.15
	CADM	-	-	-
32.9	Emond	15.7	26.2 (@ 5,136 hours)	15.3
	CADM	-	-	-
71.4	Emond	28.1	50.4 (@ 5,136 hours)	27.0
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	341	425 (@ 5,140 hours)	373
	CADM	-	-	-
7.14	Emond	1,075	1,308 (@ 3,965 hours)	1,117
	CADM	-	-	-

15.7	Emond	2,296	2,756 (@ 3,965 hours)	2,336
	CADM	-	-	-
32.9	Emond	4,696	5,597 (@ 5,141 hours)	4,712
	CADM	-	-	-
71.4	Emond	10,033	11,905 (@ 5,141 hours)	9,953
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	220	256 (@ 5,149 hours)	246
	CADM	-	-	-
7.14	Emond	501	570 (@ 4,139 hours)	542
	CADM	-	-	-
15.7	Emond	868	978 (@ 4,138 hours)	926
	CADM	-	-	-
32.9	Emond	1,476	1,657 (@ 5,145 hours)	1,558
	CADM	-	-	-
71.4	Emond	2,652	2,978 (@ 5,144 hours)	2,775
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	34.2	41.2 (@ 5,140 hours)	37.8
	CADM	-	-	-
7.14	Emond	91.6	108 (@ 3,964 hours)	96.6
	CADM	-	-	-
15.7	Emond	178	209 (@ 3,964 hours)	184
	CADM	-	-	-
32.9	Emond	339	398 (@ 5,140 hours)	346
	CADM	-	-	-
71.4	Emond	682	799 (@ 5,140 hours)	687
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	7.48	8.83 (@ 5,140 hours)	8.01
	CADM	-	-	-
7.14	Emond	15.6	17.9 (@ 3,964 hours)	16.1

	CADM	-	-	-
15.7	Emond	24.3	27.4 (@ 3,964 hours)	24.8
	CADM	-	-	-
32.9	Emond	35.7	39.6 (@ 5,140 hours)	36.0
	CADM	-	-	-
71.4	Emond	50.9	55.4 (@ 5,140 hours)	51.1
	CADM	-	-	-

C.3.1.25. *NTP (2006) 53 Weeks*

Type:	Rat	**Dose:**	0, 3, 10, 22, 46, 100 ng/kg-day
Strain:	Sprague Dawley	**Route:**	Oral gavage
Body weight:	8 weeks old (BW=215g)	**Regime:**	5 days/weeks for 105 weeks
Sex:	Female and male	**Simulation time:**	8,904 hours (53 weeks)

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	2.46	3.25 (@ 6,312 hours)	2.48
	CADM	-	-	-
7.14	Emond	5.53	7.89 (@ 3,960 hours)	5.41
	CADM	-	-	-
15.7	Emond	9.54	14.5 (@ 8,832 hours)	9.17
	CADM	-	-	-
32.9	Emond	16.2	26.3 (@ 8,832 hours)	15.3
	CADM	-	-	-
71.4	Emond	29.0	50.6 (@ 8,832 hours)	27.1
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	366	426 (@ 6,316 hours)	373
	CADM	-	-	-
7.14	Emond	1,134	1,308 (@ 3,965 hours)	1,121
	CADM	-	-	-
15.7	Emond	2,406	2,759 (@ 8,837 hours)	2,345
	CADM	-	-	-

32.9	Emond	4,902	5,612 (@ 8,837 hours)	4,727
	CADM	-	-	-
71.4	Emond	10,439	11,938 (@ 8,837 hours)	9,985
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	233	256 (@ 6,325 hours)	247
	CADM	-	-	-
7.14	Emond	524	570 (@ 4,139 hours)	544
	CADM	-	-	-
15.7	Emond	904	980 (@ 8,842 hours)	929
	CADM	-	-	-
32.9	Emond	1,533	1,661 (@ 8,841 hours)	1,562
	CADM	-	-	-
71.4	Emond	2,749	2,986 (@ 8,840 hours)	2,784
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	36.4	41.2 (@ 6,316 hours)	37.8
	CADM	-	-	-
7.14	Emond	96.1	108 (@ 3,964 hours)	96.9
	CADM	-	-	-
15.7	Emond	186	210 (@ 8,836 hours)	185
	CADM	-	-	-
32.9	Emond	353	399 (@ 8,836 hours)	347
	CADM	-	-	-
71.4	Emond	709	801 (@ 8,836 hours)	689
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	7.87	8.84 (@ 6,316 hours)	8.01
	CADM	-	-	-
7.14	Emond	16.2	17.9 (@ 3,964 hours)	16.1
	CADM	-	-	-
15.7	Emond	25.1	27.5 (@ 8,836 hours)	24.8

	CADM	-	-	-
32.9	Emond	36.6	39.7 (@ 8,836 hours)	36.1
	CADM	-	-	-
71.4	Emond	51.9	55.4 (@ 8,836 hours)	51.1
	CADM	-	-	-

C.3.1.26. *NTP (2006) 2 Years*

Type:	Rat	**Dose:**	0, 3, 10, 22, 46, 100 ng/kg-day
Strain:	Sprague Dawley	**Route:**	Oral gavage
Body weight:	8 weeks old (BW=215g)	**Regime:**	5 days/weeks for 105 weeks
Sex:	Female and male	**Simulation time:**	17,640 hours* (105 weeks)

*The CADM model simulates for 104 weeks only (17,472 hours). As a result, the terminal values from the CADM model may be underestimated compared to the Emond model, which considers the full 105 weeks of exposure.

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	2.56	3.47 (@ 17,568 hours)	2.62
	CADM	-	-	-
7.14	Emond	5.69	7.97 (@ 17,568 hours)	5.46
	CADM	-	-	-
15.7	Emond	9.79	14.6 (@ 17,568 hours)	9.22
	CADM	-	-	-
32.9	Emond	16.6	26.4 (@ 17,568 hours)	15.4
	CADM	-	-	-
71.4	Emond	29.7	50.8 (@ 17,568 hours)	27.1
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	385	460 (@ 17,572 hours)	403
	CADM	632	715	715
7.14	Emond	1,177	1,320 (@ 17,573 hours)	1,135
	CADM	2,127	2,387	2,387
15.7	Emond	2,487	2,779 (@ 17,573 hours)	2,361

	CADM	4,691	5,252	5,252
32.9	Emond	5,051	5,637 (@ 17,573 hours)	4,749
	CADM	9,822	10,984	10,984
71.4	Emond	10,734	11,976 (@ 17,573 hours)	10,018
	CADM	21,366	23,880	23,880
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	243	271 (@ 17,581 hours)	261
	CADM	302	355	277
7.14	Emond	541	575 (@ 17,579 hours)	549
	CADM	667	787	611
15.7	Emond	930	985 (@ 17,578 hours)	934
	CADM	1,242	1,463	1,138
32.9	Emond	1,574	1,667 (@ 17,577 hours)	1,568
	CADM	2,369	2,787	2,173
71.4	Emond	2,821	2,995 (@ 17,576 hours)	2,792
	CADM	4,890	5,748	4,489
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	38.1	44.0 (@ 17,572 hours)	40.4
	CADM	46.0	48.0	48.0
7.14	Emond	99.5	109 (@ 17,572 hours)	97.9
	CADM	125	130	130
15.7	Emond	192	211 (@ 17,572 hours)	186
	CADM	257	267	267
32.9	Emond	364	400 (@ 17,572 hours)	348
	CADM	520	538	538
71.4	Emond	729	804 (@ 17,572 hours)	691
	CADM	1,110	1,149	1,149
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
2.14	Emond	8.17	9.30 (@ 17,572 hours)	8.43

	CADM	-	-	-
7.14	Emond	16.6	18.0 (@ 17,572 hours)	16.2
	CADM	-	-	-
15.7	Emond	25.6	27.6 (@ 17,572 hours)	24.9
	CADM	-	-	-
32.9	Emond	37.3	39.7 (@ 17,572 hours)	36.2
	CADM	-	-	-
71.4	Emond	52.7	55.5 (@ 17,572 hours)	51.2
	CADM	-	-	-

C.3.1.27. *Sewall et al. (1995)*

Type:	Rat	**Dose:**	49, 149.8, 490, and 1750 ng/kg every two weeks or 3.5, 10.7, 35, and 125 ng/kg-day
Strain:	Sprauge-Dawley	**Route:**	Oral gavage
Body weight:	12 wk old (BW set to 250g)	**Regime:**	Once every 2 weeks for 30 weeks
Sex:	Female	**Simulation time:**	5040 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
3.5	Emond	3.29	13.7 (@ 4,704 hours)	2.88
	CADM	-	-	-
10.7	Emond	7.11	38.7 (@ 4,704 hours)	5.79
	CADM	-	-	-
35	Emond	16.6	120 (@ 4,704 hours)	12.6
	CADM	-	-	-
125	Emond	44.7	414 (@ 4,704 hours)	31.4
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
3.5	Emond	550	901 (@ 4,711 hours)	459
	CADM	-	-	-
10.7	Emond	1,605	2,632 (@ 4,712 hours)	1,229
	CADM	-	-	-
35	Emond	5,072	8,350 (@ 4,712 hours)	3,618

	CADM	-	-	-
125	Emond	17,683	29,256 (@ 4,713 hours)	12,011
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
3.5	Emond	310	383 (@ 4,765 hours)	290
	CADM	-	-	-
10.7	Emond	670	827 (@ 4,763 hours)	590
	CADM	-	-	-
35	Emond	1,569	1,957 (@ 4,760 hours)	1,304
	CADM	-	-	-
125	Emond	4,217	5,376 (@ 4,757 hours)	3,303
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
3.5	Emond	51.4	72.5 (@ 4,710 hours)	45.3
	CADM	-	-	-
10.7	Emond	130	189 (@ 4,710 hours)	106
	CADM	-	-	-
35	Emond	364	546 (@ 4,710 hours)	274
	CADM	-	-	-
125	Emond	1,164	1,793 (@ 4,710 hours)	824
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
3.5	Emond	10.2	15.8 (@ 2 hours)	9.18
	CADM	-	-	-
10.7	Emond	19.8	34.4 (@ 1 hours)	17.0
	CADM	-	-	-
35	Emond	37.0	63.2 (@ 1 hours)	31.4
	CADM	-	-	-
125	Emond	63.1	90.9 (@ 1 hours)	55.2
	CADM	-	-	-

C.3.1.28. *Shi et al. (2007) Adult Portion*

Type:	Rat	**Dose:**	1, 5, 50 and 200 ng/kg
Strain:	Sprague Dawley	**Route:**	Oral exposure
Body weight:	BW set to 4.5 g	**Regime:**	Weekly doses for 11 months
Sex:	Female	**Simulation time:**	8040 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.143	Emond	0.342	0.475 (@ 7,561 hours)	0.380
	CADM	-	-	-
0.714	Emond	1.07	1.53 (@ 7,560 hours)	1.09
	CADM	-	-	-
7.14	Emond	5.23	9.12 (@ 7,560 hours)	4.86
	CADM	-	-	-
28.6	Emond	13.9	29.2 (@ 7,560 hours)	12.4
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.143	Emond	26.1	36.5 (@ 7,564 hours)	29.6
	CADM	-	-	-
0.714	Emond	118	159 (@ 7,564 hours)	120
	CADM	-	-	-
7.14	Emond	1,068	1,415 (@ 7,565 hours)	970
	CADM	-	-	-
28.6	Emond	4,119	5,450 (@ 7,565 hours)	3,574
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.143	Emond	32.5	40.0 (@ 7,583 hours)	36.7
	CADM	-	-	-
0.714	Emond	102	120 (@ 7,584 hours)	106
	CADM	-	-	-

7.14	Emond	497	571 (@ 7,584 hours)	475
	CADM	-	-	-
28.6	Emond	1,322	1,527 (@ 7,584 hours)	1,217
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.143	Emond	3.94	4.99 (@ 7,566 hours)	4.45
	CADM	-	-	-
0.714	Emond	14.0	17.2 (@ 7,566 hours)	14.5
	CADM	-	-	-
7.14	Emond	90.8	112 (@ 7,566 hours)	84.4
	CADM	-	-	-
28.6	Emond	300	374 (@ 7,566 hours)	266
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.143	Emond	1.18	1.60 (@ 7,563 hours)	1.31
	CADM	-	-	-
0.714	Emond	3.62	4.75 (@ 7,563 hours)	3.70
	CADM	-	-	-
7.14	Emond	15.6	19.7 (@ 7,564 hours)	14.7
	CADM	-	-	-
28.6	Emond	33.5	40.7 (@ 7,564 hours)	31.2
	CADM	-	-	-

C.3.1.29. *Smialowicz et al. (2008)*

Type:	Mice	**Dose:**	0, 1.5, 15, 150, 450 ng/kg-day
Strain:	B6C3F1	**Route:**	Oral gavage
Body weight:	13 wk old (BW set to 28g)	**Regime:**	5 days/week for 13 weeks
Sex:	Female	**Simulation time:**	2184

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.07	Emond	0.438	0.815 (@ 2,112 hours)	0.557
	CADM	-	-	-
10.7	Emond	2.46	5.12 (@ 2,112 hours)	2.65
	CADM	-	-	-
107	Emond	13.4	36.4 (@ 2,112 hours)	12.7
	CADM	-	-	-
321	Emond	31.6	98.6 (@ 2,112 hours)	28.4
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.07	Emond	67.1	107 (@ 2,116 hours)	91.5
	CADM	59.0	92.0	88.0
10.7	Emond	683	971 (@ 2,117 hours)	787
	CADM	767	1,000	907
107	Emond	6,784	9,010 (@ 2,117 hours)	7,043
	CADM	8,349	10,306	8,998
321	Emond	20,218	26,379 (@ 2,117 hours)	20,405
	CADM	25,344	31,006	26,967
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.07	Emond	156	229 (@ 2,130 hours)	225
	CADM	151	210	204
10.7	Emond	885	1,155 (@ 2,124 hours)	1,111
	CADM	689	815	774
107	Emond	4,831	5,979 (@ 2,120 hours)	5,591
	CADM	2,771	3,224	2,937
321	Emond	11,420	14,037 (@ 2,119 hours)	12,920
	CADM	6,337	7,509	6,688

BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.07	Emond	17.0	25.5 (@ 2,116 hours)	23.9
	CADM	21.0	29.0	29.0
10.7	Emond	117	159 (@ 2,116 hours)	141
	CADM	119	145	135
107	Emond	852	1,103 (@ 2,116 hours)	923
	CADM	727	875	778
321	Emond	2,304	2,958 (@ 2,116 hours)	2,419
	CADM	1,961	2,370	2,080
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1.07	Emond	1.48	2.17 (@ 2,116 hours)	1.90
	CADM	-	-	-
10.7	Emond	7.60	9.86 (@ 2,116 hours)	8.42
	CADM	-	-	-
107	Emond	30.3	36.0 (@ 2,117 hours)	31.1
	CADM	-	-	-
321	Emond	51.1	58.1 (@ 2,117 hours)	51.8
	CADM	-	-	-

C.3.1.30. *Toth et al., 1 Year (1979)*

Type:	Mice	**Dose:**	7, 700, 7000 ng/kg/week
Strain:	Swiss/H/Riop	**Route:**	Oral gavage In gastric tube
Body weight:	10 weeks old (BW= 27g)	**Regime:**	1/week for 1 year (365 days)
Sex:	Female and male	**Simulation time:**	8,760 hours

We did not simulate the scenario using the CADM model because this model can only be run for a maximum of 123 days.

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	0.573	1.61 (@ 8,736 hours)	0.682
	CADM	-	-	-
100	Emond	14.2	116 (@ 8,736 hours)	15.7
	CADM	-	-	-
1,000	Emond	91.2	1,108 (@ 8,736 hours)	99.3
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	94.2	131 (@ 8,743 hours)	123
	CADM	-	-	-
100	Emond	7,343	10,134 (@ 8,745 hours)	9,604
	CADM	-	-	-
1,000	Emond	70,243	97,658 (@ 8,745 hours)	92,506
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	215	247 (@ 8,613 hours)	245
	CADM	-	-	-
100	Emond	5,339	5,914 (@ 8,760 hours)	5,914
	CADM	-	-	-
1,000	Emond	34,249	38,828 (@ 8,756 hours)	38,807
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	23.4	28.4 (@ 8,742 hours)	27.9
	CADM	-	-	-
100	Emond	929	1,189 (@ 8,742 hours)	1,132
	CADM	-	-	-

1,000	Emond	7,569	10,045 (@ 8,742 hours)	9,471
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
1	Emond	1.93	2.65 (@ 8,741 hours)	2.35
	CADM	-	-	-
100	Emond	31.8	58.4 (@ 2 hours)	36.7
	CADM	-	-	-
1,000	Emond	78.6	103 (@ 2 hours)	84.8
	CADM	-	-	-

C.3.1.31. *Van Birgelen et al. (1995)*

Type:	Rat	**Dose:**	0, 13.5, 26.4, 46.9, 320, 1024 ng/kg-day
Strain:	Sprague Dawley	**Route:**	Oral gavage
Body weight:	150 g	**Regime:**	Once per day for 13 weeks
Sex:	Female	**Simulation time:**	2184 hours (13 weeks)

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
13.5	Emond	7.20	11.1 (@ 2,160 hours)	8.47
	CADM	-	-	-
26.4	Emond	11.8	18.6 (@ 2,160 hours)	13.5
	CADM	-	-	-
46.9	Emond	18.1	29.6 (@ 2,160 hours)	20.5
	CADM	-	-	-
320	Emond	86.4	156 (@ 2,160 hours)	95.4
	CADM	-	-	-
1024	Emond	250	470 (@ 2,160 hours)	275
	CADM	-	-	-

LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
13.5	Emond	1,655	2,208 (@ 2,164 hours)	2,107
	CADM	-	-	-
26.4	Emond	3,228	4,216 (@ 2,164 hours)	4,017
	CADM	-	-	-
46.9	Emond	5,719	7,366 (@ 2,164 hours)	7,008
	CADM	-	-	-
320	Emond	38,484	47,999 (@ 2,164 hours)	45,537
	CADM	-	-	-
1024	Emond	121,640	150,410 (@ 2,164 hours)	142,510
	CADM	-	-	-
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
13.5	Emond	669	843 (@ 2,167 hours)	835
	CADM	-	-	-
26.4	Emond	1,092	1,357 (@ 2,166 hours)	1,342
	CADM	-	-	-
46.9	Emond	1,680	2,071 (@ 2,166 hours)	2,045
	CADM	-	-	-
320	Emond	8,027	9,816 (@ 2,165 hours)	9,639
	CADM	-	-	-
1024	Emond	23,234	28,519 (@ 2,165 hours)	27,954
	CADM	-	-	-
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
13.5	Emond	132	173 (@ 2,164 hours)	167
	CADM	-	-	-
26.4	Emond	240	308 (@ 2,164 hours)	296
	CADM	-	-	-
46.9	Emond	404	513 (@ 2,164 hours)	492
	CADM	-	-	-

320	Emond	2,437	3,031 (@ 2,164 hours)	2,887
	CADM	-	-	-
1024	Emond	7,521	9,310 (@ 2,164 hours)	8,846
	CADM	-	-	-
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
13.5	Emond	19.9	24.2 (@ 2,164 hours)	23.4
	CADM	-	-	-
26.4	Emond	29.0	34.3 (@ 2,164 hours)	33.2
	CADM	-	-	-
46.9	Emond	38.8	45.0 (@ 2,164 hours)	43.7
	CADM	-	-	-
320	Emond	79.1	85.2 (@ 2,164 hours)	84.1
	CADM	-	-	-
1024	Emond	97.5	101 (@ 2,164 hours)	101
	CADM	-	-	-

C.3.1.32. *Vanden Heuvel et al. (1994)*

Type:	Rat	**Dose:**	0.05, 0.1, 1, 10, 100, 1000, 10000 ng/kg/d
Strain:	Sprague Dawley	**Route:**	Oral gavage
Body weight:	10 weeks old (BW 225 to 275g, set to 250g)	**Regime:**	Single dose
Sex:	Female	**Simulation time:**	24 hours *

* 1 week is the minimum that can be simulated with the CADM model, so the CADM model was not used.

WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.05	Emond	0.01	0.011 (@ 0 hours)	0.0039
	CADM	-	-	-
0.1	Emond	0.0113	0.022 (@ 0 hours)	0.008
	CADM	-	-	-
1	Emond	0.106	0.215 (@ 0 hours)	0.0723
	CADM	-	-	-

10	Emond	0.883	2.15 (@ 0 hours)	0.583
	CADM	-	-	-
100	Emond	6.45	21.5 (@ 0 hours)	3.85
	CADM	-	-	-
1000	Emond	48.3	216 (@ 0 hours)	23.9
	CADM	-	-	-
10000	Emond	435	2,166 (@ 0 hours)	186
	CADM	-	-	-
LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.05	Emond	0.232	0.315 (@ 3 hours)	0.173
	CADM	-	-	0.0140
0.1	Emond	0.469	0.631 (@ 3 hours)	0.353
	CADM	-	-	0.0320
1	Emond	5.08	6.42 (@ 4 hours)	4.08
	CADM	-	-	0.950
10	Emond	60.2	68.7 (@ 5 hours)	54.1
	CADM	-	-	52.7
100	Emond	730	800 (@ 9 hours)	719
	CADM	-	-	1,342
1000	Emond	8,186	8,919 (@ 11 hours)	8,442
	CADM	-	-	15,967
10000	Emond	84,254	91,675 (@ 11 hours)	88,230
	CADM	-	-	162,773
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.05	Emond	0.138	0.215 (@ 24 hours)	0.215
	CADM	-	-	0.780
0.1	Emond	0.274	0.427 (@ 24 hours)	0.427
	CADM	-	-	1.57
1	Emond	2.58	3.97 (@ 24 hours)	3.97
	CADM	-	-	15.3

10	Emond	22.1	32.8 (@ 24 hours)	32.8
	CADM	-	-	125
100	Emond	170	235 (@ 24 hours)	235
	CADM	-	-	739
1000	Emond	1,348	1,720 (@ 24 hours)	1,720
	CADM	-	-	5,779
10000	Emond	12,500	15,265 (@ 24 hours)	15,265
	CADM	-	-	55,825
BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.05	Emond	0.0269	0.028 (@ 9 hours)	0.0283
	CADM	-	-	0.0450
0.1	Emond	0.0538	0.057 (@ 9 hours)	0.0565
	CADM	-	-	0.0900
1	Emond	0.536	0.568 (@ 9 hours)	0.562
	CADM	-	-	0.900
10	Emond	5.32	5.65 (@ 8 hours)	5.55
	CADM	-	-	9.00
100	Emond	52.8	56.3 (@ 7 hours)	54.4
	CADM	-	-	90.0
1000	Emond	525	562 (@ 7 hours)	538
	CADM	-	-	900
10000	Emond	5,238	5,610 (@ 7 hours)	5,353
	CADM	-	-	9,000
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
0.05	Emond	0.0194	0.027 (@ 3 hours)	0.0142
	CADM	-	-	-
0.1	Emond	0.0383	0.054 (@ 3 hours)	0.0281
	CADM	-	-	-
1	Emond	0.353	0.506 (@ 3 hours)	0.261
	CADM	-	-	-

10	Emond	2.77	4.24 (@ 2 hours)	2.08
	CADM	-	-	-
100	Emond	16.1	26.4 (@ 2 hours)	12.4
	CADM	-	-	-
1000	Emond	57.4	80.2 (@ 1 hours)	48.5
	CADM	-	-	-
10000	Emond	100	108 (@ 1 hours)	96.1
	CADM	-	-	-

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3 **C.3.1.33.** ***White et al. (1986)***

Type:	Mice	**Dose:**	10, 50, 100, 500, 1000, 2000 ng/kg-day
Strain:	B6C3F1	**Route:**	Oral gavage
Body weight:	7 weeks old (BW set to 23g)	**Regime:**	1/day for 14 days
Sex:	Female	**Simulation time:**	336 hours

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WHOLE BLOOD CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	1.09	2.73 (@ 312 hours)	1.42
	CADM	-	-	-
50	Emond	4.08	11.6 (@ 312 hours)	4.98
	CADM	-	-	-
100	Emond	7.14	21.7 (@ 312 hours)	8.44
	CADM	-	-	-
500	Emond	26.8	96.5 (@ 312 hours)	29.8
	CADM	-	-	-
1,000	Emond	48.7	187 (@ 312 hours)	53.1
	CADM	-	-	-
2,000	Emond	90.6	365 (@ 312 hours)	97.5
	CADM	-	-	-

LIVER CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	216	375 (@ 317 hours)	343
	CADM	217	468 (336h)	463
50	Emond	1,279	2,164 (@ 317 hours)	1,997
	CADM	1,775	3,261 (336h)	3,261
100	Emond	2,707	4,525 (@ 317 hours)	4,184
	CADM	3,999	6,923 (336h)	6,923
500	Emond	14,802	24,165 (@ 317 hours)	22,383
	CADM	22,705	36,362 (336h)	36,362
1,000	Emond	30,278	49,034 (@ 317 hours)	45,414
	CADM	46,309	73,145 (336h)	73,145
2,000	Emond	61,381	98,703 (@ 317 hours)	91,363
	CADM	93,577	146,695 (336h)	146,695
FAT CONCENTRATIONS (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	279	507 (@ 336 hours)	507
	CADM	316	537 (336h)	537
50	Emond	1,056	1,846 (@ 336 hours)	1,846
	CADM	1,029	1,564 (336h)	1,564
100	Emond	1,854	3,195 (@ 333 hours)	3,195
	CADM	1,662	2,470 (336h)	2,470
500	Emond	7,008	11,868 (@ 324 hours)	11,816
	CADM	5,711	8,594 (336h)	8,594
1,000	Emond	12,746	21,566 (@ 323 hours)	21,424
	CADM	10,498	15,993 (336h)	15,993
2,000	Emond	23,691	40,177 (@ 322 hours)	39,843
	CADM	19,990	30,726 (336h)	30,726

BODY BURDEN (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	37.7	65.9 (@ 317 hours)	63.8
	CADM	47.9	85.9 (336h)	85.9
50	Emond	175	297 (@ 317 hours)	284
	CADM	207	342 (336h)	342
100	Emond	338	570 (@ 316 hours)	542
	CADM	388	624 (336h)	624
500	Emond	1,597	2,637 (@ 316 hours)	2,480
	CADM	1,761	2,754 (336h)	2,754
1,000	Emond	3,137	5,153 (@ 316 hours)	4,830
	CADM	3,455	5,387 (336h)	5,387
2,000	Emond	6,186	10,118 (@ 316 hours)	9,459
	CADM	6,836	10,643 (336h)	10,643
BOUND LIVER (ng/kg)				
Dose (ng/kg-day) Adjusted dose	**Model**	**Metric**		
		Time-weighted Ave	**Max**	**Terminal**
10	Emond	3.49	5.32 (@ 316 hours)	4.82
	CADM	-	-	-
50	Emond	11.4	16.4 (@ 317 hours)	15.1
	CADM	-	-	-
100	Emond	18.1	25.1 (@ 317 hours)	23.4
	CADM	-	-	-
500	Emond	44.2	56.2 (@ 317 hours)	53.8
	CADM	-	-	-
1,000	Emond	59.3	71.9 (@ 317 hours)	69.7
	CADM	-	-	-
2,000	Emond	74.4	86.1 (@ 317 hours)	84.3
	CADM	-	-	-

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C.3.2. Gestational Studies

C.3.2.1. *Bell et al. (2007)*

Type:	Rat	**Dose:**	2.4, 8, and 46 ng/kg-day with a 0.03 ng/kg-day background
Strain:	Han/Wistar	**Route:**	Diet oral dose
Body weight:	6 weeks (BW= 85g)	**Regime:**	Once per day for 12 weeks prior to mating, during the two week mating period, and during gestation
Sex:	Female	**Simulation time:**	2,352 hr (98 days) prior to gestation + 504 hr (21 days) during gestation for a total simulation of 2,856 hours

* Time averages are computed during the gestation period only.

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2.43	2.20	6,295	3.10 (@ 2,352 hours)	2.20
8.03	5.14	14,674	7.31 (@ 2,352 hours)	5.08
46.03	18.4	52,584	28.1 (@ 2,352 hours)	18.1
LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2.43	320	914,290	437 (@ 2,356 hours)	321
8.03	1,040	2,969,800	1,349 (@ 2,356 hours)	1,042
46.03	5,892	16,829,000	7,289 (@ 2,356 hours)	6,007
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2.43	205	585,530	263 (@ 2,336 hours)	211
8.03	478	1,365,100	589 (@ 2,335 hours)	486
46.03	1,713	4,891,500	2,045 (@ 2,334 hours)	1,745

BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2.43	33.0	94,390	44.4 (@ 2,836 hours)	43.4
8.03	90.4	258,110	117 (@ 2,836 hours)	114
46.03	422	1,206,500	531 (@ 2,836 hours)	511
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2.43	3.03	8,648	39.6 (@ 2,530 hours)	6.48
8.03	6.65	18,999	86.7 (@ 2,529 hours)	14.4
46.03	20.9	59,794	272 (@ 2,527 hours)	46.0
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2.43	7.10	20,289	8.98 (@ 2,356 hours)	7.23
8.03	15.1	43,242	18.2 (@ 2,356 hours)	15.4
46.03	39.6	113,070	44.8 (@ 2,356 hours)	40.6

C.3.2.2. *Haavisto et al. (2006)*

Type:	Rat	**Dose:**	20, 400, and 1,000 ng/kg
Strain:	Sprague Dawley	**Route:**	Oral exposure
Body weight	BW = 190 g	**Regime:**	Single dose on GD13
Sex:	Female	**Simulation time**	336 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	2.86	68.9	8.01 (@ 312 hours)	1.73
400	11.3	273	40.1 (@ 312 hours)	6.28
1000	46.9	1,129	202 (@ 312 hours)	22.8

LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	265	6,371	298 (@ 319 hours)	244
400	1,497	36,005	1,653 (@ 320 hours)	1,462
1000	8,061	193,860	8,832 (@ 321 hours)	8,147
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	56.3	1,354	81.9 (@ 336 hours)	81.9
400	232	5,584	321 (@ 336 hours)	321
1000	1,002	24,084	1,313 (@ 336 hours)	1,313
BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	21.1	508	22.5 (@ 319 hours)	21.9
400	105	2,528	112 (@ 319 hours)	108
1000	524	12,612	561 (@ 319 hours)	538
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	8.47	203	11.3 (@ 336 hours)	11.3
400	31.2	751	40.3 (@ 336 hours)	40.3
1000	112	2,689	139 (@ 336 hours)	139
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	8.20	197	13.5 (@ 314 hours)	6.03
400	24.9	598	40.8 (@ 313 hours)	19.1
1000	57.1	1,373	80.1 (@ 313 hours)	47.7

C.3.2.3. *Hojo et al. (2002)*

Type:	Rat	**Dose:**	20, 60 and 180 ng/kg
Strain:	Sprague Dawley	**Route:**	Oral exposure
Body weight	20 ng/kg BW = 271g 60 ng/kg BW = 275g 180 ng/kg BW = 262g	**Regime:**	Single dose on GD8
Sex:	Female	**Simulation time**	216 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	1.62	39.1	4.47 (@ 192 hours)	1.02
60	4.17	100	13.3 (@ 192 hours)	2.50
180	10.7	258	40.3 (@ 192 hours)	5.96
LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	128	20,554	144 (@ 198 hours)	43.2
60	420	72,340	465 (@ 200 hours)	147
180	1,364	250,820	1,497 (@ 201 hours)	497
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	32.5	17,253	63.0 (@ 281 hours)	49.4
60	86.4	44,093	161 (@ 284 hours)	124
180	226	108,730	398 (@ 286 hours)	301
BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	10.6	3,054	11.3 (@ 200 hours)	8.67
60	31.8	8,702	33.8 (@ 199 hours)	23.6
180	95.0	24,747	101 (@ 199 hours)	63.4

FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	15.9	2,334	18.4 (@ 206 hours)	1.64
60	39.8	5,829	45.7 (@ 205 hours)	4.10
180	96.3	13,866	110 (@ 203 hours)	9.72
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	4.88	759	7.74 (@ 194 hours)	1.75
60	11.2	1,848	18.5 (@ 194 hours)	4.26
180	23.6	4,157	38.5 (@ 193 hours)	9.65

C.3.2.4. *Ikeda et al. (2005)*

Type:	Rat	**Dose:**	400 ng/kg single dose and 80 ng/kg weekly maintenance dose
Strain:	Sprague Dawley	**Route:**	Oral gavage
Body weight:	10 weeks (BW= 250g)	**Regime:**	400 ng/kg single dose, two weekly maintenance doses prior to gestation and weekly maintenance doses during gestation
Sex:	Female	**Simulation time:**	504 hr (21 days) prior to gestation + 504 hr (21 days) during gestation for a total simulation of 1,008 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
16.5	22.9	23,086	101 (@ 144 hours)	10.1
LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
16.5	7,755	7,817,300	17,016 (@ 150 hours)	2,698

FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
16.5	2,087	2,103,900	3,663 (@ 184 hours)	1,028
BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
16.5	548	552,590	1,085 (@ 149 hours)	262
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
16.5	45.9	46,290	245 (@ 679 hours)	30.2
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
16.5	44.0	44,361	63.8 (@ 149 hours)	26.8

C.3.2.5. *Kattainen et al. (2001)*

Type:	Rat	**Dose:**	30, 100, 300, and 1,000 ng/kg
Strain:	Han/Wistar (Kuopio) and Long/Evans (Turku/AB) crossing.	**Route:**	Oral exposure
Body weight:	BW no specify (BW set to 190g)*	**Regime:**	Single dose in the GD15
Sex:	Female	**Simulation time:**	360 hours

*Derelanko and Hollinger (1995).

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	2.23	53.7	5.95 (@ 336 hours)	1.36
100	6.25	150	19.8 (@ 336 hours)	3.62

300	16.1	387	59.8 (@ 336 hours)	8.62
1,000	46.9	1,128	200 (@ 336 hours)	22.7
LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	193	4,648	219 (@ 342 hours)	175
100	713	17,141	793 (@ 344 hours)	680
300	2,298	55,266	2,533 (@ 345 hours)	2,267
1,000	8,055	193,720	8,831 (@ 345 hours)	8,134
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	42.8	1,027	62.8 (@ 360 hours)	62.8
100	123	2,964	175 (@ 360 hours)	175
300	327	7,853	446 (@ 360 hours)	446
1,000	981	23,588	1,289 (@ 360 hours)	1,289
BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	15.9	382	16.9 (@ 343 hours)	16.4
100	52.7	1,266	56.2 (@ 343 hours)	54.3
300	158	3,791	168 (@ 343 hours)	162
1,000	524	12,612	561 (@ 343 hours)	538
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	4.86	117	6.66 (@ 360 hours)	6.66
100	13.2	317	17.6 (@ 360 hours)	17.6
300	31.5	758	41.2 (@ 360 hours)	41.2
1,000	82.2	1,975	104 (@ 360 hours)	104

BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	6.57	158	10.7 (@ 338 hours)	4.80
100	15.8	381	26.3 (@ 338 hours)	11.9
300	31.6	760	50.6 (@ 337 hours)	24.7
1,000	57.1	1,373	80.1 (@ 337 hours)	47.7

C.3.2.6. *Keller et al. (2007)*

Type:	Mouse	**Dose:**	10, 100, and 1000 ng/kg
Strain:	CBA/J and C3H/HeJ	**Route:**	Oral
Body weight:	Not specified (24 g used in the simulation)	**Regime:**	Single dose at gestation day 13
Sex:	Female	**Simulation time:**	336 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
10	0.537	12.9	1.43 (@ 312 hours)	0.269
100	4.29	103	14.3 (@ 312 hours)	1.95
1,000	34.1	820	143 (@ 312 hours)	12.3
LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
10	30.6	737	39.8 (@ 316 hours)	22.2
100	371	8,922	421 (@ 319 hours)	317
1,000	4,214	101,360	4,697 (@ 321 hours)	3,940
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
10	22.4	538	33.3 (@ 336 hours)	33.3
100	188	4,523	264 (@ 336 hours)	264
1,000	1,591	38,233	2,080 (@ 336 hours)	2,080

BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
10	5.57	134	5.99 (@ 319 hours)	5.72
100	54.3	1,306	59.0 (@ 318 hours)	54.7
1,000	530	12,747	581 (@ 318 hours)	524
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
10	2.57	61.7	3.80 (@ 336 hours)	3.80
100	21.7	522	30.0 (@ 334 hours)	29.9
1,000	179	4,312	233 (@ 329 hours)	225
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
10	1.74	41.8	3.14 (@ 315 hours)	1.01
100	11.5	276	23.5 (@ 314 hours)	6.99
1,000	46.7	1,123	79.8 (@ 314 hours)	32.9

C.3.2.7. *Li et al. (2006) 3-Day*

Type:	Mouse	**Dose:**	2, 50, and 100 ng/kg-day
Strain:	NIH	**Route:**	Oral
Body weight:	25-28 g (used 27 g in the simulation)	**Regime:**	Daily exposure from gestation day 1 to gestation day 8
Sex:	Female	**Simulation time:**	72 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2	0.159	11.4	0.392 (@ 48 hours)	0.136
50	2.84	205	8.90 (@ 48 hours)	2.38
100	5.12	369	17.3 (@ 48 hours)	4.20

LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2	8.98	647	15.1 (@ 52 hours)	9.10
50	333	23,971	539 (@ 53 hours)	402
100	718	51,738	1,156 (@ 53 hours)	888
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2	17.0	1,227	31.1 (@ 72 hours)	31.1
50	315	22,704	548 (@ 72 hours)	548
100	576	41,460	984 (@ 72 hours)	984
BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2	2.29	165	3.51 (@ 55 hours)	3.43
50	53.6	3,863	82.2 (@ 54 hours)	77.1
100	105	7,598	162 (@ 53 hours)	150
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2	0.00	0	0.000 (@ 72 hours)	0.00
50	0.0	0	0.000 (@ 72 hours)	0.00
100	0.0	0	0.000 (@ 72 hours)	0.00
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
2	0.538	38.8	0.864 (@ 51 hours)	0.498
50	8.24	594	13.5 (@ 2 hours)	8.16
100	13.6	981	23.7 (@ 2 hours)	13.6

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C.3.2.8. *Markowski et al. (2001)*

Type:	Rat	**Dose:**	20, 60 and 180 ng/kg
Strain:	Holtzman rats	**Route:**	Oral exposure
Body weight:	BW no specify (BW set to 190g)*	**Regime:**	Single dose in the GD18
Sex:	Female	**Simulation time:**	432 hours

*Derelanko and Hollinger (1995).

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	1.56	37.5	3.82 (@ 408 hours)	0.958
60	4.03	97.0	11.5 (@ 408 hours)	2.38
180	10.3	248	34.8 (@ 408 hours)	5.72
LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	123	2,959	141 (@ 414 hours)	109
60	409	9,843	459 (@ 415 hours)	382
180	1,334	32,086	1,479 (@ 416 hours)	1,295
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	27.9	670	41.6 (@ 432 hours)	41.6
60	74.0	1,778	107 (@ 432 hours)	107
180	195	4,685	273 (@ 432 hours)	273
BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	10.6	254	11.2 (@ 415 hours)	10.9
60	31.7	762	33.8 (@ 415 hours)	32.7
180	94.7	2,278	101 (@ 415 hours)	97.5

FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	1.26	30.2	1.80 (@ 432 hours)	1.80
60	3.21	77.2	4.49 (@ 432 hours)	4.49
180	7.81	188	10.7 (@ 432 hours)	10.7
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
20	4.74	114	7.59 (@ 410 hours)	3.43
60	11.0	265	18.2 (@ 410 hours)	8.16
180	23.2	559	38.1 (@ 409 hours)	17.7

C.3.2.9. *Mietinnen et al. (2006)*

Type:	Rat	**Dose:**	30, 100, 300 and 1000 ng/kg
Strain:	cross-breeding of Han/Wistar and Long-Evans rats	**Route:**	Oral exposure
Body weight:	BW 11 weeks (BW set to 180g)	**Regime:**	Single dose in the GD15
Sex:	Female	**Simulation time:**	360 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	2.22	53.4	5.87 (@ 336 hours)	1.36
100	6.23	150	19.6 (@ 336 hours)	3.61
300	16.0	386	59.0 (@ 336 hours)	8.61
1,000	46.6	1,123	198 (@ 336 hours)	22.7

LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	193	4,631	219 (@ 342 hours)	174
100	711	17,096	791 (@ 344 hours)	677
300	2,294	55,166	2,530 (@ 345 hours)	2,260
1,000	8,042	193,410	8,820 (@ 345 hours)	8,114
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	43.0	1,034	63.2 (@ 360 hours)	63.2
100	124	2,984	176 (@ 360 hours)	176
300	329	7,905	449 (@ 360 hours)	449
1,000	987	23,729	1,296 (@ 360 hours)	1,296
BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	15.9	381	16.9 (@ 343 hours)	16.4
100	52.6	1,266	56.1 (@ 343 hours)	54.3
300	158	3,791	168 (@ 343 hours)	162
1,000	524	12,609	561 (@ 343 hours)	538
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	4.83	116	6.62 (@ 360 hours)	6.62
100	13.1	315	17.5 (@ 360 hours)	17.5
300	31.3	753	41.0 (@ 360 hours)	41.0
1,000	81.7	1,963	104 (@ 360 hours)	104
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
30	6.56	158	10.7 (@ 338 hours)	4.78

100	15.8	381	26.3 (@ 338 hours)	11.9
300	31.6	760	50.5 (@ 337 hours)	24.6
1,000	57.0	1,372	80.1 (@ 337 hours)	47.6

C.3.2.10. *Nohara et al. (2000)*

Type:	Rat	**Dose:**	12.5, 50, 200 or 800 ng TCDD/kg
Strain:	Holtzman rats	**Route:**	Oral exposure
Body weight:	BW no specify (BW set to 190g)*	**Regime:**	Single dose in the GD15
Sex:	Female	**Simulation time:**	360 hours

*Derelanko and Hollinger (1995).

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	1.03	24.8	2.44 (@ 336 hours)	0.645
50	3.45	82.9	9.78 (@ 336 hours)	2.07
200	11.3	271	39.2 (@ 336 hours)	6.25
800	38.1	918	158 (@ 336 hours)	18.9
LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	73.8	1,776	86.1 (@ 341 hours)	63.6
50	336	8,084	378 (@ 343 hours)	311
200	1,492	35,890	1,651 (@ 344 hours)	1,454
800	6,389	153,640	7,012 (@ 345 hours)	6,423
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	19.7	473	29.5 (@ 360 hours)	29.5
50	67.6	1,624	97.8 (@ 360 hours)	97.8
200	229	5,504	317 (@ 360 hours)	317
800	803	19,292	1,061 (@ 360 hours)	1,061

BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	6.62	159	7.04 (@ 343 hours)	6.88
50	26.4	635	28.1 (@ 343 hours)	27.3
200	105	2,528	112 (@ 343 hours)	108
800	420	10,092	449 (@ 343 hours)	430
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	2.25	54.0	3.14 (@ 360 hours)	3.14
50	7.43	179	10.1 (@ 360 hours)	10.1
200	22.8	548	30.1 (@ 360 hours)	30.1
800	68.1	1,638	87.0 (@ 360 hours)	87.0
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	3.24	77.9	5.12 (@ 338 hours)	2.32
50	9.66	232	16.0 (@ 338 hours)	7.12
200	24.8	597	40.7 (@ 337 hours)	19.0
800	51.9	1,248	75.0 (@ 337 hours)	42.7

C.3.2.11. *Ohsako et al. (2001)*

Type:	Rat	**Dose:**	12.5, 50, 200, and 800 ng/kg-day
Strain:	Holtzmann	**Route:**	Oral exposure on GD15
Body weight	10 weeks (200g)	**Regime:**	Single dose
Sex:	Female	**Simulation time**	384 hours

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	1.04	25.0	2.48 (@ 360 hours)	0.649
50	3.47	83.6	9.93 (@ 360 hours)	2.07
200	11.4	273	39.9 (@ 360 hours)	6.26
800	38.4	925	161 (@ 360 hours)	18.9
LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	74.3	1,788	86.5 (@ 365 hours)	64.2
50	338	8,126	379 (@ 367 hours)	314
200	1,497	36,006	1,655 (@ 368 hours)	1,461
800	6,402	153,960	7,025 (@ 369 hours)	6,443
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	19.0	457	28.6 (@ 384 hours)	28.6
50	65.3	1,569	94.7 (@ 384 hours)	94.7
200	221	5,321	307 (@ 384 hours)	307
800	777	18,671	1,029 (@ 384 hours)	1,029
BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	6.63	159	7.05 (@ 367 hours)	6.89
50	26.4	635	28.2 (@ 367 hours)	27.3
200	105	2,529	112 (@ 367 hours)	108
800	420	10,093	449 (@ 367 hours)	430
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	1.65	39.5	2.33 (@ 384 hours)	2.33

50	5.44	131	7.48 (@ 384 hours)	7.48
200	16.7	401	22.3 (@ 384 hours)	22.3
800	49.9	1,200	64.6 (@ 384 hours)	64.6
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
12.5	3.25	78.3	5.13 (@ 362 hours)	2.34
50	9.69	233	16.0 (@ 362 hours)	7.16
200	24.9	598	40.7 (@ 361 hours)	19.1
800	51.9	1,249	75.0 (@ 361 hours)	42.8

C.3.2.12. *Schantz et al. (1996) and Amin et al. (2000)*

Type:	Rat	**Dose:**	25 and 100 ng/kg-day
Strain:	Sprague Dawley	**Route:**	Oral exposure
Body weight:	BW not specified (BW set to 250g)	**Regime:**	Daily doses from GD 10 - 16
Sex:	Female	**Simulation time:**	384 hours; time averages are calculated from the beginning of the dosing

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	3.38	487	8.63 (@ 360 hours)	4.03
100	10.6	1,522	31.1 (@ 360 hours)	12.3
LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	512	73,686	871 (@ 365 hours)	778
100	2,374	341,960	4,012 (@ 366 hours)	3,665
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	169	24,323	306 (@ 384 hours)	306

100	532	76,675	950 (@ 384 hours)	950
BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	45.1	6,490	76.6 (@ 365 hours)	74.3
100	177	25,438	298 (@ 365 hours)	287
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	25.2	3,627	30.4 (@ 343 hours)	27.3
100	74.1	10,672	88.1 (@ 342 hours)	77.9
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	9.99	1,439	14.4 (@ 364 hours)	12.8
100	25.2	3,632	34.2 (@ 364 hours)	31.6

C.3.2.13. *Seo et al. (1995)*

Type:	Rat	**Dose:**	25 and 100 ng/kg-day
Strain:	Sprague Dawley	**Route:**	Oral exposure
Body weight:	BW not specified (BW set to 190g)	**Regime:**	Daily doses from GD 10 - 16
Sex:	Female	**Simulation time:**	384 hours; time averages are calculated from the beginning of the dosing

WHOLE BLOOD CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	3.33	479	8.25 (@ 360 hours)	4.00
100	10.4	1,498	29.6 (@ 360 hours)	12.2

LIVER CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	504	72,592	861 (@ 365 hours)	767
100	2,347	337,970	3,978 (@ 365 hours)	3,627
FAT CONCENTRATIONS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	172	24,807	310 (@ 384 hours)	310
100	542	78,097	962 (@ 384 hours)	962
BODY BURDEN (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	45.0	6,486	76.5 (@ 365 hours)	74.2
100	176	25,387	298 (@ 365 hours)	287
FETUS (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	24.7	3,551	29.8 (@ 343 hours)	26.8
100	72.6	10,456	86.6 (@ 342 hours)	76.8
BOUND LIVER (ng/kg) and AUC ((ng/kg) • hr)				
Dose (ng/kg-day) Adjusted dose	**Metric**			
	Time-weighted Ave	**Area Under the Curve**	**Max**	**Terminal**
25	9.90	1,426	14.3 (@ 364 hours)	12.7
100	25.0	3,607	34.1 (@ 364 hours)	31.4

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Table C-1. Model input parameters potentially addressed by selected articles

Articles	Model input parameters potentially addressed										
	Absorption	**Desorption**	**Distribution**	**Elimination**	**Kinetics**	**Induction CYP1A1**	**Interspecies differences**	**Age Differences**	**Aryl hydrocarbon receptor (AhR)**	**Mode of action**	**Partition coefficient**
Aylward et al., 2004	●	●	●	●	●						
Aylward et al., 2005a, b	●	●	●	●	●						
Aylward et al., 2009				●							
Bohonowych and Denison, 2007						●	●		●		
Boverhof et al., 2005						●	●				
Connor and Aylward, 2006							●	●	●		
Heinzl et al., 2007			●						●		
Irigaray et al., 2005			●				●				
Kerger et al., 2006			●		●			●			
Kerger et al., 2007								●			
Kim et al., 2003			●								
Korenaga et al., 2007						●	●				
Korkalainen et al., 2004							●	●			
Kransler et al., 2007							●	●			
Maruyama et al., 2002	●		●	●							
Maruyama et al., 2003	●		●	●							
Maruyama and Aoki, 2006	●		●	●							
Millbrath et al., 2009			●	●	●		●				
Moser and McLachlan, 2002		●		●							
Mullerova and Kopecky, 2007			●								
Nadal et al., 2009				●	●						
Nohara et al., 2006							●		●		
Olsman et al., 2007									●		
Saghir et al., 2005			●	●	●						
Schecter et al., 2003				●				●			
Staskal et al., 2005						●			●		
Toyoshiba et al., 2004			●			●			●		
Wilkes et al., 2008						●					

Partition coefficient estimates and CYP parameter value estimates were derived from Wang et al. (1997, 2000) and Santostefano et al. (1998).

C.4. RESPONSE SURFACE TABLES

In order to calculate human equivalent doses, the human model must be run with a daily intake which gives average blood concentrations which match the average concentrations in the rodent models. However, such calculation can require numerous human model runs with repeated intake adjustments in order to reach the target blood concentrations. To facilitate this process, a response surface was created for the human model. In the response surface, numerous intakes were run and the blood, fat, and body burden average concentrations were recorded. These tables can then be used to estimate the intake which would give a target blood concentration. The two closest intakes are found and the intake is estimated by linearly interpolating between the two doses. Then, this intake is run through the human model to confirm that the average blood concentration is within a specified tolerance of the target blood concentration.

For the current analysis, three different response surfaces were created: non-gestational lifetime to be used with long-term animal bioassays, nongestational five year average runs to be used with shorter term animal bioassays, and gestationsl to be used with gestational animal bioassays. All three response sufraces are shown in the following tables.

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C.4.1. Nongestational Lifetime

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
1.00E-09	2.39E-05	8.58E-06	2.52E-07
1.33E-09	3.18E-05	1.14E-05	3.35E-07
1.67E-09	3.98E-05	1.43E-05	4.19E-07
2.00E-09	4.77E-05	1.72E-05	5.03E-07
2.33E-09	5.57E-05	2.00E-05	5.87E-07
2.67E-09	6.36E-05	2.29E-05	6.70E-07
3.00E-09	7.16E-05	2.57E-05	7.54E-07
3.33E-09	7.95E-05	2.86E-05	8.38E-07
3.67E-09	8.74E-05	3.14E-05	9.22E-07
4.00E-09	9.54E-05	3.43E-05	1.01E-06
4.33E-09	1.03E-04	3.72E-05	1.09E-06
4.67E-09	1.11E-04	4.00E-05	1.17E-06
5.00E-09	1.19E-04	4.29E-05	1.26E-06
5.33E-09	1.27E-04	4.57E-05	1.34E-06
5.67E-09	1.35E-04	4.86E-05	1.42E-06
6.00E-09	1.43E-04	5.14E-05	1.51E-06
6.33E-09	1.51E-04	5.43E-05	1.59E-06
6.67E-09	1.59E-04	5.71E-05	1.68E-06
7.00E-09	1.67E-04	6.00E-05	1.76E-06
7.33E-09	1.75E-04	6.29E-05	1.84E-06
7.67E-09	1.83E-04	6.57E-05	1.93E-06
8.00E-09	1.91E-04	6.86E-05	2.01E-06
8.33E-09	1.99E-04	7.14E-05	2.09E-06
8.67E-09	2.07E-04	7.43E-05	2.18E-06
9.00E-09	2.14E-04	7.71E-05	2.26E-06
9.33E-09	2.22E-04	8.00E-05	2.34E-06
9.67E-09	2.30E-04	8.28E-05	2.43E-06
1.00E-08	2.38E-04	8.57E-05	2.51E-06

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
1.33E-08	3.17E-04	1.14E-04	3.34E-06
1.67E-08	3.96E-04	1.43E-04	4.18E-06
2.00E-08	4.75E-04	1.71E-04	5.01E-06
2.33E-08	5.54E-04	1.99E-04	5.84E-06
2.67E-08	6.33E-04	2.28E-04	6.67E-06
3.00E-08	7.12E-04	2.56E-04	7.50E-06
3.33E-08	7.91E-04	2.85E-04	8.34E-06
3.67E-08	8.70E-04	3.13E-04	9.17E-06
4.00E-08	9.49E-04	3.41E-04	1.00E-05
4.33E-08	1.03E-03	3.70E-04	1.08E-05
4.67E-08	1.11E-03	3.98E-04	1.17E-05
5.00E-08	1.19E-03	4.27E-04	1.25E-05
5.33E-08	1.26E-03	4.55E-04	1.33E-05
5.67E-08	1.34E-03	4.83E-04	1.41E-05
6.00E-08	1.42E-03	5.12E-04	1.50E-05
6.33E-08	1.50E-03	5.40E-04	1.58E-05
6.67E-08	1.58E-03	5.68E-04	1.66E-05
7.00E-08	1.66E-03	5.96E-04	1.75E-05
7.33E-08	1.73E-03	6.25E-04	1.83E-05
7.67E-08	1.81E-03	6.53E-04	1.91E-05
8.00E-08	1.89E-03	6.81E-04	1.99E-05
8.33E-08	1.97E-03	7.10E-04	2.08E-05
8.67E-08	2.05E-03	7.38E-04	2.16E-05
9.00E-08	2.13E-03	7.66E-04	2.24E-05
9.33E-08	2.21E-03	7.94E-04	2.32E-05
9.67E-08	2.28E-03	8.23E-04	2.41E-05
1.00E-07	2.36E-03	8.51E-04	2.49E-05
1.33E-07	3.14E-03	1.13E-03	3.31E-05
1.67E-07	3.92E-03	1.41E-03	4.13E-05

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
2.00E-07	4.70E-03	1.70E-03	4.96E-05
2.33E-07	5.48E-03	1.98E-03	5.78E-05
2.67E-07	6.26E-03	2.26E-03	6.60E-05
3.00E-07	7.04E-03	2.54E-03	7.42E-05
3.33E-07	7.82E-03	2.82E-03	8.24E-05
3.67E-07	8.60E-03	3.10E-03	9.06E-05
4.00E-07	9.38E-03	3.38E-03	9.89E-05
4.33E-07	1.02E-02	3.66E-03	1.07E-04
4.67E-07	1.09E-02	3.95E-03	1.15E-04
5.00E-07	1.17E-02	4.23E-03	1.24E-04
5.33E-07	1.25E-02	4.50E-03	1.31E-04
5.66E-07	1.32E-02	4.78E-03	1.39E-04
5.99E-07	1.40E-02	5.05E-03	1.47E-04
6.33E-07	1.47E-02	5.32E-03	1.55E-04
6.66E-07	1.55E-02	5.60E-03	1.63E-04
6.99E-07	1.63E-02	5.87E-03	1.71E-04
7.32E-07	1.70E-02	6.15E-03	1.79E-04
7.65E-07	1.78E-02	6.42E-03	1.87E-04
7.98E-07	1.85E-02	6.69E-03	1.95E-04
8.32E-07	1.93E-02	6.97E-03	2.03E-04
8.65E-07	2.00E-02	7.24E-03	2.11E-04
8.98E-07	2.08E-02	7.52E-03	2.19E-04
9.31E-07	2.16E-02	7.79E-03	2.27E-04
9.64E-07	2.23E-02	8.07E-03	2.35E-04
9.97E-07	2.31E-02	8.34E-03	2.43E-04
1.01E-06	2.34E-02	8.46E-03	2.47E-04
1.03E-06	2.37E-02	8.59E-03	2.50E-04
1.04E-06	2.41E-02	8.71E-03	2.54E-04
1.06E-06	2.44E-02	8.84E-03	2.58E-04

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
1.07E-06	2.48E-02	8.97E-03	2.61E-04
1.09E-06	2.52E-02	9.10E-03	2.65E-04
1.11E-06	2.55E-02	9.23E-03	2.69E-04
1.12E-06	2.59E-02	9.37E-03	2.73E-04
1.14E-06	2.63E-02	9.51E-03	2.77E-04
1.16E-06	2.67E-02	9.65E-03	2.81E-04
1.17E-06	2.70E-02	9.79E-03	2.85E-04
1.19E-06	2.74E-02	9.93E-03	2.89E-04
1.21E-06	2.78E-02	1.01E-02	2.93E-04
1.23E-06	2.82E-02	1.02E-02	2.98E-04
1.24E-06	2.87E-02	1.04E-02	3.02E-04
1.26E-06	2.91E-02	1.05E-02	3.06E-04
1.28E-06	2.95E-02	1.07E-02	3.11E-04
1.30E-06	2.99E-02	1.08E-02	3.15E-04
1.32E-06	3.04E-02	1.10E-02	3.20E-04
1.34E-06	3.08E-02	1.12E-02	3.25E-04
1.36E-06	3.13E-02	1.13E-02	3.29E-04
1.38E-06	3.17E-02	1.15E-02	3.34E-04
1.40E-06	3.22E-02	1.16E-02	3.39E-04
1.42E-06	3.26E-02	1.18E-02	3.44E-04
1.44E-06	3.31E-02	1.20E-02	3.49E-04
1.46E-06	3.36E-02	1.22E-02	3.54E-04
1.49E-06	3.41E-02	1.24E-02	3.59E-04
1.53E-06	3.51E-02	1.27E-02	3.70E-04
1.58E-06	3.61E-02	1.31E-02	3.81E-04
1.62E-06	3.72E-02	1.35E-02	3.92E-04
1.67E-06	3.83E-02	1.39E-02	4.03E-04
1.72E-06	3.94E-02	1.43E-02	4.15E-04
1.77E-06	4.05E-02	1.47E-02	4.27E-04

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
1.83E-06	4.17E-02	1.51E-02	4.39E-04
1.88E-06	4.29E-02	1.56E-02	4.52E-04
1.94E-06	4.41E-02	1.60E-02	4.65E-04
2.00E-06	4.54E-02	1.65E-02	4.79E-04
2.06E-06	4.67E-02	1.70E-02	4.93E-04
2.12E-06	4.81E-02	1.75E-02	5.07E-04
2.18E-06	4.95E-02	1.80E-02	5.22E-04
2.25E-06	5.09E-02	1.85E-02	5.37E-04
2.32E-06	5.24E-02	1.90E-02	5.52E-04
2.39E-06	5.39E-02	1.96E-02	5.68E-04
2.46E-06	5.55E-02	2.02E-02	5.85E-04
2.53E-06	5.71E-02	2.07E-02	6.02E-04
2.61E-06	5.87E-02	2.13E-02	6.19E-04
2.68E-06	6.04E-02	2.20E-02	6.37E-04
2.76E-06	6.22E-02	2.26E-02	6.55E-04
2.85E-06	6.40E-02	2.33E-02	6.74E-04
2.93E-06	6.58E-02	2.39E-02	6.93E-04
3.02E-06	6.77E-02	2.46E-02	7.13E-04
3.11E-06	6.96E-02	2.53E-02	7.34E-04
3.21E-06	7.16E-02	2.61E-02	7.55E-04
3.30E-06	7.37E-02	2.68E-02	7.76E-04
3.40E-06	7.58E-02	2.76E-02	7.99E-04
3.50E-06	7.80E-02	2.84E-02	8.22E-04
3.61E-06	8.02E-02	2.92E-02	8.45E-04
3.72E-06	8.25E-02	3.01E-02	8.69E-04
3.83E-06	8.48E-02	3.09E-02	8.94E-04
3.94E-06	8.73E-02	3.18E-02	9.20E-04
4.06E-06	8.98E-02	3.27E-02	9.46E-04
4.18E-06	9.23E-02	3.37E-02	9.73E-04

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
4.31E-06	9.49E-02	3.47E-02	1.00E-03
4.44E-06	9.76E-02	3.57E-02	1.03E-03
4.57E-06	1.00E-01	3.67E-02	1.06E-03
4.71E-06	1.03E-01	3.77E-02	1.09E-03
4.85E-06	1.06E-01	3.88E-02	1.12E-03
4.99E-06	1.09E-01	3.99E-02	1.15E-03
5.14E-06	1.12E-01	4.11E-02	1.18E-03
5.30E-06	1.15E-01	4.22E-02	1.22E-03
5.46E-06	1.19E-01	4.34E-02	1.25E-03
5.62E-06	1.22E-01	4.47E-02	1.29E-03
5.79E-06	1.25E-01	4.59E-02	1.32E-03
5.96E-06	1.29E-01	4.73E-02	1.36E-03
6.14E-06	1.33E-01	4.86E-02	1.40E-03
6.33E-06	1.36E-01	5.00E-02	1.44E-03
6.52E-06	1.40E-01	5.14E-02	1.48E-03
6.71E-06	1.44E-01	5.28E-02	1.52E-03
6.91E-06	1.48E-01	5.43E-02	1.56E-03
7.12E-06	1.52E-01	5.58E-02	1.60E-03
7.33E-06	1.56E-01	5.74E-02	1.65E-03
7.55E-06	1.61E-01	5.90E-02	1.69E-03
7.78E-06	1.65E-01	6.06E-02	1.74E-03
8.01E-06	1.70E-01	6.23E-02	1.79E-03
8.25E-06	1.74E-01	6.41E-02	1.84E-03
8.50E-06	1.79E-01	6.59E-02	1.89E-03
8.76E-06	1.84E-01	6.77E-02	1.94E-03
9.02E-06	1.89E-01	6.96E-02	1.99E-03
9.29E-06	1.94E-01	7.15E-02	2.05E-03
9.57E-06	2.00E-01	7.35E-02	2.10E-03
9.86E-06	2.05E-01	7.56E-02	2.16E-03

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Nongestational Lifetime			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.02E-05	2.11E-01	7.77E-02	2.22E-03
1.05E-05	2.16E-01	7.98E-02	2.28E-03
1.08E-05	2.22E-01	8.20E-02	2.34E-03
1.11E-05	2.28E-01	8.43E-02	2.41E-03
1.14E-05	2.34E-01	8.67E-02	2.47E-03
1.18E-05	2.41E-01	8.91E-02	2.54E-03
1.21E-05	2.47E-01	9.15E-02	2.61E-03
1.25E-05	2.54E-01	9.41E-02	2.68E-03
1.29E-05	2.61E-01	9.67E-02	2.75E-03
1.32E-05	2.68E-01	9.93E-02	2.82E-03
1.36E-05	2.75E-01	1.02E-01	2.90E-03
1.41E-05	2.83E-01	1.05E-01	2.98E-03
1.45E-05	2.90E-01	1.08E-01	3.06E-03
1.49E-05	2.98E-01	1.11E-01	3.14E-03
1.54E-05	3.06E-01	1.14E-01	3.22E-03
1.58E-05	3.14E-01	1.17E-01	3.31E-03
1.63E-05	3.23E-01	1.20E-01	3.40E-03
1.68E-05	3.31E-01	1.23E-01	3.49E-03
1.73E-05	3.40E-01	1.27E-01	3.58E-03
1.78E-05	3.49E-01	1.30E-01	3.68E-03
1.83E-05	3.58E-01	1.34E-01	3.78E-03
1.89E-05	3.68E-01	1.37E-01	3.88E-03
1.95E-05	3.78E-01	1.41E-01	3.98E-03
2.00E-05	3.88E-01	1.45E-01	4.09E-03
2.06E-05	3.98E-01	1.49E-01	4.20E-03
2.13E-05	4.09E-01	1.53E-01	4.31E-03
2.19E-05	4.20E-01	1.57E-01	4.42E-03
2.25E-05	4.31E-01	1.61E-01	4.54E-03
2.32E-05	4.42E-01	1.66E-01	4.66E-03

Nongestational Lifetime			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
2.39E-05	4.54E-01	1.70E-01	4.78E-03
2.46E-05	4.66E-01	1.75E-01	4.91E-03
2.54E-05	4.78E-01	1.80E-01	5.04E-03
2.61E-05	4.91E-01	1.84E-01	5.17E-03
2.69E-05	5.04E-01	1.89E-01	5.31E-03
2.77E-05	5.17E-01	1.95E-01	5.45E-03
2.86E-05	5.31E-01	2.00E-01	5.59E-03
2.94E-05	5.45E-01	2.05E-01	5.74E-03
3.03E-05	5.59E-01	2.11E-01	5.89E-03
3.12E-05	5.74E-01	2.16E-01	6.05E-03
3.21E-05	5.89E-01	2.22E-01	6.20E-03
3.31E-05	6.06E-01	2.29E-01	6.38E-03
3.41E-05	6.22E-01	2.35E-01	6.54E-03
3.51E-05	6.38E-01	2.41E-01	6.72E-03
3.62E-05	6.54E-01	2.48E-01	6.89E-03
3.73E-05	6.71E-01	2.54E-01	7.08E-03
3.84E-05	6.89E-01	2.61E-01	7.25E-03
3.95E-05	7.07E-01	2.68E-01	7.45E-03
4.07E-05	7.23E-01	2.74E-01	7.62E-03
4.19E-05	7.41E-01	2.82E-01	7.82E-03
4.32E-05	7.60E-01	2.89E-01	8.01E-03
4.45E-05	7.80E-01	2.97E-01	8.22E-03
4.58E-05	8.00E-01	3.05E-01	8.43E-03
4.72E-05	8.20E-01	3.13E-01	8.64E-03
4.86E-05	8.41E-01	3.21E-01	8.86E-03
5.01E-05	8.63E-01	3.29E-01	9.09E-03
5.16E-05	8.84E-01	3.38E-01	9.32E-03
5.31E-05	9.07E-01	3.47E-01	9.55E-03
5.47E-05	9.30E-01	3.56E-01	9.80E-03

Nongestational Lifetime			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
5.64E-05	9.53E-01	3.65E-01	1.00E-02
5.81E-05	9.77E-01	3.75E-01	1.03E-02
5.98E-05	1.00E+00	3.84E-01	1.06E-02
6.16E-05	1.03E+00	3.95E-01	1.08E-02
6.34E-05	1.05E+00	4.05E-01	1.11E-02
6.54E-05	1.08E+00	4.15E-01	1.14E-02
6.73E-05	1.11E+00	4.26E-01	1.17E-02
6.93E-05	1.13E+00	4.37E-01	1.19E-02
7.14E-05	1.16E+00	4.48E-01	1.22E-02
7.36E-05	1.19E+00	4.58E-01	1.25E-02
7.58E-05	1.22E+00	4.70E-01	1.28E-02
7.80E-05	1.25E+00	4.82E-01	1.31E-02
8.04E-05	1.28E+00	4.94E-01	1.34E-02
8.28E-05	1.31E+00	5.07E-01	1.38E-02
8.53E-05	1.34E+00	5.20E-01	1.41E-02
8.78E-05	1.37E+00	5.33E-01	1.45E-02
9.05E-05	1.41E+00	5.47E-01	1.48E-02
9.32E-05	1.44E+00	5.61E-01	1.52E-02
9.60E-05	1.48E+00	5.75E-01	1.55E-02
9.89E-05	1.51E+00	5.90E-01	1.59E-02
1.02E-04	1.55E+00	6.05E-01	1.63E-02
1.05E-04	1.59E+00	6.20E-01	1.67E-02
1.08E-04	1.62E+00	6.36E-01	1.71E-02
1.11E-04	1.66E+00	6.52E-01	1.75E-02
1.15E-04	1.70E+00	6.69E-01	1.79E-02
1.18E-04	1.75E+00	6.86E-01	1.84E-02
1.22E-04	1.79E+00	7.03E-01	1.88E-02
1.25E-04	1.83E+00	7.20E-01	1.93E-02
1.29E-04	1.87E+00	7.39E-01	1.97E-02

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Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
1.33E-04	1.92E+00	7.57E-01	2.02E-02
1.37E-04	1.97E+00	7.76E-01	2.07E-02
1.41E-04	2.01E+00	7.96E-01	2.12E-02
1.45E-04	2.08E+00	8.23E-01	2.19E-02
1.50E-04	2.11E+00	8.36E-01	2.22E-02
1.54E-04	2.16E+00	8.57E-01	2.27E-02
1.59E-04	2.23E+00	8.88E-01	2.35E-02
1.63E-04	2.29E+00	9.10E-01	2.41E-02
1.68E-04	2.32E+00	9.24E-01	2.44E-02
1.73E-04	2.37E+00	9.47E-01	2.50E-02
1.79E-04	2.43E+00	9.71E-01	2.56E-02
1.84E-04	2.49E+00	9.96E-01	2.62E-02
1.89E-04	2.55E+00	1.02E+00	2.68E-02
1.95E-04	2.61E+00	1.05E+00	2.75E-02
2.01E-04	2.67E+00	1.07E+00	2.81E-02
2.07E-04	2.76E+00	1.11E+00	2.91E-02
2.13E-04	2.80E+00	1.13E+00	2.94E-02
2.20E-04	2.86E+00	1.16E+00	3.01E-02
2.26E-04	2.95E+00	1.19E+00	3.11E-02
2.33E-04	3.02E+00	1.22E+00	3.18E-02
2.40E-04	3.09E+00	1.25E+00	3.26E-02
2.47E-04	3.14E+00	1.27E+00	3.30E-02
2.55E-04	3.21E+00	1.31E+00	3.38E-02
2.62E-04	3.29E+00	1.34E+00	3.46E-02
2.70E-04	3.39E+00	1.38E+00	3.57E-02
2.78E-04	3.47E+00	1.42E+00	3.65E-02
2.86E-04	3.55E+00	1.45E+00	3.74E-02
2.95E-04	3.61E+00	1.48E+00	3.80E-02
3.04E-04	3.72E+00	1.53E+00	3.91E-02

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
3.13E-04	3.80E+00	1.56E+00	4.00E-02
3.22E-04	3.89E+00	1.60E+00	4.10E-02
3.32E-04	3.98E+00	1.64E+00	4.19E-02
3.42E-04	4.07E+00	1.68E+00	4.29E-02
3.52E-04	4.16E+00	1.72E+00	4.38E-02
3.63E-04	4.26E+00	1.77E+00	4.48E-02
3.74E-04	4.35E+00	1.81E+00	4.58E-02
3.85E-04	4.45E+00	1.85E+00	4.69E-02
3.97E-04	4.55E+00	1.90E+00	4.80E-02
4.08E-04	4.66E+00	1.94E+00	4.90E-02
4.21E-04	4.76E+00	1.99E+00	5.01E-02
4.33E-04	4.87E+00	2.04E+00	5.13E-02
4.46E-04	4.98E+00	2.09E+00	5.24E-02
4.60E-04	5.09E+00	2.14E+00	5.36E-02
4.74E-04	5.20E+00	2.19E+00	5.48E-02
4.88E-04	5.32E+00	2.24E+00	5.60E-02
5.02E-04	5.43E+00	2.30E+00	5.72E-02
5.17E-04	5.55E+00	2.35E+00	5.85E-02
5.33E-04	5.68E+00	2.41E+00	5.98E-02
5.49E-04	5.80E+00	2.47E+00	6.11E-02
5.65E-04	5.93E+00	2.53E+00	6.24E-02
5.82E-04	6.06E+00	2.59E+00	6.38E-02
6.00E-04	6.19E+00	2.65E+00	6.52E-02
6.18E-04	6.33E+00	2.71E+00	6.66E-02
6.36E-04	6.46E+00	2.78E+00	6.80E-02
6.55E-04	6.60E+00	2.84E+00	6.95E-02
6.75E-04	6.75E+00	2.91E+00	7.10E-02
6.95E-04	6.89E+00	2.98E+00	7.26E-02
7.16E-04	7.04E+00	3.05E+00	7.41E-02

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
7.38E-04	7.20E+00	3.13E+00	7.58E-02
7.60E-04	7.35E+00	3.20E+00	7.74E-02
7.83E-04	7.51E+00	3.28E+00	7.91E-02
8.06E-04	7.61E+00	3.33E+00	8.01E-02
8.30E-04	7.77E+00	3.41E+00	8.19E-02
8.55E-04	7.94E+00	3.49E+00	8.36E-02
8.81E-04	8.11E+00	3.58E+00	8.54E-02
9.07E-04	8.30E+00	3.67E+00	8.74E-02
9.21E-04	8.37E+00	3.70E+00	8.81E-02
9.35E-04	8.46E+00	3.75E+00	8.90E-02
9.49E-04	9.14E+00	4.12E+00	9.62E-02
9.63E-04	9.54E+00	4.33E+00	1.00E-01
9.69E-04	9.70E+00	4.42E+00	1.02E-01
9.77E-04	9.87E+00	4.51E+00	1.04E-01
1.17E-03	1.01E+01	4.58E+00	1.07E-01
1.18E-03	1.02E+01	4.63E+00	1.08E-01
1.20E-03	1.03E+01	4.68E+00	1.09E-01
1.22E-03	1.04E+01	4.73E+00	1.10E-01
1.24E-03	1.05E+01	4.75E+00	1.10E-01
1.26E-03	1.06E+01	4.81E+00	1.11E-01
1.27E-03	1.07E+01	4.86E+00	1.12E-01
1.29E-03	1.08E+01	4.92E+00	1.14E-01
1.31E-03	1.09E+01	4.97E+00	1.15E-01
1.33E-03	1.10E+01	5.03E+00	1.16E-01
1.35E-03	1.11E+01	5.08E+00	1.17E-01
1.37E-03	1.12E+01	5.13E+00	1.18E-01
1.39E-03	1.13E+01	5.18E+00	1.19E-01
1.41E-03	1.14E+01	5.23E+00	1.20E-01
1.43E-03	1.15E+01	5.29E+00	1.21E-01

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Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
1.46E-03	1.16E+01	5.34E+00	1.22E-01
1.48E-03	1.17E+01	5.40E+00	1.23E-01
1.50E-03	1.18E+01	5.47E+00	1.25E-01
1.52E-03	1.20E+01	5.54E+00	1.26E-01
1.54E-03	1.21E+01	5.61E+00	1.28E-01
1.57E-03	1.22E+01	5.66E+00	1.29E-01
1.59E-03	1.24E+01	5.73E+00	1.30E-01
1.61E-03	1.25E+01	5.82E+00	1.32E-01
1.64E-03	1.27E+01	5.88E+00	1.33E-01
1.66E-03	1.28E+01	5.95E+00	1.35E-01
1.69E-03	1.29E+01	6.02E+00	1.36E-01
1.71E-03	1.31E+01	6.10E+00	1.37E-01
1.74E-03	1.32E+01	6.17E+00	1.39E-01
1.76E-03	1.33E+01	6.24E+00	1.40E-01
1.79E-03	1.35E+01	6.32E+00	1.42E-01
1.82E-03	1.36E+01	6.39E+00	1.43E-01
1.84E-03	1.38E+01	6.46E+00	1.45E-01
1.87E-03	1.40E+01	6.59E+00	1.47E-01
1.90E-03	1.46E+01	6.95E+00	1.54E-01
2.02E-03	1.50E+01	7.16E+00	1.58E-01
2.08E-03	1.51E+01	7.23E+00	1.59E-01
2.14E-03	1.53E+01	7.31E+00	1.61E-01
2.20E-03	1.56E+01	7.47E+00	1.64E-01
2.27E-03	1.59E+01	7.65E+00	1.68E-01
2.34E-03	1.62E+01	7.82E+00	1.71E-01
2.41E-03	1.66E+01	8.00E+00	1.74E-01
2.48E-03	1.69E+01	8.19E+00	1.78E-01
2.55E-03	1.72E+01	8.38E+00	1.81E-01
2.63E-03	1.76E+01	8.57E+00	1.85E-01

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
2.71E-03	1.79E+01	8.77E+00	1.89E-01
2.79E-03	1.83E+01	8.98E+00	1.92E-01
2.87E-03	1.87E+01	9.19E+00	1.96E-01
2.96E-03	1.90E+01	9.41E+00	2.00E-01
3.05E-03	1.94E+01	9.62E+00	2.04E-01
3.14E-03	1.98E+01	9.85E+00	2.08E-01
3.23E-03	2.02E+01	1.01E+01	2.13E-01
3.33E-03	2.06E+01	1.03E+01	2.17E-01
3.43E-03	2.10E+01	1.06E+01	2.21E-01
3.53E-03	2.14E+01	1.08E+01	2.25E-01
3.64E-03	2.18E+01	1.11E+01	2.30E-01
3.75E-03	2.25E+01	1.15E+01	2.37E-01
3.98E-03	2.29E+01	1.17E+01	2.41E-01
4.10E-03	2.32E+01	1.18E+01	2.44E-01
4.22E-03	2.35E+01	1.20E+01	2.48E-01
4.35E-03	2.40E+01	1.23E+01	2.52E-01
4.48E-03	2.44E+01	1.26E+01	2.57E-01
4.61E-03	2.49E+01	1.29E+01	2.63E-01
4.75E-03	2.55E+01	1.33E+01	2.69E-01
4.89E-03	2.61E+01	1.36E+01	2.74E-01
5.04E-03	2.69E+01	1.41E+01	2.83E-01
5.19E-03	2.75E+01	1.45E+01	2.90E-01
5.35E-03	2.83E+01	1.51E+01	2.98E-01
5.51E-03	2.91E+01	1.55E+01	3.06E-01
5.67E-03	2.97E+01	1.59E+01	3.13E-01
5.84E-03	3.03E+01	1.63E+01	3.19E-01
5.93E-03	3.04E+01	1.64E+01	3.20E-01
6.02E-03	3.07E+01	1.65E+01	3.23E-01
6.20E-03	3.15E+01	1.71E+01	3.31E-01

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
6.38E-03	3.22E+01	1.76E+01	3.39E-01
6.57E-03	3.28E+01	1.80E+01	3.46E-01
6.77E-03	3.35E+01	1.84E+01	3.53E-01
6.98E-03	3.42E+01	1.89E+01	3.60E-01
7.18E-03	3.50E+01	1.94E+01	3.68E-01
7.40E-03	3.57E+01	1.99E+01	3.76E-01
7.51E-03	3.61E+01	2.02E+01	3.80E-01
7.62E-03	3.63E+01	2.03E+01	3.82E-01
7.85E-03	3.67E+01	2.06E+01	3.87E-01
8.09E-03	3.70E+01	2.07E+01	3.89E-01
8.33E-03	3.75E+01	2.10E+01	3.94E-01
8.58E-03	3.89E+01	2.21E+01	4.09E-01
8.71E-03	3.93E+01	2.24E+01	4.14E-01
8.84E-03	3.97E+01	2.26E+01	4.18E-01
9.10E-03	4.04E+01	2.31E+01	4.25E-01
9.37E-03	4.13E+01	2.38E+01	4.35E-01
9.66E-03	4.21E+01	2.43E+01	4.44E-01
9.94E-03	4.31E+01	2.50E+01	4.53E-01
1.02E-02	4.39E+01	2.56E+01	4.62E-01
1.06E-02	4.47E+01	2.62E+01	4.71E-01
1.09E-02	4.56E+01	2.68E+01	4.80E-01
1.12E-02	4.66E+01	2.75E+01	4.90E-01
1.15E-02	4.75E+01	2.82E+01	5.00E-01
1.19E-02	4.82E+01	2.87E+01	5.07E-01
1.22E-02	4.91E+01	2.94E+01	5.17E-01
1.26E-02	5.00E+01	3.00E+01	5.26E-01
1.30E-02	5.12E+01	3.09E+01	5.39E-01
1.34E-02	5.24E+01	3.19E+01	5.52E-01
1.38E-02	5.36E+01	3.28E+01	5.65E-01

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Nongestational Lifetime			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.42E-02	5.48E+01	3.37E+01	5.77E-01
1.46E-02	5.57E+01	3.44E+01	5.87E-01
1.50E-02	5.68E+01	3.52E+01	5.97E-01
1.55E-02	5.78E+01	3.60E+01	6.08E-01
1.60E-02	5.88E+01	3.67E+01	6.19E-01
1.64E-02	5.97E+01	3.75E+01	6.29E-01
1.69E-02	6.10E+01	3.85E+01	6.42E-01
1.74E-02	6.22E+01	3.95E+01	6.55E-01
1.80E-02	6.34E+01	4.04E+01	6.68E-01
1.85E-02	6.47E+01	4.14E+01	6.81E-01
1.91E-02	6.60E+01	4.25E+01	6.94E-01
1.96E-02	6.73E+01	4.35E+01	7.08E-01
2.02E-02	6.86E+01	4.46E+01	7.22E-01
2.08E-02	7.00E+01	4.57E+01	7.36E-01
2.14E-02	7.13E+01	4.69E+01	7.51E-01
2.21E-02	7.28E+01	4.81E+01	7.66E-01
2.28E-02	7.42E+01	4.93E+01	7.81E-01
2.34E-02	7.57E+01	5.05E+01	7.97E-01
2.41E-02	7.71E+01	5.18E+01	8.12E-01
2.49E-02	7.87E+01	5.31E+01	8.28E-01
2.56E-02	8.02E+01	5.44E+01	8.44E-01
2.64E-02	8.18E+01	5.58E+01	8.61E-01
2.72E-02	8.33E+01	5.71E+01	8.77E-01
2.80E-02	8.50E+01	5.86E+01	8.95E-01
2.88E-02	8.67E+01	6.01E+01	9.12E-01
2.97E-02	8.83E+01	6.16E+01	9.30E-01
3.06E-02	9.03E+01	6.34E+01	9.50E-01
3.15E-02	9.21E+01	6.50E+01	9.69E-01
3.24E-02	9.40E+01	6.67E+01	9.89E-01

Nongestational Lifetime			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
3.34E-02	9.57E+01	6.83E+01	1.01E+00
3.44E-02	9.74E+01	6.99E+01	1.03E+00
3.54E-02	9.92E+01	7.15E+01	1.04E+00
3.65E-02	1.01E+02	7.32E+01	1.06E+00
3.76E-02	1.03E+02	7.51E+01	1.08E+00
3.87E-02	1.05E+02	7.69E+01	1.10E+00
3.99E-02	1.07E+02	7.89E+01	1.13E+00
4.11E-02	1.09E+02	8.09E+01	1.15E+00
4.23E-02	1.11E+02	8.30E+01	1.17E+00
4.36E-02	1.14E+02	8.53E+01	1.20E+00
4.49E-02	1.16E+02	8.76E+01	1.22E+00
4.63E-02	1.18E+02	8.99E+01	1.24E+00
4.76E-02	1.21E+02	9.22E+01	1.27E+00
4.91E-02	1.23E+02	9.46E+01	1.29E+00
5.05E-02	1.25E+02	9.70E+01	1.32E+00
5.21E-02	1.28E+02	9.95E+01	1.34E+00
5.36E-02	1.30E+02	1.02E+02	1.37E+00
5.52E-02	1.33E+02	1.05E+02	1.40E+00
5.69E-02	1.35E+02	1.07E+02	1.43E+00
5.86E-02	1.38E+02	1.10E+02	1.45E+00
6.03E-02	1.41E+02	1.13E+02	1.48E+00
6.22E-02	1.43E+02	1.16E+02	1.51E+00
6.40E-02	1.46E+02	1.19E+02	1.54E+00
6.59E-02	1.49E+02	1.22E+02	1.57E+00
6.79E-02	1.52E+02	1.25E+02	1.60E+00
7.00E-02	1.55E+02	1.28E+02	1.63E+00
7.21E-02	1.58E+02	1.31E+02	1.66E+00
7.42E-02	1.61E+02	1.35E+02	1.69E+00
7.64E-02	1.64E+02	1.38E+02	1.73E+00

Nongestational Lifetime			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
7.87E-02	1.67E+02	1.42E+02	1.76E+00
8.11E-02	1.71E+02	1.46E+02	1.80E+00
8.35E-02	1.74E+02	1.50E+02	1.83E+00
8.60E-02	1.78E+02	1.54E+02	1.87E+00
8.86E-02	1.81E+02	1.58E+02	1.90E+00
9.13E-02	1.85E+02	1.62E+02	1.94E+00
9.40E-02	1.88E+02	1.66E+02	1.98E+00
9.68E-02	1.92E+02	1.70E+02	2.02E+00
9.97E-02	1.96E+02	1.75E+02	2.06E+00
1.03E-01	1.99E+02	1.79E+02	2.10E+00
1.06E-01	2.03E+02	1.84E+02	2.14E+00
1.09E-01	2.07E+02	1.89E+02	2.18E+00
1.12E-01	2.11E+02	1.94E+02	2.22E+00
1.16E-01	2.15E+02	1.99E+02	2.27E+00
1.19E-01	2.20E+02	2.04E+02	2.31E+00
1.23E-01	2.24E+02	2.10E+02	2.36E+00
1.26E-01	2.28E+02	2.15E+02	2.40E+00
1.30E-01	2.33E+02	2.21E+02	2.45E+00
1.34E-01	2.38E+02	2.27E+02	2.50E+00
1.38E-01	2.42E+02	2.33E+02	2.55E+00
1.42E-01	2.47E+02	2.39E+02	2.60E+00
1.46E-01	2.52E+02	2.46E+02	2.65E+00
1.51E-01	2.57E+02	2.52E+02	2.70E+00
1.55E-01	2.62E+02	2.59E+02	2.75E+00
1.60E-01	2.67E+02	2.66E+02	2.81E+00
1.65E-01	2.72E+02	2.73E+02	2.86E+00
1.70E-01	2.78E+02	2.80E+02	2.92E+00
1.75E-01	2.83E+02	2.88E+02	2.98E+00
1.80E-01	2.89E+02	2.95E+02	3.04E+00

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Nongestational Lifetime			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.86E-01	2.94E+02	3.03E+02	3.10E+00
1.91E-01	3.00E+02	3.12E+02	3.16E+00
1.97E-01	3.06E+02	3.20E+02	3.22E+00
2.03E-01	3.12E+02	3.28E+02	3.28E+00
2.09E-01	3.18E+02	3.37E+02	3.35E+00
2.15E-01	3.25E+02	3.46E+02	3.42E+00
2.22E-01	3.31E+02	3.56E+02	3.48E+00
2.28E-01	3.38E+02	3.65E+02	3.55E+00
2.35E-01	3.44E+02	3.75E+02	3.62E+00
2.42E-01	3.51E+02	3.86E+02	3.70E+00
2.49E-01	3.58E+02	3.96E+02	3.77E+00
2.57E-01	3.65E+02	4.07E+02	3.85E+00
2.65E-01	3.73E+02	4.18E+02	3.92E+00
2.72E-01	3.80E+02	4.29E+02	4.00E+00
2.81E-01	3.88E+02	4.41E+02	4.08E+00
2.89E-01	3.95E+02	4.53E+02	4.16E+00
2.98E-01	4.03E+02	4.65E+02	4.24E+00
3.07E-01	4.11E+02	4.77E+02	4.33E+00
3.16E-01	4.19E+02	4.90E+02	4.41E+00
3.25E-01	4.28E+02	5.04E+02	4.50E+00
3.35E-01	4.36E+02	5.18E+02	4.59E+00
3.45E-01	4.45E+02	5.32E+02	4.68E+00
3.56E-01	4.54E+02	5.47E+02	4.78E+00
3.66E-01	4.63E+02	5.62E+02	4.87E+00
3.77E-01	4.72E+02	5.77E+02	4.97E+00
3.89E-01	4.82E+02	5.93E+02	5.07E+00
4.00E-01	4.91E+02	6.09E+02	5.17E+00
4.12E-01	5.01E+02	6.26E+02	5.28E+00
4.25E-01	5.11E+02	6.43E+02	5.38E+00

Nongestational Lifetime			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
4.37E-01	5.22E+02	6.61E+02	5.49E+00
4.50E-01	5.32E+02	6.79E+02	5.60E+00
4.64E-01	5.43E+02	6.98E+02	5.71E+00
4.92E-01	5.65E+02	7.37E+02	5.95E+00
5.07E-01	5.76E+02	7.57E+02	6.07E+00
5.22E-01	5.88E+02	7.78E+02	6.19E+00
5.54E-01	6.12E+02	8.22E+02	6.44E+00
5.71E-01	6.25E+02	8.44E+02	6.58E+00
5.88E-01	6.37E+02	8.68E+02	6.71E+00
6.05E-01	6.50E+02	8.92E+02	6.84E+00
6.23E-01	6.64E+02	9.17E+02	6.98E+00
6.61E-01	6.91E+02	9.68E+02	7.27E+00
6.81E-01	7.05E+02	9.95E+02	7.42E+00
7.02E-01	7.20E+02	1.02E+03	7.57E+00
7.23E-01	7.34E+02	1.05E+03	7.73E+00
7.44E-01	7.49E+02	1.08E+03	7.89E+00
7.67E-01	7.65E+02	1.11E+03	8.05E+00
7.90E-01	7.80E+02	1.14E+03	8.21E+00
8.13E-01	7.97E+02	1.17E+03	8.38E+00
8.38E-01	8.13E+02	1.21E+03	8.56E+00
8.63E-01	8.30E+02	1.24E+03	8.73E+00
8.89E-01	8.47E+02	1.28E+03	8.91E+00
9.16E-01	8.65E+02	1.31E+03	9.10E+00
9.43E-01	8.83E+02	1.35E+03	9.29E+00
9.71E-01	9.01E+02	1.39E+03	9.48E+00
1.00E+00	9.20E+02	1.43E+03	9.68E+00
1.06E+00	9.58E+02	1.51E+03	1.01E+01
1.09E+00	9.78E+02	1.55E+03	1.03E+01
1.13E+00	9.99E+02	1.59E+03	1.05E+01

Nongestational Lifetime			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.16E+00	1.02E+03	1.64E+03	1.07E+01
1.19E+00	1.04E+03	1.68E+03	1.10E+01
1.23E+00	1.06E+03	1.73E+03	1.12E+01
1.27E+00	1.09E+03	1.78E+03	1.14E+01
1.31E+00	1.11E+03	1.83E+03	1.17E+01
1.34E+00	1.13E+03	1.88E+03	1.19E+01
1.38E+00	1.16E+03	1.94E+03	1.22E+01
1.43E+00	1.18E+03	1.99E+03	1.24E+01
1.47E+00	1.21E+03	2.05E+03	1.27E+01
1.51E+00	1.23E+03	2.11E+03	1.30E+01
1.56E+00	1.26E+03	2.17E+03	1.32E+01
1.61E+00	1.28E+03	2.23E+03	1.35E+01
1.65E+00	1.31E+03	2.29E+03	1.38E+01
1.70E+00	1.34E+03	2.36E+03	1.41E+01
1.75E+00	1.37E+03	2.42E+03	1.44E+01
1.81E+00	1.40E+03	2.49E+03	1.47E+01
1.86E+00	1.43E+03	2.56E+03	1.50E+01
1.92E+00	1.46E+03	2.64E+03	1.54E+01
1.97E+00	1.49E+03	2.71E+03	1.57E+01
2.03E+00	1.52E+03	2.79E+03	1.60E+01
2.09E+00	1.56E+03	2.87E+03	1.64E+01
2.16E+00	1.59E+03	2.95E+03	1.67E+01
2.22E+00	1.62E+03	3.03E+03	1.71E+01
2.29E+00	1.66E+03	3.12E+03	1.75E+01
2.36E+00	1.70E+03	3.21E+03	1.79E+01
2.43E+00	1.73E+03	3.30E+03	1.82E+01
2.50E+00	1.77E+03	3.40E+03	1.86E+01
2.58E+00	1.81E+03	3.49E+03	1.91E+01
2.65E+00	1.85E+03	3.59E+03	1.95E+01

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Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
2.73E+00	1.89E+03	3.70E+03	1.99E+01
2.82E+00	1.93E+03	3.80E+03	2.04E+01
2.90E+00	1.98E+03	3.91E+03	2.08E+01
2.99E+00	2.02E+03	4.03E+03	2.13E+01
3.08E+00	2.07E+03	4.14E+03	2.17E+01
3.17E+00	2.11E+03	4.26E+03	2.22E+01
3.26E+00	2.16E+03	4.38E+03	2.27E+01
3.36E+00	2.21E+03	4.51E+03	2.32E+01
3.46E+00	2.26E+03	4.64E+03	2.38E+01
3.57E+00	2.31E+03	4.77E+03	2.43E+01
3.67E+00	2.36E+03	4.91E+03	2.49E+01
3.78E+00	2.42E+03	5.05E+03	2.54E+01
3.90E+00	2.47E+03	5.20E+03	2.60E+01
4.01E+00	2.53E+03	5.35E+03	2.66E+01
4.13E+00	2.58E+03	5.50E+03	2.72E+01
4.26E+00	2.64E+03	5.66E+03	2.78E+01
4.39E+00	2.70E+03	5.83E+03	2.85E+01
4.52E+00	2.77E+03	6.00E+03	2.91E+01
4.65E+00	2.83E+03	6.17E+03	2.98E+01
4.79E+00	2.90E+03	6.35E+03	3.05E+01
4.94E+00	2.96E+03	6.53E+03	3.12E+01
5.08E+00	3.03E+03	6.72E+03	3.19E+01
5.24E+00	3.10E+03	6.92E+03	3.27E+01
5.39E+00	3.18E+03	7.12E+03	3.34E+01
5.56E+00	3.25E+03	7.33E+03	3.42E+01
5.72E+00	3.33E+03	7.54E+03	3.50E+01
5.89E+00	3.41E+03	7.76E+03	3.58E+01
6.07E+00	3.49E+03	7.98E+03	3.67E+01
6.25E+00	3.57E+03	8.22E+03	3.76E+01

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
6.44E+00	3.65E+03	8.45E+03	3.85E+01
6.63E+00	3.74E+03	8.70E+03	3.94E+01
6.83E+00	3.83E+03	8.95E+03	4.03E+01
7.04E+00	3.92E+03	9.21E+03	4.13E+01
7.25E+00	4.02E+03	9.48E+03	4.23E+01
7.47E+00	4.11E+03	9.76E+03	4.33E+01
7.69E+00	4.21E+03	1.00E+04	4.43E+01
7.92E+00	4.32E+03	1.03E+04	4.54E+01
8.16E+00	4.42E+03	1.06E+04	4.65E+01
8.40E+00	4.53E+03	1.10E+04	4.77E+01
8.66E+00	4.64E+03	1.13E+04	4.88E+01
8.92E+00	4.75E+03	1.16E+04	5.00E+01
9.18E+00	4.87E+03	1.19E+04	5.13E+01
9.46E+00	4.99E+03	1.23E+04	5.25E+01
9.74E+00	5.11E+03	1.26E+04	5.38E+01
1.00E+01	5.22E+03	1.30E+04	5.50E+01
1.00E+01	5.24E+03	1.30E+04	5.51E+01
1.34E+01	6.64E+03	1.72E+04	6.99E+01
1.67E+01	8.04E+03	2.14E+04	8.47E+01
2.00E+01	9.45E+03	2.56E+04	9.94E+01
2.33E+01	1.08E+04	2.97E+04	1.14E+02
2.67E+01	1.22E+04	3.39E+04	1.28E+02
3.00E+01	1.36E+04	3.81E+04	1.43E+02
3.33E+01	1.49E+04	4.22E+04	1.57E+02
3.67E+01	1.63E+04	4.63E+04	1.72E+02
4.00E+01	1.77E+04	5.05E+04	1.86E+02
4.33E+01	1.90E+04	5.46E+04	2.00E+02
4.67E+01	2.04E+04	5.87E+04	2.15E+02
5.00E+01	2.17E+04	6.28E+04	2.29E+02

Nongestational Lifetime			
Intake (ng/kg/day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
5.33E+01	2.31E+04	6.69E+04	2.43E+02
5.67E+01	2.45E+04	7.10E+04	2.57E+02
6.00E+01	2.58E+04	7.51E+04	2.72E+02
6.33E+01	2.72E+04	7.92E+04	2.86E+02
6.67E+01	2.85E+04	8.32E+04	3.00E+02
7.00E+01	2.99E+04	8.73E+04	3.14E+02
7.33E+01	3.12E+04	9.13E+04	3.29E+02
7.67E+01	3.26E+04	9.54E+04	3.43E+02
8.00E+01	3.39E+04	9.94E+04	3.57E+02
8.33E+01	3.53E+04	1.03E+05	3.71E+02
8.67E+01	3.66E+04	1.07E+05	3.86E+02
9.00E+01	3.80E+04	1.12E+05	4.00E+02
9.33E+01	3.94E+04	1.16E+05	4.14E+02
9.67E+01	4.07E+04	1.20E+05	4.28E+02
1.00E+02	4.21E+04	1.24E+05	4.43E+02

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C.4.2. Nongestational 5-Year Average

Non-gestational 5-year Average			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.00E-09	5.18E-05	1.87E-05	5.45E-07
1.33E-09	6.90E-05	2.50E-05	7.26E-07
1.67E-09	8.62E-05	3.12E-05	9.07E-07
2.00E-09	1.03E-04	3.74E-05	1.09E-06
2.33E-09	1.21E-04	4.36E-05	1.27E-06
2.67E-09	1.38E-04	4.99E-05	1.45E-06
3.00E-09	1.55E-04	5.61E-05	1.63E-06
3.33E-09	1.72E-04	6.23E-05	1.81E-06
3.67E-09	1.90E-04	6.86E-05	1.99E-06
4.00E-09	2.07E-04	7.48E-05	2.17E-06
4.33E-09	2.24E-04	8.10E-05	2.36E-06
4.67E-09	2.41E-04	8.72E-05	2.54E-06
5.00E-09	2.58E-04	9.35E-05	2.72E-06
5.33E-09	2.76E-04	9.97E-05	2.90E-06
5.67E-09	2.93E-04	1.06E-04	3.08E-06
6.00E-09	3.10E-04	1.12E-04	3.26E-06
6.33E-09	3.27E-04	1.18E-04	3.44E-06
6.67E-09	3.44E-04	1.25E-04	3.62E-06
7.00E-09	3.61E-04	1.31E-04	3.80E-06
7.33E-09	3.79E-04	1.37E-04	3.98E-06
7.67E-09	3.96E-04	1.43E-04	4.16E-06
8.00E-09	4.13E-04	1.49E-04	4.34E-06
8.33E-09	4.30E-04	1.56E-04	4.52E-06
8.67E-09	4.47E-04	1.62E-04	4.70E-06
9.00E-09	4.65E-04	1.68E-04	4.89E-06
9.33E-09	4.82E-04	1.74E-04	5.07E-06
9.67E-09	4.99E-04	1.80E-04	5.25E-06
1.00E-08	5.16E-04	1.87E-04	5.43E-06
1.33E-08	6.87E-04	2.48E-04	7.22E-06
1.67E-08	8.57E-04	3.10E-04	9.01E-06
2.00E-08	1.03E-03	3.72E-04	1.08E-05
2.33E-08	1.20E-03	4.34E-04	1.26E-05
2.67E-08	1.37E-03	4.96E-04	1.44E-05
3.00E-08	1.54E-03	5.57E-04	1.62E-05
3.33E-08	1.71E-03	6.19E-04	1.80E-05

Non-gestational 5-year Average			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
3.67E-08	1.88E-03	6.81E-04	1.98E-05
4.00E-08	2.05E-03	7.43E-04	2.16E-05
4.33E-08	2.22E-03	8.04E-04	2.34E-05
4.67E-08	2.39E-03	8.66E-04	2.51E-05
5.00E-08	2.56E-03	9.28E-04	2.69E-05
5.33E-08	2.73E-03	9.89E-04	2.87E-05
5.67E-08	2.90E-03	1.05E-03	3.05E-05
6.00E-08	3.07E-03	1.11E-03	3.23E-05
6.33E-08	3.24E-03	1.17E-03	3.40E-05
6.67E-08	3.41E-03	1.23E-03	3.58E-05
7.00E-08	3.57E-03	1.30E-03	3.76E-05
7.33E-08	3.74E-03	1.36E-03	3.94E-05
7.67E-08	3.91E-03	1.42E-03	4.11E-05
8.00E-08	4.08E-03	1.48E-03	4.29E-05
8.33E-08	4.25E-03	1.54E-03	4.47E-05
8.67E-08	4.42E-03	1.60E-03	4.65E-05
9.00E-08	4.59E-03	1.66E-03	4.82E-05
9.33E-08	4.76E-03	1.72E-03	5.00E-05
9.67E-08	4.93E-03	1.79E-03	5.18E-05
1.00E-07	5.09E-03	1.85E-03	5.36E-05
1.33E-07	6.74E-03	2.45E-03	7.09E-05
1.67E-07	8.39E-03	3.05E-03	8.82E-05
2.00E-07	1.00E-02	3.65E-03	1.06E-04
2.33E-07	1.17E-02	4.25E-03	1.23E-04
2.67E-07	1.33E-02	4.85E-03	1.40E-04
3.00E-07	1.50E-02	5.45E-03	1.57E-04
3.33E-07	1.66E-02	6.05E-03	1.75E-04
3.67E-07	1.83E-02	6.65E-03	1.92E-04
4.00E-07	1.99E-02	7.25E-03	2.09E-04
4.33E-07	2.16E-02	7.85E-03	2.27E-04
4.67E-07	2.32E-02	8.45E-03	2.44E-04
5.00E-07	2.49E-02	9.05E-03	2.61E-04
5.33E-07	2.64E-02	9.63E-03	2.78E-04
5.66E-07	2.80E-02	1.02E-02	2.94E-04
5.99E-07	2.96E-02	1.08E-02	3.11E-04
6.33E-07	3.11E-02	1.14E-02	3.28E-04
6.66E-07	3.27E-02	1.19E-02	3.44E-04
6.99E-07	3.43E-02	1.25E-02	3.61E-04

Non-gestational 5-year Average			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
7.32E-07	3.59E-02	1.31E-02	3.77E-04
7.65E-07	3.74E-02	1.37E-02	3.94E-04
7.98E-07	3.90E-02	1.42E-02	4.10E-04
8.32E-07	4.06E-02	1.48E-02	4.27E-04
8.65E-07	4.22E-02	1.54E-02	4.43E-04
8.98E-07	4.37E-02	1.60E-02	4.60E-04
9.31E-07	4.53E-02	1.66E-02	4.77E-04
9.64E-07	4.69E-02	1.71E-02	4.93E-04
9.97E-07	4.85E-02	1.77E-02	5.10E-04
1.01E-06	4.92E-02	1.80E-02	5.17E-04
1.03E-06	4.99E-02	1.82E-02	5.24E-04
1.04E-06	5.06E-02	1.85E-02	5.32E-04
1.06E-06	5.13E-02	1.88E-02	5.40E-04
1.07E-06	5.20E-02	1.90E-02	5.47E-04
1.09E-06	5.28E-02	1.93E-02	5.55E-04
1.11E-06	5.35E-02	1.96E-02	5.63E-04
1.12E-06	5.43E-02	1.99E-02	5.71E-04
1.14E-06	5.51E-02	2.01E-02	5.79E-04
1.16E-06	5.59E-02	2.04E-02	5.88E-04
1.17E-06	5.67E-02	2.07E-02	5.96E-04
1.19E-06	5.75E-02	2.10E-02	6.05E-04
1.21E-06	5.83E-02	2.13E-02	6.13E-04
1.23E-06	5.92E-02	2.16E-02	6.22E-04
1.24E-06	6.00E-02	2.20E-02	6.31E-04
1.26E-06	6.09E-02	2.23E-02	6.40E-04
1.28E-06	6.17E-02	2.26E-02	6.49E-04
1.30E-06	6.26E-02	2.29E-02	6.58E-04
1.32E-06	6.35E-02	2.32E-02	6.68E-04
1.34E-06	6.44E-02	2.36E-02	6.77E-04
1.36E-06	6.53E-02	2.39E-02	6.87E-04
1.38E-06	6.63E-02	2.43E-02	6.97E-04
1.40E-06	6.72E-02	2.46E-02	7.07E-04
1.42E-06	6.82E-02	2.50E-02	7.17E-04
1.44E-06	6.91E-02	2.53E-02	7.27E-04
1.46E-06	7.02E-02	2.57E-02	7.38E-04
1.49E-06	7.12E-02	2.61E-02	7.48E-04
1.53E-06	7.32E-02	2.68E-02	7.70E-04
1.58E-06	7.53E-02	2.76E-02	7.92E-04

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Non-gestational 5-year Average			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.62E-06	7.74E-02	2.84E-02	8.14E-04
1.67E-06	7.96E-02	2.92E-02	8.37E-04
1.72E-06	8.19E-02	3.00E-02	8.61E-04
1.77E-06	8.42E-02	3.09E-02	8.86E-04
1.83E-06	8.66E-02	3.18E-02	9.11E-04
1.88E-06	8.91E-02	3.27E-02	9.37E-04
1.94E-06	9.16E-02	3.36E-02	9.63E-04
2.00E-06	9.42E-02	3.46E-02	9.91E-04
2.06E-06	9.69E-02	3.56E-02	1.02E-03
2.12E-06	9.96E-02	3.66E-02	1.05E-03
2.18E-06	1.02E-01	3.77E-02	1.08E-03
2.25E-06	1.05E-01	3.87E-02	1.11E-03
2.32E-06	1.08E-01	3.98E-02	1.14E-03
2.39E-06	1.11E-01	4.10E-02	1.17E-03
2.46E-06	1.15E-01	4.21E-02	1.20E-03
2.53E-06	1.18E-01	4.33E-02	1.24E-03
2.61E-06	1.21E-01	4.46E-02	1.27E-03
2.68E-06	1.24E-01	4.58E-02	1.31E-03
2.76E-06	1.28E-01	4.71E-02	1.35E-03
2.85E-06	1.32E-01	4.85E-02	1.38E-03
2.93E-06	1.35E-01	4.98E-02	1.42E-03
3.02E-06	1.39E-01	5.13E-02	1.46E-03
3.11E-06	1.43E-01	5.27E-02	1.50E-03
3.21E-06	1.47E-01	5.42E-02	1.54E-03
3.30E-06	1.51E-01	5.57E-02	1.59E-03
3.40E-06	1.55E-01	5.73E-02	1.63E-03
3.50E-06	1.59E-01	5.89E-02	1.68E-03
3.61E-06	1.64E-01	6.05E-02	1.72E-03
3.72E-06	1.68E-01	6.22E-02	1.77E-03
3.83E-06	1.73E-01	6.40E-02	1.82E-03
3.94E-06	1.78E-01	6.58E-02	1.87E-03
4.06E-06	1.83E-01	6.76E-02	1.92E-03
4.18E-06	1.88E-01	6.95E-02	1.97E-03
4.31E-06	1.93E-01	7.15E-02	2.03E-03
4.44E-06	1.98E-01	7.34E-02	2.08E-03
4.57E-06	2.04E-01	7.55E-02	2.14E-03
4.71E-06	2.09E-01	7.76E-02	2.20E-03
4.85E-06	2.15E-01	7.98E-02	2.26E-03

Non-gestational 5-year Average			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
4.99E-06	2.21E-01	8.20E-02	2.32E-03
5.14E-06	2.27E-01	8.42E-02	2.39E-03
5.30E-06	2.33E-01	8.66E-02	2.45E-03
5.46E-06	2.39E-01	8.90E-02	2.52E-03
5.62E-06	2.46E-01	9.14E-02	2.59E-03
5.79E-06	2.53E-01	9.39E-02	2.66E-03
5.96E-06	2.59E-01	9.65E-02	2.73E-03
6.14E-06	2.66E-01	9.92E-02	2.80E-03
6.33E-06	2.74E-01	1.02E-01	2.88E-03
6.52E-06	2.81E-01	1.05E-01	2.95E-03
6.71E-06	2.88E-01	1.07E-01	3.03E-03
6.91E-06	2.96E-01	1.10E-01	3.11E-03
7.12E-06	3.04E-01	1.13E-01	3.19E-03
7.33E-06	3.12E-01	1.16E-01	3.28E-03
7.55E-06	3.20E-01	1.19E-01	3.36E-03
7.78E-06	3.28E-01	1.23E-01	3.45E-03
8.01E-06	3.37E-01	1.26E-01	3.54E-03
8.25E-06	3.46E-01	1.29E-01	3.64E-03
8.50E-06	3.55E-01	1.33E-01	3.73E-03
8.76E-06	3.64E-01	1.36E-01	3.83E-03
9.02E-06	3.74E-01	1.40E-01	3.93E-03
9.29E-06	3.84E-01	1.44E-01	4.04E-03
9.57E-06	3.94E-01	1.48E-01	4.15E-03
9.86E-06	4.05E-01	1.52E-01	4.25E-03
1.02E-05	4.15E-01	1.56E-01	4.36E-03
1.05E-05	4.26E-01	1.60E-01	4.48E-03
1.08E-05	4.37E-01	1.64E-01	4.59E-03
1.11E-05	4.48E-01	1.68E-01	4.71E-03
1.14E-05	4.60E-01	1.73E-01	4.83E-03
1.18E-05	4.72E-01	1.78E-01	4.96E-03
1.21E-05	4.84E-01	1.82E-01	5.08E-03
1.25E-05	4.96E-01	1.87E-01	5.21E-03
1.29E-05	5.09E-01	1.92E-01	5.35E-03
1.32E-05	5.22E-01	1.97E-01	5.49E-03
1.36E-05	5.35E-01	2.02E-01	5.63E-03
1.41E-05	5.49E-01	2.08E-01	5.77E-03
1.45E-05	5.63E-01	2.13E-01	5.92E-03
1.49E-05	5.77E-01	2.18E-01	6.07E-03

Non-gestational 5-year Average			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.54E-05	5.92E-01	2.24E-01	6.23E-03
1.58E-05	6.07E-01	2.30E-01	6.38E-03
1.63E-05	6.23E-01	2.36E-01	6.55E-03
1.68E-05	6.38E-01	2.42E-01	6.71E-03
1.73E-05	6.54E-01	2.49E-01	6.88E-03
1.78E-05	6.71E-01	2.55E-01	7.05E-03
1.83E-05	6.88E-01	2.62E-01	7.23E-03
1.89E-05	7.05E-01	2.69E-01	7.41E-03
1.95E-05	7.23E-01	2.75E-01	7.60E-03
2.00E-05	7.41E-01	2.83E-01	7.79E-03
2.06E-05	7.60E-01	2.90E-01	7.99E-03
2.13E-05	7.79E-01	2.97E-01	8.18E-03
2.19E-05	7.98E-01	3.05E-01	8.39E-03
2.25E-05	8.18E-01	3.13E-01	8.60E-03
2.32E-05	8.38E-01	3.21E-01	8.81E-03
2.39E-05	8.59E-01	3.29E-01	9.03E-03
2.46E-05	8.80E-01	3.38E-01	9.25E-03
2.54E-05	9.02E-01	3.46E-01	9.48E-03
2.61E-05	9.24E-01	3.55E-01	9.71E-03
2.69E-05	9.47E-01	3.64E-01	9.95E-03
2.77E-05	9.70E-01	3.73E-01	1.02E-02
2.86E-05	9.94E-01	3.83E-01	1.04E-02
2.94E-05	1.02E+00	3.92E-01	1.07E-02
3.03E-05	1.04E+00	4.02E-01	1.10E-02
3.12E-05	1.07E+00	4.12E-01	1.12E-02
3.21E-05	1.09E+00	4.23E-01	1.15E-02
3.31E-05	1.12E+00	4.35E-01	1.18E-02
3.41E-05	1.15E+00	4.46E-01	1.21E-02
3.51E-05	1.18E+00	4.57E-01	1.23E-02
3.62E-05	1.21E+00	4.68E-01	1.27E-02
3.73E-05	1.24E+00	4.80E-01	1.30E-02
3.84E-05	1.26E+00	4.92E-01	1.33E-02
3.95E-05	1.29E+00	5.04E-01	1.35E-02
4.07E-05	1.32E+00	5.14E-01	1.39E-02
4.19E-05	1.35E+00	5.26E-01	1.42E-02
4.32E-05	1.38E+00	5.39E-01	1.45E-02
4.45E-05	1.41E+00	5.52E-01	1.49E-02
4.58E-05	1.45E+00	5.66E-01	1.52E-02

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Non-gestational 5-year Average			
Intake (ng/kg/ day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
4.72E-05	1.48E+00	5.80E-01	1.56E-02
4.86E-05	1.52E+00	5.94E-01	1.59E-02
5.01E-05	1.55E+00	6.08E-01	1.63E-02
5.16E-05	1.59E+00	6.23E-01	1.67E-02
5.31E-05	1.62E+00	6.38E-01	1.71E-02
5.47E-05	1.66E+00	6.53E-01	1.75E-02
5.64E-05	1.70E+00	6.69E-01	1.79E-02
5.81E-05	1.74E+00	6.85E-01	1.83E-02
5.98E-05	1.78E+00	7.02E-01	1.87E-02
6.16E-05	1.82E+00	7.19E-01	1.91E-02
6.34E-05	1.86E+00	7.36E-01	1.96E-02
6.54E-05	1.90E+00	7.53E-01	2.00E-02
6.73E-05	1.95E+00	7.71E-01	2.05E-02
6.93E-05	1.99E+00	7.90E-01	2.09E-02
7.14E-05	2.04E+00	8.08E-01	2.14E-02
7.36E-05	2.06E+00	8.18E-01	2.16E-02
7.58E-05	2.11E+00	8.37E-01	2.21E-02
7.80E-05	2.15E+00	8.57E-01	2.26E-02
8.04E-05	2.20E+00	8.77E-01	2.31E-02
8.28E-05	2.25E+00	8.98E-01	2.36E-02
8.53E-05	2.30E+00	9.19E-01	2.42E-02
8.78E-05	2.35E+00	9.40E-01	2.47E-02
9.05E-05	2.40E+00	9.62E-01	2.52E-02
9.32E-05	2.46E+00	9.84E-01	2.58E-02
9.60E-05	2.51E+00	1.01E+00	2.64E-02
9.89E-05	2.57E+00	1.03E+00	2.69E-02
1.02E-04	2.62E+00	1.05E+00	2.75E-02
1.05E-04	2.68E+00	1.08E+00	2.81E-02
1.08E-04	2.74E+00	1.10E+00	2.88E-02
1.11E-04	2.80E+00	1.13E+00	2.94E-02
1.15E-04	2.86E+00	1.15E+00	3.00E-02
1.18E-04	2.92E+00	1.18E+00	3.07E-02
1.22E-04	2.98E+00	1.21E+00	3.13E-02
1.25E-04	3.05E+00	1.24E+00	3.20E-02
1.29E-04	3.11E+00	1.26E+00	3.27E-02
1.33E-04	3.18E+00	1.29E+00	3.34E-02
1.37E-04	3.25E+00	1.32E+00	3.41E-02
1.41E-04	3.32E+00	1.35E+00	3.48E-02

Non-gestational 5-year Average			
Intake (ng/kg/ day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
1.45E-04	3.45E+00	1.41E+00	3.62E-02
1.50E-04	3.46E+00	1.41E+00	3.63E-02
1.54E-04	3.53E+00	1.45E+00	3.71E-02
1.59E-04	3.67E+00	1.51E+00	3.86E-02
1.63E-04	3.75E+00	1.54E+00	3.94E-02
1.68E-04	3.77E+00	1.55E+00	3.96E-02
1.73E-04	3.86E+00	1.59E+00	4.06E-02
1.79E-04	3.95E+00	1.63E+00	4.15E-02
1.84E-04	4.04E+00	1.67E+00	4.24E-02
1.89E-04	4.13E+00	1.71E+00	4.33E-02
1.95E-04	4.22E+00	1.75E+00	4.43E-02
2.01E-04	4.31E+00	1.79E+00	4.52E-02
2.07E-04	4.44E+00	1.84E+00	4.66E-02
2.13E-04	4.49E+00	1.87E+00	4.72E-02
2.20E-04	4.59E+00	1.92E+00	4.82E-02
2.26E-04	4.72E+00	1.97E+00	4.95E-02
2.33E-04	4.81E+00	2.02E+00	5.05E-02
2.40E-04	4.91E+00	2.06E+00	5.16E-02
2.47E-04	5.00E+00	2.10E+00	5.24E-02
2.55E-04	5.10E+00	2.15E+00	5.35E-02
2.62E-04	5.21E+00	2.19E+00	5.47E-02
2.70E-04	5.33E+00	2.25E+00	5.60E-02
2.78E-04	5.44E+00	2.30E+00	5.71E-02
2.86E-04	5.55E+00	2.35E+00	5.83E-02
2.95E-04	5.66E+00	2.40E+00	5.94E-02
3.04E-04	5.78E+00	2.46E+00	6.07E-02
3.13E-04	5.90E+00	2.51E+00	6.19E-02
3.22E-04	6.02E+00	2.57E+00	6.32E-02
3.32E-04	6.14E+00	2.63E+00	6.44E-02
3.42E-04	6.26E+00	2.68E+00	6.57E-02
3.52E-04	6.39E+00	2.74E+00	6.71E-02
3.63E-04	6.52E+00	2.80E+00	6.84E-02
3.74E-04	6.65E+00	2.87E+00	6.98E-02
3.85E-04	6.78E+00	2.93E+00	7.12E-02
3.97E-04	6.92E+00	3.00E+00	7.26E-02
4.08E-04	7.06E+00	3.06E+00	7.41E-02
4.21E-04	7.20E+00	3.13E+00	7.56E-02
4.33E-04	7.34E+00	3.20E+00	7.71E-02

Non-gestational 5-year Average			
Intake (ng/kg/ day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
4.46E-04	7.49E+00	3.27E+00	7.86E-02
4.60E-04	7.64E+00	3.34E+00	8.02E-02
4.74E-04	7.79E+00	3.42E+00	8.18E-02
4.88E-04	7.95E+00	3.49E+00	8.34E-02
5.02E-04	8.10E+00	3.57E+00	8.50E-02
5.17E-04	8.26E+00	3.65E+00	8.67E-02
5.33E-04	8.43E+00	3.73E+00	8.84E-02
5.49E-04	8.59E+00	3.81E+00	9.02E-02
5.65E-04	8.76E+00	3.89E+00	9.19E-02
5.82E-04	8.93E+00	3.98E+00	9.37E-02
6.00E-04	9.11E+00	4.07E+00	9.56E-02
6.18E-04	9.29E+00	4.16E+00	9.74E-02
6.36E-04	9.47E+00	4.25E+00	9.94E-02
6.55E-04	9.65E+00	4.34E+00	1.01E-01
6.75E-04	9.84E+00	4.44E+00	1.03E-01
6.95E-04	1.00E+01	4.54E+00	1.05E-01
7.16E-04	1.02E+01	4.64E+00	1.07E-01
7.38E-04	1.04E+01	4.74E+00	1.09E-01
7.60E-04	1.06E+01	4.84E+00	1.12E-01
7.83E-04	1.08E+01	4.95E+00	1.14E-01
8.06E-04	1.10E+01	5.06E+00	1.16E-01
8.30E-04	1.13E+01	5.17E+00	1.18E-01
8.55E-04	1.15E+01	5.28E+00	1.20E-01
8.81E-04	1.17E+01	5.40E+00	1.23E-01
9.07E-04	1.19E+01	5.52E+00	1.25E-01
9.21E-04	1.20E+01	5.58E+00	1.26E-01
9.35E-04	1.22E+01	5.64E+00	1.27E-01
9.49E-04	1.30E+01	6.23E+00	1.37E-01
9.63E-04	1.38E+01	6.92E+00	1.45E-01
9.69E-04	1.43E+01	7.14E+00	1.50E-01
9.77E-04	1.48E+01	7.34E+00	1.55E-01
9.84E-04	1.52E+01	7.50E+00	1.59E-01
9.91E-04	1.55E+01	7.64E+00	1.63E-01
1.37E-03	1.56E+01	7.50E+00	1.63E-01
1.39E-03	1.57E+01	7.58E+00	1.65E-01
1.41E-03	1.59E+01	7.66E+00	1.66E-01
1.43E-03	1.60E+01	7.75E+00	1.68E-01
1.46E-03	1.62E+01	7.83E+00	1.69E-01

Non-gestational 5-year Average			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.48E-03	1.63E+01	7.92E+00	1.71E-01
1.50E-03	1.65E+01	8.00E+00	1.73E-01
1.52E-03	1.66E+01	8.09E+00	1.74E-01
1.54E-03	1.68E+01	8.18E+00	1.76E-01
1.57E-03	1.69E+01	8.27E+00	1.78E-01
1.59E-03	1.71E+01	8.36E+00	1.79E-01
1.61E-03	1.73E+01	8.46E+00	1.81E-01
1.64E-03	1.74E+01	8.55E+00	1.83E-01
1.66E-03	1.76E+01	8.64E+00	1.84E-01
1.69E-03	1.78E+01	8.74E+00	1.86E-01
1.71E-03	1.79E+01	8.83E+00	1.88E-01
1.74E-03	1.81E+01	8.93E+00	1.90E-01
1.76E-03	1.83E+01	9.03E+00	1.92E-01
1.79E-03	1.84E+01	9.13E+00	1.93E-01
1.82E-03	1.86E+01	9.23E+00	1.95E-01
1.84E-03	1.88E+01	9.33E+00	1.97E-01
1.87E-03	1.91E+01	9.53E+00	2.00E-01
1.90E-03	1.98E+01	1.01E+01	2.08E-01
1.93E-03	2.05E+01	1.08E+01	2.14E-01
1.96E-03	2.05E+01	1.05E+01	2.14E-01
2.27E-03	2.14E+01	1.09E+01	2.25E-01
2.34E-03	2.18E+01	1.11E+01	2.29E-01
2.41E-03	2.22E+01	1.14E+01	2.33E-01
2.48E-03	2.26E+01	1.16E+01	2.37E-01
2.55E-03	2.31E+01	1.19E+01	2.42E-01
2.63E-03	2.35E+01	1.22E+01	2.46E-01
2.71E-03	2.39E+01	1.24E+01	2.51E-01
2.79E-03	2.44E+01	1.27E+01	2.56E-01
2.87E-03	2.49E+01	1.30E+01	2.61E-01
2.96E-03	2.53E+01	1.33E+01	2.66E-01
3.05E-03	2.58E+01	1.36E+01	2.71E-01
3.14E-03	2.63E+01	1.39E+01	2.76E-01
3.23E-03	2.68E+01	1.42E+01	2.81E-01
3.33E-03	2.73E+01	1.45E+01	2.86E-01
3.43E-03	2.78E+01	1.49E+01	2.91E-01
3.53E-03	2.83E+01	1.52E+01	2.97E-01
3.64E-03	2.88E+01	1.55E+01	3.02E-01
3.75E-03	2.96E+01	1.61E+01	3.10E-01

Non-gestational 5-year Average			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
3.81E-03	2.99E+01	1.63E+01	3.14E-01
3.86E-03	3.00E+01	1.63E+01	3.14E-01
4.22E-03	3.04E+01	1.66E+01	3.19E-01
4.35E-03	3.10E+01	1.69E+01	3.25E-01
4.48E-03	3.16E+01	1.73E+01	3.31E-01
4.61E-03	3.21E+01	1.77E+01	3.37E-01
4.75E-03	3.28E+01	1.81E+01	3.44E-01
4.89E-03	3.34E+01	1.86E+01	3.50E-01
5.04E-03	3.44E+01	1.94E+01	3.60E-01
5.19E-03	3.57E+01	2.06E+01	3.74E-01
5.35E-03	3.72E+01	2.12E+01	3.90E-01
5.51E-03	3.81E+01	2.17E+01	3.99E-01
5.67E-03	3.88E+01	2.23E+01	4.07E-01
5.84E-03	3.95E+01	2.28E+01	4.14E-01
5.93E-03	3.98E+01	2.30E+01	4.18E-01
6.02E-03	4.00E+01	2.33E+01	4.20E-01
6.20E-03	4.10E+01	2.38E+01	4.30E-01
6.38E-03	4.18E+01	2.44E+01	4.38E-01
6.57E-03	4.26E+01	2.49E+01	4.46E-01
6.77E-03	4.34E+01	2.55E+01	4.55E-01
6.98E-03	4.42E+01	2.61E+01	4.63E-01
7.18E-03	4.50E+01	2.67E+01	4.72E-01
7.40E-03	4.59E+01	2.73E+01	4.81E-01
7.51E-03	4.63E+01	2.77E+01	4.85E-01
7.62E-03	4.66E+01	2.78E+01	4.88E-01
7.85E-03	4.71E+01	2.81E+01	4.94E-01
8.09E-03	4.72E+01	2.79E+01	4.95E-01
8.33E-03	4.74E+01	2.83E+01	4.97E-01
8.58E-03	4.93E+01	2.99E+01	5.17E-01
8.71E-03	4.98E+01	3.03E+01	5.22E-01
8.84E-03	5.03E+01	3.06E+01	5.27E-01
9.10E-03	5.13E+01	3.15E+01	5.38E-01
9.37E-03	5.23E+01	3.22E+01	5.49E-01
9.66E-03	5.33E+01	3.29E+01	5.59E-01
9.94E-03	5.44E+01	3.38E+01	5.70E-01
1.02E-02	5.54E+01	3.46E+01	5.81E-01
1.06E-02	5.64E+01	3.54E+01	5.92E-01
1.09E-02	5.75E+01	3.62E+01	6.03E-01

Non-gestational 5-year Average			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.12E-02	5.86E+01	3.71E+01	6.14E-01
1.15E-02	5.96E+01	3.79E+01	6.25E-01
1.19E-02	6.05E+01	3.85E+01	6.35E-01
1.22E-02	6.14E+01	3.92E+01	6.43E-01
1.26E-02	6.24E+01	4.01E+01	6.54E-01
1.30E-02	6.38E+01	4.15E+01	6.69E-01
1.34E-02	6.56E+01	4.31E+01	6.87E-01
1.38E-02	6.74E+01	4.42E+01	7.07E-01
1.42E-02	6.87E+01	4.53E+01	7.20E-01
1.46E-02	6.94E+01	4.59E+01	7.28E-01
1.50E-02	7.06E+01	4.69E+01	7.40E-01
1.55E-02	7.19E+01	4.78E+01	7.54E-01
1.60E-02	7.30E+01	4.87E+01	7.66E-01
1.64E-02	7.38E+01	4.96E+01	7.74E-01
1.69E-02	7.55E+01	5.11E+01	7.92E-01
1.74E-02	7.69E+01	5.23E+01	8.07E-01
1.80E-02	7.84E+01	5.36E+01	8.22E-01
1.85E-02	7.99E+01	5.49E+01	8.37E-01
1.91E-02	8.13E+01	5.62E+01	8.53E-01
1.96E-02	8.29E+01	5.75E+01	8.69E-01
2.02E-02	8.44E+01	5.89E+01	8.85E-01
2.08E-02	8.60E+01	6.03E+01	9.01E-01
2.14E-02	8.76E+01	6.18E+01	9.18E-01
2.21E-02	8.92E+01	6.32E+01	9.35E-01
2.28E-02	9.09E+01	6.48E+01	9.53E-01
2.34E-02	9.26E+01	6.63E+01	9.71E-01
2.41E-02	9.44E+01	6.80E+01	9.89E-01
2.49E-02	9.64E+01	6.98E+01	1.01E+00
2.56E-02	9.79E+01	7.13E+01	1.03E+00
2.64E-02	9.98E+01	7.30E+01	1.05E+00
2.72E-02	1.02E+02	7.48E+01	1.07E+00
2.80E-02	1.04E+02	7.66E+01	1.09E+00
2.88E-02	1.06E+02	7.85E+01	1.11E+00
2.97E-02	1.07E+02	8.04E+01	1.13E+00
3.06E-02	1.10E+02	8.28E+01	1.15E+00
3.15E-02	1.12E+02	8.51E+01	1.17E+00
3.24E-02	1.14E+02	8.69E+01	1.20E+00
3.34E-02	1.16E+02	8.88E+01	1.22E+00

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Non-gestational 5-year Average			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
3.44E-02	1.18E+02	9.08E+01	1.24E+00
3.54E-02	1.20E+02	9.28E+01	1.26E+00
3.65E-02	1.22E+02	9.47E+01	1.28E+00
3.76E-02	1.24E+02	9.73E+01	1.30E+00
3.87E-02	1.27E+02	9.96E+01	1.33E+00
3.99E-02	1.29E+02	1.02E+02	1.35E+00
4.11E-02	1.32E+02	1.04E+02	1.38E+00
4.23E-02	1.34E+02	1.07E+02	1.40E+00
4.36E-02	1.37E+02	1.10E+02	1.43E+00
4.49E-02	1.40E+02	1.13E+02	1.47E+00
4.63E-02	1.43E+02	1.16E+02	1.49E+00
4.76E-02	1.45E+02	1.19E+02	1.52E+00
4.91E-02	1.48E+02	1.22E+02	1.55E+00
5.05E-02	1.51E+02	1.25E+02	1.58E+00
5.21E-02	1.53E+02	1.28E+02	1.61E+00
5.36E-02	1.56E+02	1.31E+02	1.64E+00
5.52E-02	1.59E+02	1.34E+02	1.67E+00
5.69E-02	1.62E+02	1.38E+02	1.70E+00
5.86E-02	1.65E+02	1.41E+02	1.73E+00
6.03E-02	1.69E+02	1.45E+02	1.77E+00
6.22E-02	1.72E+02	1.48E+02	1.80E+00
6.40E-02	1.74E+02	1.52E+02	1.83E+00
6.59E-02	1.78E+02	1.55E+02	1.86E+00
6.79E-02	1.81E+02	1.59E+02	1.90E+00
7.00E-02	1.84E+02	1.63E+02	1.93E+00
7.21E-02	1.88E+02	1.67E+02	1.97E+00
7.42E-02	1.91E+02	1.71E+02	2.01E+00
7.64E-02	1.95E+02	1.76E+02	2.05E+00
7.87E-02	1.99E+02	1.81E+02	2.09E+00
8.11E-02	2.03E+02	1.86E+02	2.13E+00
8.35E-02	2.07E+02	1.90E+02	2.17E+00
8.60E-02	2.11E+02	1.95E+02	2.21E+00
8.86E-02	2.15E+02	2.00E+02	2.25E+00
9.13E-02	2.19E+02	2.05E+02	2.30E+00
9.40E-02	2.23E+02	2.10E+02	2.34E+00
9.68E-02	2.27E+02	2.16E+02	2.38E+00
9.97E-02	2.32E+02	2.22E+02	2.43E+00
1.03E-01	2.36E+02	2.27E+02	2.48E+00

Non-gestational 5-year Average			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.06E-01	2.41E+02	2.33E+02	2.52E+00
1.09E-01	2.45E+02	2.39E+02	2.57E+00
1.12E-01	2.50E+02	2.44E+02	2.62E+00
1.16E-01	2.55E+02	2.51E+02	2.67E+00
1.19E-01	2.60E+02	2.57E+02	2.72E+00
1.23E-01	2.65E+02	2.64E+02	2.77E+00
1.26E-01	2.70E+02	2.71E+02	2.83E+00
1.30E-01	2.75E+02	2.78E+02	2.88E+00
1.34E-01	2.80E+02	2.86E+02	2.94E+00
1.38E-01	2.86E+02	2.93E+02	3.00E+00
1.42E-01	2.92E+02	3.01E+02	3.06E+00
1.46E-01	2.97E+02	3.09E+02	3.11E+00
1.51E-01	3.03E+02	3.16E+02	3.17E+00
1.55E-01	3.08E+02	3.24E+02	3.23E+00
1.60E-01	3.14E+02	3.33E+02	3.29E+00
1.65E-01	3.20E+02	3.42E+02	3.36E+00
1.70E-01	3.27E+02	3.51E+02	3.42E+00
1.75E-01	3.33E+02	3.60E+02	3.49E+00
1.80E-01	3.39E+02	3.69E+02	3.56E+00
1.86E-01	3.46E+02	3.79E+02	3.63E+00
1.91E-01	3.53E+02	3.89E+02	3.70E+00
1.97E-01	3.60E+02	3.99E+02	3.77E+00
2.03E-01	3.66E+02	4.09E+02	3.84E+00
2.09E-01	3.73E+02	4.20E+02	3.91E+00
2.15E-01	3.81E+02	4.31E+02	3.99E+00
2.22E-01	3.88E+02	4.43E+02	4.07E+00
2.28E-01	3.96E+02	4.55E+02	4.15E+00
2.35E-01	4.03E+02	4.67E+02	4.23E+00
2.42E-01	4.11E+02	4.79E+02	4.31E+00
2.49E-01	4.20E+02	4.92E+02	4.40E+00
2.57E-01	4.28E+02	5.05E+02	4.48E+00
2.65E-01	4.36E+02	5.19E+02	4.57E+00
2.72E-01	4.45E+02	5.32E+02	4.66E+00
2.81E-01	4.53E+02	5.46E+02	4.75E+00
2.89E-01	4.62E+02	5.61E+02	4.84E+00
2.98E-01	4.71E+02	5.75E+02	4.93E+00
3.07E-01	4.80E+02	5.91E+02	5.03E+00
3.16E-01	4.90E+02	6.07E+02	5.13E+00

Non-gestational 5-year Average			
Intake (ng/kg/day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
3.25E-01	4.99E+02	6.23E+02	5.23E+00
3.35E-01	5.09E+02	6.40E+02	5.34E+00
3.45E-01	5.19E+02	6.57E+02	5.44E+00
3.56E-01	5.30E+02	6.75E+02	5.55E+00
3.66E-01	5.40E+02	6.93E+02	5.66E+00
3.77E-01	5.51E+02	7.12E+02	5.77E+00
3.89E-01	5.62E+02	7.31E+02	5.89E+00
4.00E-01	5.73E+02	7.51E+02	6.00E+00
4.12E-01	5.84E+02	7.71E+02	6.12E+00
4.25E-01	5.96E+02	7.92E+02	6.25E+00
4.37E-01	6.08E+02	8.13E+02	6.37E+00
4.50E-01	6.20E+02	8.35E+02	6.50E+00
4.64E-01	6.32E+02	8.58E+02	6.63E+00
4.92E-01	6.58E+02	9.05E+02	6.89E+00
5.07E-01	6.71E+02	9.29E+02	7.03E+00
5.22E-01	6.85E+02	9.55E+02	7.17E+00
5.54E-01	7.12E+02	1.01E+03	7.46E+00
5.71E-01	7.27E+02	1.04E+03	7.61E+00
5.88E-01	7.41E+02	1.06E+03	7.77E+00
6.05E-01	7.56E+02	1.09E+03	7.92E+00
6.23E-01	7.71E+02	1.12E+03	8.08E+00
6.61E-01	8.03E+02	1.18E+03	8.41E+00
6.81E-01	8.19E+02	1.22E+03	8.58E+00
7.02E-01	8.36E+02	1.25E+03	8.76E+00
7.23E-01	8.53E+02	1.28E+03	8.94E+00
7.44E-01	8.70E+02	1.32E+03	9.12E+00
7.67E-01	8.88E+02	1.36E+03	9.31E+00
7.90E-01	9.06E+02	1.39E+03	9.50E+00
8.13E-01	9.25E+02	1.43E+03	9.69E+00
8.38E-01	9.44E+02	1.47E+03	9.89E+00
8.63E-01	9.63E+02	1.51E+03	1.01E+01
8.89E-01	9.83E+02	1.55E+03	1.03E+01
9.16E-01	1.00E+03	1.60E+03	1.05E+01
9.43E-01	1.02E+03	1.64E+03	1.07E+01
9.71E-01	1.05E+03	1.69E+03	1.10E+01
1.00E+00	1.07E+03	1.73E+03	1.12E+01
1.06E+00	1.11E+03	1.83E+03	1.16E+01
1.09E+00	1.14E+03	1.88E+03	1.19E+01

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Non-gestational 5-year Average			
Intake (ng/kg/ day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
1.13E+00	1.16E+03	1.94E+03	1.21E+01
1.16E+00	1.18E+03	1.99E+03	1.24E+01
1.19E+00	1.21E+03	2.04E+03	1.27E+01
1.23E+00	1.23E+03	2.10E+03	1.29E+01
1.27E+00	1.26E+03	2.16E+03	1.32E+01
1.31E+00	1.29E+03	2.22E+03	1.35E+01
1.34E+00	1.31E+03	2.28E+03	1.38E+01
1.38E+00	1.34E+03	2.35E+03	1.40E+01
1.43E+00	1.37E+03	2.41E+03	1.43E+01
1.47E+00	1.40E+03	2.48E+03	1.46E+01
1.51E+00	1.43E+03	2.55E+03	1.50E+01
1.56E+00	1.46E+03	2.62E+03	1.53E+01
1.61E+00	1.49E+03	2.69E+03	1.56E+01
1.65E+00	1.52E+03	2.77E+03	1.59E+01
1.70E+00	1.55E+03	2.85E+03	1.63E+01
1.75E+00	1.59E+03	2.93E+03	1.66E+01
1.81E+00	1.62E+03	3.01E+03	1.70E+01
1.86E+00	1.66E+03	3.10E+03	1.74E+01
1.92E+00	1.69E+03	3.18E+03	1.77E+01
1.97E+00	1.73E+03	3.27E+03	1.81E+01
2.03E+00	1.77E+03	3.37E+03	1.85E+01
2.09E+00	1.80E+03	3.46E+03	1.89E+01
2.16E+00	1.84E+03	3.56E+03	1.93E+01
2.22E+00	1.88E+03	3.66E+03	1.97E+01
2.29E+00	1.92E+03	3.76E+03	2.02E+01
2.36E+00	1.97E+03	3.87E+03	2.06E+01
2.43E+00	2.01E+03	3.98E+03	2.11E+01
2.50E+00	2.05E+03	4.09E+03	2.15E+01
2.58E+00	2.10E+03	4.21E+03	2.20E+01
2.65E+00	2.15E+03	4.33E+03	2.25E+01
2.73E+00	2.19E+03	4.45E+03	2.30E+01
2.82E+00	2.24E+03	4.58E+03	2.35E+01
2.90E+00	2.29E+03	4.71E+03	2.40E+01
2.99E+00	2.34E+03	4.85E+03	2.46E+01
3.08E+00	2.40E+03	4.98E+03	2.51E+01
3.17E+00	2.45E+03	5.13E+03	2.57E+01
3.26E+00	2.51E+03	5.27E+03	2.63E+01
3.36E+00	2.56E+03	5.42E+03	2.69E+01

Non-gestational 5-year Average			
Intake (ng/kg/ day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
3.46E+00	2.62E+03	5.58E+03	2.75E+01
3.57E+00	2.68E+03	5.74E+03	2.81E+01
3.67E+00	2.74E+03	5.90E+03	2.87E+01
3.78E+00	2.80E+03	6.07E+03	2.94E+01
3.90E+00	2.87E+03	6.25E+03	3.01E+01
4.01E+00	2.93E+03	6.42E+03	3.07E+01
4.13E+00	3.00E+03	6.61E+03	3.15E+01
4.26E+00	3.07E+03	6.80E+03	3.22E+01
4.39E+00	3.14E+03	6.99E+03	3.29E+01
4.52E+00	3.22E+03	7.20E+03	3.37E+01
4.65E+00	3.29E+03	7.40E+03	3.45E+01
4.79E+00	3.37E+03	7.62E+03	3.53E+01
4.94E+00	3.45E+03	7.83E+03	3.61E+01
5.08E+00	3.53E+03	8.06E+03	3.69E+01
5.24E+00	3.61E+03	8.29E+03	3.78E+01
5.39E+00	3.69E+03	8.53E+03	3.87E+01
5.56E+00	3.78E+03	8.78E+03	3.96E+01
5.72E+00	3.87E+03	9.03E+03	4.06E+01
5.89E+00	3.96E+03	9.29E+03	4.15E+01
6.07E+00	4.06E+03	9.56E+03	4.25E+01
6.25E+00	4.15E+03	9.84E+03	4.35E+01
6.44E+00	4.25E+03	1.01E+04	4.46E+01
6.63E+00	4.36E+03	1.04E+04	4.56E+01
6.83E+00	4.46E+03	1.07E+04	4.67E+01
7.04E+00	4.57E+03	1.10E+04	4.79E+01
7.25E+00	4.68E+03	1.13E+04	4.90E+01
7.47E+00	4.79E+03	1.17E+04	5.02E+01
7.69E+00	4.91E+03	1.20E+04	5.15E+01
7.92E+00	5.03E+03	1.24E+04	5.27E+01
8.16E+00	5.15E+03	1.27E+04	5.40E+01
8.40E+00	5.28E+03	1.31E+04	5.53E+01
8.66E+00	5.41E+03	1.35E+04	5.67E+01
8.92E+00	5.54E+03	1.39E+04	5.81E+01
9.18E+00	5.68E+03	1.43E+04	5.95E+01
9.46E+00	5.82E+03	1.47E+04	6.10E+01
9.74E+00	5.97E+03	1.51E+04	6.25E+01
1.00E+01	6.10E+03	1.55E+04	6.39E+01
1.00E+01	6.12E+03	1.56E+04	6.41E+01

Non-gestational 5-year Average			
Intake (ng/kg/ day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
1.34E+01	7.77E+03	2.05E+04	8.15E+01
1.67E+01	9.43E+03	2.55E+04	9.88E+01
2.00E+01	1.11E+04	3.05E+04	1.16E+02
2.33E+01	1.27E+04	3.54E+04	1.33E+02
2.67E+01	1.43E+04	4.03E+04	1.50E+02
3.00E+01	1.60E+04	4.53E+04	1.67E+02
3.33E+01	1.76E+04	5.02E+04	1.84E+02
3.67E+01	1.92E+04	5.51E+04	2.01E+02
4.00E+01	2.08E+04	6.00E+04	2.18E+02
4.33E+01	2.24E+04	6.49E+04	2.35E+02
4.67E+01	2.40E+04	6.97E+04	2.52E+02
5.00E+01	2.57E+04	7.46E+04	2.69E+02
5.33E+01	2.73E+04	7.94E+04	2.86E+02
5.67E+01	2.89E+04	8.43E+04	3.03E+02
6.00E+01	3.05E+04	8.91E+04	3.19E+02
6.33E+01	3.21E+04	9.39E+04	3.36E+02
6.67E+01	3.37E+04	9.87E+04	3.53E+02
7.00E+01	3.53E+04	1.04E+05	3.70E+02
7.33E+01	3.69E+04	1.08E+05	3.87E+02
7.67E+01	3.85E+04	1.13E+05	4.04E+02
8.00E+01	4.01E+04	1.18E+05	4.20E+02
8.33E+01	4.17E+04	1.23E+05	4.37E+02
8.67E+01	4.33E+04	1.27E+05	4.54E+02
9.00E+01	4.49E+04	1.32E+05	4.71E+02
9.33E+01	4.65E+04	1.37E+05	4.88E+02
9.67E+01	4.81E+04	1.41E+05	5.04E+02
1.00E+02	4.97E+04	1.46E+05	5.21E+02
1.10E+02	5.45E+04	1.60E+05	5.72E+02
1.20E+02	5.94E+04	1.74E+05	6.22E+02

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C.4.3. Gestational

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.00E-09	2.81E-05	1.11E-05	2.96E-07
1.33E-09	3.74E-05	1.47E-05	3.94E-07
1.67E-09	4.68E-05	1.84E-05	4.92E-07
2.00E-09	5.61E-05	2.21E-05	5.91E-07
2.33E-09	6.55E-05	2.58E-05	6.89E-07
2.67E-09	7.48E-05	2.95E-05	7.88E-07
3.00E-09	8.42E-05	3.32E-05	8.86E-07
3.33E-09	9.35E-05	3.69E-05	9.84E-07
3.67E-09	1.03E-04	4.05E-05	1.08E-06
4.00E-09	1.12E-04	4.42E-05	1.18E-06
4.33E-09	1.22E-04	4.79E-05	1.28E-06
4.67E-09	1.31E-04	5.16E-05	1.38E-06
5.00E-09	1.40E-04	5.53E-05	1.48E-06
5.33E-09	1.50E-04	5.90E-05	1.57E-06
5.67E-09	1.59E-04	6.26E-05	1.67E-06
6.00E-09	1.68E-04	6.63E-05	1.77E-06
6.33E-09	1.78E-04	7.00E-05	1.87E-06
6.67E-09	1.87E-04	7.37E-05	1.97E-06
7.00E-09	1.96E-04	7.74E-05	2.07E-06
7.33E-09	2.06E-04	8.11E-05	2.16E-06
7.67E-09	2.15E-04	8.47E-05	2.26E-06
8.00E-09	2.24E-04	8.84E-05	2.36E-06
8.33E-09	2.34E-04	9.21E-05	2.46E-06
8.67E-09	2.43E-04	9.58E-05	2.56E-06
9.00E-09	2.52E-04	9.95E-05	2.66E-06
9.33E-09	2.62E-04	1.03E-04	2.75E-06
9.67E-09	2.71E-04	1.07E-04	2.85E-06
1.00E-08	2.80E-04	1.11E-04	2.95E-06
1.33E-08	3.73E-04	1.47E-04	3.93E-06
1.67E-08	4.66E-04	1.84E-04	4.91E-06
2.00E-08	5.59E-04	2.21E-04	5.89E-06
2.33E-08	6.52E-04	2.57E-04	6.87E-06
2.67E-08	7.46E-04	2.94E-04	7.85E-06
3.00E-08	8.39E-04	3.31E-04	8.83E-06
3.33E-08	9.32E-04	3.67E-04	9.81E-06
3.67E-08	1.02E-03	4.04E-04	1.08E-05
4.00E-08	1.12E-03	4.41E-04	1.18E-05

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
4.33E-08	1.21E-03	4.78E-04	1.27E-05
4.67E-08	1.30E-03	5.14E-04	1.37E-05
5.00E-08	1.40E-03	5.51E-04	1.47E-05
5.33E-08	1.49E-03	5.88E-04	1.57E-05
5.67E-08	1.58E-03	6.24E-04	1.67E-05
6.00E-08	1.67E-03	6.61E-04	1.76E-05
6.33E-08	1.77E-03	6.97E-04	1.86E-05
6.67E-08	1.86E-03	7.34E-04	1.96E-05
7.00E-08	1.95E-03	7.70E-04	2.05E-05
7.33E-08	2.04E-03	8.07E-04	2.15E-05
7.67E-08	2.14E-03	8.43E-04	2.25E-05
8.00E-08	2.23E-03	8.80E-04	2.35E-05
8.33E-08	2.32E-03	9.17E-04	2.44E-05
8.67E-08	2.41E-03	9.53E-04	2.54E-05
9.00E-08	2.51E-03	9.90E-04	2.64E-05
9.33E-08	2.60E-03	1.03E-03	2.74E-05
9.67E-08	2.69E-03	1.06E-03	2.83E-05
1.00E-07	2.79E-03	1.10E-03	2.93E-05
1.33E-07	3.70E-03	1.46E-03	3.90E-05
1.67E-07	4.62E-03	1.83E-03	4.86E-05
2.00E-07	5.54E-03	2.19E-03	5.83E-05
2.33E-07	6.46E-03	2.55E-03	6.80E-05
2.67E-07	7.37E-03	2.92E-03	7.76E-05
3.00E-07	8.29E-03	3.28E-03	8.73E-05
3.33E-07	9.21E-03	3.64E-03	9.69E-05
3.67E-07	1.01E-02	4.01E-03	1.07E-04
4.00E-07	1.10E-02	4.37E-03	1.16E-04
4.33E-07	1.20E-02	4.74E-03	1.26E-04
4.67E-07	1.29E-02	5.10E-03	1.36E-04
5.00E-07	1.38E-02	5.46E-03	1.45E-04
5.33E-07	1.47E-02	5.82E-03	1.55E-04
5.66E-07	1.56E-02	6.17E-03	1.64E-04
5.99E-07	1.65E-02	6.53E-03	1.73E-04
6.33E-07	1.74E-02	6.88E-03	1.83E-04
6.66E-07	1.83E-02	7.24E-03	1.92E-04
6.99E-07	1.92E-02	7.59E-03	2.02E-04
7.32E-07	2.01E-02	7.95E-03	2.11E-04
7.65E-07	2.09E-02	8.30E-03	2.20E-04

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
7.98E-07	2.18E-02	8.66E-03	2.30E-04
8.32E-07	2.27E-02	9.01E-03	2.39E-04
8.65E-07	2.36E-02	9.37E-03	2.49E-04
8.98E-07	2.45E-02	9.72E-03	2.58E-04
9.31E-07	2.54E-02	1.01E-02	2.67E-04
9.64E-07	2.63E-02	1.04E-02	2.77E-04
9.97E-07	2.72E-02	1.08E-02	2.86E-04
1.01E-06	2.76E-02	1.09E-02	2.90E-04
1.03E-06	2.80E-02	1.11E-02	2.95E-04
1.04E-06	2.84E-02	1.13E-02	2.99E-04
1.06E-06	2.88E-02	1.14E-02	3.03E-04
1.07E-06	2.93E-02	1.16E-02	3.08E-04
1.09E-06	2.97E-02	1.18E-02	3.12E-04
1.11E-06	3.01E-02	1.20E-02	3.17E-04
1.12E-06	3.06E-02	1.21E-02	3.22E-04
1.14E-06	3.10E-02	1.23E-02	3.26E-04
1.16E-06	3.15E-02	1.25E-02	3.31E-04
1.17E-06	3.19E-02	1.27E-02	3.36E-04
1.19E-06	3.24E-02	1.29E-02	3.41E-04
1.21E-06	3.29E-02	1.31E-02	3.46E-04
1.23E-06	3.34E-02	1.32E-02	3.51E-04
1.24E-06	3.38E-02	1.34E-02	3.56E-04
1.26E-06	3.43E-02	1.36E-02	3.61E-04
1.28E-06	3.48E-02	1.38E-02	3.67E-04
1.30E-06	3.54E-02	1.40E-02	3.72E-04
1.32E-06	3.59E-02	1.42E-02	3.77E-04
1.34E-06	3.64E-02	1.45E-02	3.83E-04
1.36E-06	3.69E-02	1.47E-02	3.89E-04
1.38E-06	3.75E-02	1.49E-02	3.94E-04
1.40E-06	3.80E-02	1.51E-02	4.00E-04
1.42E-06	3.86E-02	1.53E-02	4.06E-04
1.44E-06	3.92E-02	1.56E-02	4.12E-04
1.46E-06	3.98E-02	1.58E-02	4.18E-04
1.49E-06	4.03E-02	1.60E-02	4.25E-04
1.53E-06	4.15E-02	1.65E-02	4.37E-04
1.58E-06	4.27E-02	1.70E-02	4.50E-04
1.62E-06	4.40E-02	1.75E-02	4.63E-04
1.67E-06	4.53E-02	1.80E-02	4.76E-04

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Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.72E-06	4.66E-02	1.85E-02	4.90E-04
1.77E-06	4.80E-02	1.91E-02	5.05E-04
1.83E-06	4.94E-02	1.96E-02	5.20E-04
1.88E-06	5.08E-02	2.02E-02	5.35E-04
1.94E-06	5.23E-02	2.08E-02	5.50E-04
2.00E-06	5.38E-02	2.14E-02	5.66E-04
2.06E-06	5.54E-02	2.21E-02	5.83E-04
2.12E-06	5.70E-02	2.27E-02	6.00E-04
2.18E-06	5.87E-02	2.34E-02	6.17E-04
2.25E-06	6.04E-02	2.41E-02	6.35E-04
2.32E-06	6.22E-02	2.48E-02	6.54E-04
2.39E-06	6.40E-02	2.55E-02	6.73E-04
2.46E-06	6.58E-02	2.62E-02	6.93E-04
2.53E-06	6.77E-02	2.70E-02	7.13E-04
2.61E-06	6.97E-02	2.78E-02	7.33E-04
2.68E-06	7.17E-02	2.86E-02	7.55E-04
2.76E-06	7.38E-02	2.94E-02	7.77E-04
2.85E-06	7.60E-02	3.03E-02	8.00E-04
2.93E-06	7.82E-02	3.12E-02	8.22E-04
3.02E-06	8.04E-02	3.21E-02	8.46E-04
3.11E-06	8.27E-02	3.30E-02	8.71E-04
3.21E-06	8.51E-02	3.40E-02	8.96E-04
3.30E-06	8.76E-02	3.50E-02	9.22E-04
3.40E-06	9.01E-02	3.60E-02	9.48E-04
3.50E-06	9.27E-02	3.71E-02	9.76E-04
3.61E-06	9.54E-02	3.81E-02	1.00E-03
3.72E-06	9.82E-02	3.93E-02	1.03E-03
3.83E-06	1.01E-01	4.04E-02	1.06E-03
3.94E-06	1.04E-01	4.16E-02	1.09E-03
4.06E-06	1.07E-01	4.28E-02	1.12E-03
4.18E-06	1.10E-01	4.40E-02	1.16E-03
4.31E-06	1.13E-01	4.53E-02	1.19E-03
4.44E-06	1.16E-01	4.66E-02	1.22E-03
4.57E-06	1.20E-01	4.79E-02	1.26E-03
4.71E-06	1.23E-01	4.93E-02	1.30E-03
4.85E-06	1.27E-01	5.08E-02	1.33E-03
4.99E-06	1.30E-01	5.22E-02	1.37E-03
5.14E-06	1.34E-01	5.37E-02	1.41E-03

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
5.30E-06	1.38E-01	5.53E-02	1.45E-03
5.46E-06	1.42E-01	5.69E-02	1.49E-03
5.62E-06	1.46E-01	5.85E-02	1.53E-03
5.79E-06	1.50E-01	6.02E-02	1.58E-03
5.96E-06	1.54E-01	6.19E-02	1.62E-03
6.14E-06	1.59E-01	6.37E-02	1.67E-03
6.33E-06	1.63E-01	6.55E-02	1.72E-03
6.52E-06	1.68E-01	6.74E-02	1.76E-03
6.71E-06	1.72E-01	6.93E-02	1.81E-03
6.91E-06	1.77E-01	7.13E-02	1.86E-03
7.12E-06	1.82E-01	7.33E-02	1.92E-03
7.33E-06	1.87E-01	7.54E-02	1.97E-03
7.55E-06	1.93E-01	7.75E-02	2.03E-03
7.78E-06	1.98E-01	7.97E-02	2.08E-03
8.01E-06	2.03E-01	8.20E-02	2.14E-03
8.25E-06	2.09E-01	8.43E-02	2.20E-03
8.50E-06	2.15E-01	8.67E-02	2.26E-03
8.76E-06	2.21E-01	8.92E-02	2.33E-03
9.02E-06	2.27E-01	9.17E-02	2.39E-03
9.29E-06	2.34E-01	9.43E-02	2.46E-03
9.57E-06	2.40E-01	9.70E-02	2.53E-03
9.86E-06	2.47E-01	9.97E-02	2.60E-03
1.02E-05	2.54E-01	1.03E-01	2.67E-03
1.05E-05	2.61E-01	1.05E-01	2.74E-03
1.08E-05	2.68E-01	1.08E-01	2.82E-03
1.11E-05	2.75E-01	1.11E-01	2.90E-03
1.14E-05	2.83E-01	1.15E-01	2.98E-03
1.18E-05	2.91E-01	1.18E-01	3.06E-03
1.21E-05	2.99E-01	1.21E-01	3.14E-03
1.25E-05	3.07E-01	1.25E-01	3.23E-03
1.29E-05	3.16E-01	1.28E-01	3.32E-03
1.32E-05	3.24E-01	1.32E-01	3.41E-03
1.36E-05	3.33E-01	1.35E-01	3.51E-03
1.41E-05	3.42E-01	1.39E-01	3.60E-03
1.45E-05	3.52E-01	1.43E-01	3.70E-03
1.49E-05	3.61E-01	1.47E-01	3.80E-03
1.54E-05	3.71E-01	1.51E-01	3.90E-03
1.58E-05	3.81E-01	1.55E-01	4.01E-03

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.63E-05	3.92E-01	1.60E-01	4.12E-03
1.68E-05	4.03E-01	1.64E-01	4.23E-03
1.73E-05	4.13E-01	1.69E-01	4.35E-03
1.78E-05	4.25E-01	1.73E-01	4.47E-03
1.83E-05	4.36E-01	1.78E-01	4.59E-03
1.89E-05	4.48E-01	1.83E-01	4.71E-03
1.95E-05	4.60E-01	1.88E-01	4.84E-03
2.00E-05	4.72E-01	1.93E-01	4.97E-03
2.06E-05	4.85E-01	1.98E-01	5.10E-03
2.13E-05	4.98E-01	2.04E-01	5.24E-03
2.19E-05	5.12E-01	2.10E-01	5.38E-03
2.25E-05	5.25E-01	2.15E-01	5.53E-03
2.32E-05	5.40E-01	2.21E-01	5.68E-03
2.39E-05	5.54E-01	2.27E-01	5.83E-03
2.46E-05	5.69E-01	2.34E-01	5.98E-03
2.54E-05	5.84E-01	2.40E-01	6.14E-03
2.61E-05	6.00E-01	2.47E-01	6.31E-03
2.69E-05	6.16E-01	2.53E-01	6.48E-03
2.77E-05	6.32E-01	2.60E-01	6.65E-03
2.86E-05	6.49E-01	2.67E-01	6.82E-03
2.94E-05	6.66E-01	2.75E-01	7.01E-03
3.03E-05	6.84E-01	2.82E-01	7.19E-03
3.12E-05	7.02E-01	2.90E-01	7.38E-03
3.21E-05	7.20E-01	2.98E-01	7.58E-03
3.31E-05	7.42E-01	3.07E-01	7.80E-03
3.41E-05	7.62E-01	3.15E-01	8.01E-03
3.51E-05	7.82E-01	3.24E-01	8.22E-03
3.62E-05	8.03E-01	3.33E-01	8.44E-03
3.73E-05	8.24E-01	3.42E-01	8.68E-03
3.84E-05	8.45E-01	3.51E-01	8.89E-03
3.95E-05	8.68E-01	3.61E-01	9.12E-03
4.07E-05	8.88E-01	3.69E-01	9.34E-03
4.19E-05	9.11E-01	3.79E-01	9.59E-03
4.32E-05	9.35E-01	3.89E-01	9.83E-03
4.45E-05	9.59E-01	4.00E-01	1.01E-02
4.58E-05	9.83E-01	4.10E-01	1.03E-02
4.72E-05	1.01E+00	4.21E-01	1.06E-02
4.86E-05	1.04E+00	4.33E-01	1.09E-02

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Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
5.01E-05	1.06E+00	4.44E-01	1.12E-02
5.16E-05	1.09E+00	4.56E-01	1.14E-02
5.31E-05	1.12E+00	4.68E-01	1.17E-02
5.47E-05	1.15E+00	4.81E-01	1.21E-02
5.64E-05	1.18E+00	4.93E-01	1.24E-02
5.81E-05	1.21E+00	5.06E-01	1.27E-02
5.98E-05	1.24E+00	5.20E-01	1.30E-02
6.16E-05	1.27E+00	5.34E-01	1.33E-02
6.34E-05	1.30E+00	5.48E-01	1.37E-02
6.54E-05	1.33E+00	5.62E-01	1.40E-02
6.73E-05	1.37E+00	5.77E-01	1.44E-02
6.93E-05	1.40E+00	5.92E-01	1.47E-02
7.14E-05	1.44E+00	6.08E-01	1.51E-02
7.36E-05	1.47E+00	6.24E-01	1.55E-02
7.58E-05	1.51E+00	6.40E-01	1.59E-02
7.80E-05	1.55E+00	6.57E-01	1.63E-02
8.04E-05	1.59E+00	6.74E-01	1.67E-02
8.28E-05	1.63E+00	6.92E-01	1.71E-02
8.53E-05	1.67E+00	7.10E-01	1.75E-02
8.78E-05	1.71E+00	7.28E-01	1.79E-02
9.05E-05	1.75E+00	7.47E-01	1.84E-02
9.32E-05	1.79E+00	7.66E-01	1.88E-02
9.60E-05	1.84E+00	7.86E-01	1.93E-02
9.89E-05	1.88E+00	8.07E-01	1.98E-02
1.02E-04	1.93E+00	8.28E-01	2.03E-02
1.05E-04	1.98E+00	8.49E-01	2.08E-02
1.08E-04	2.03E+00	8.71E-01	2.13E-02
1.11E-04	2.08E+00	8.93E-01	2.18E-02
1.15E-04	2.13E+00	9.16E-01	2.24E-02
1.18E-04	2.18E+00	9.39E-01	2.29E-02
1.22E-04	2.23E+00	9.63E-01	2.34E-02
1.25E-04	2.28E+00	9.87E-01	2.40E-02
1.29E-04	2.34E+00	1.01E+00	2.46E-02
1.33E-04	2.40E+00	1.04E+00	2.52E-02
1.37E-04	2.45E+00	1.06E+00	2.58E-02
1.41E-04	2.51E+00	1.09E+00	2.64E-02
1.45E-04	2.58E+00	1.12E+00	2.72E-02
1.50E-04	2.63E+00	1.15E+00	2.77E-02

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.54E-04	2.70E+00	1.18E+00	2.83E-02
1.59E-04	2.78E+00	1.21E+00	2.92E-02
1.63E-04	2.84E+00	1.24E+00	2.99E-02
1.68E-04	2.89E+00	1.27E+00	3.04E-02
1.73E-04	2.96E+00	1.30E+00	3.11E-02
1.79E-04	3.03E+00	1.33E+00	3.18E-02
1.84E-04	3.10E+00	1.36E+00	3.26E-02
1.89E-04	3.17E+00	1.40E+00	3.33E-02
1.95E-04	3.25E+00	1.43E+00	3.41E-02
2.01E-04	3.32E+00	1.47E+00	3.49E-02
2.07E-04	3.43E+00	1.52E+00	3.61E-02
2.13E-04	3.51E+00	1.56E+00	3.69E-02
2.20E-04	3.57E+00	1.59E+00	3.75E-02
2.26E-04	3.67E+00	1.63E+00	3.85E-02
2.33E-04	3.77E+00	1.68E+00	3.96E-02
2.40E-04	3.86E+00	1.72E+00	4.05E-02
2.47E-04	3.95E+00	1.76E+00	4.15E-02
2.55E-04	4.04E+00	1.81E+00	4.24E-02
2.62E-04	4.13E+00	1.85E+00	4.34E-02
2.70E-04	4.22E+00	1.90E+00	4.44E-02
2.78E-04	4.32E+00	1.94E+00	4.54E-02
2.86E-04	4.42E+00	1.99E+00	4.64E-02
2.95E-04	4.52E+00	2.04E+00	4.75E-02
3.04E-04	4.62E+00	2.09E+00	4.86E-02
3.13E-04	4.73E+00	2.14E+00	4.97E-02
3.22E-04	4.84E+00	2.20E+00	5.08E-02
3.32E-04	4.95E+00	2.25E+00	5.20E-02
3.42E-04	5.06E+00	2.30E+00	5.31E-02
3.52E-04	5.17E+00	2.36E+00	5.43E-02
3.63E-04	5.29E+00	2.42E+00	5.56E-02
3.74E-04	5.41E+00	2.48E+00	5.68E-02
3.85E-04	5.53E+00	2.54E+00	5.81E-02
3.97E-04	5.65E+00	2.60E+00	5.94E-02
4.08E-04	5.78E+00	2.66E+00	6.07E-02
4.21E-04	5.91E+00	2.73E+00	6.20E-02
4.33E-04	6.04E+00	2.79E+00	6.34E-02
4.46E-04	6.17E+00	2.86E+00	6.48E-02
4.60E-04	6.31E+00	2.93E+00	6.63E-02

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
4.74E-04	6.45E+00	3.00E+00	6.77E-02
4.88E-04	6.59E+00	3.07E+00	6.92E-02
5.02E-04	6.74E+00	3.15E+00	7.07E-02
5.17E-04	6.88E+00	3.22E+00	7.23E-02
5.33E-04	7.03E+00	3.30E+00	7.39E-02
5.49E-04	7.19E+00	3.38E+00	7.55E-02
5.65E-04	7.34E+00	3.46E+00	7.71E-02
5.82E-04	7.50E+00	3.54E+00	7.88E-02
6.00E-04	7.67E+00	3.63E+00	8.05E-02
6.18E-04	7.83E+00	3.71E+00	8.22E-02
6.36E-04	8.00E+00	3.80E+00	8.40E-02
6.55E-04	8.17E+00	3.89E+00	8.58E-02
6.75E-04	8.35E+00	3.98E+00	8.77E-02
6.95E-04	8.53E+00	4.08E+00	8.95E-02
7.16E-04	8.70E+00	4.17E+00	9.14E-02
7.38E-04	8.89E+00	4.27E+00	9.33E-02
7.60E-04	9.08E+00	4.37E+00	9.53E-02
7.83E-04	9.27E+00	4.47E+00	9.74E-02
8.06E-04	9.47E+00	4.58E+00	9.94E-02
8.30E-04	9.67E+00	4.69E+00	1.02E-01
8.55E-04	9.88E+00	4.80E+00	1.04E-01
8.81E-04	1.01E+01	4.91E+00	1.06E-01
9.07E-04	1.03E+01	5.03E+00	1.08E-01
9.21E-04	1.04E+01	5.09E+00	1.09E-01
9.35E-04	1.05E+01	5.14E+00	1.10E-01
9.49E-04	1.26E+01	6.31E+00	1.32E-01
1.37E-03	1.38E+01	6.99E+00	1.45E-01
1.39E-03	1.40E+01	7.07E+00	1.46E-01
1.41E-03	1.41E+01	7.15E+00	1.48E-01
1.43E-03	1.42E+01	7.23E+00	1.49E-01
1.46E-03	1.44E+01	7.31E+00	1.51E-01
1.48E-03	1.45E+01	7.39E+00	1.52E-01
1.50E-03	1.46E+01	7.47E+00	1.54E-01
1.52E-03	1.48E+01	7.55E+00	1.55E-01
1.54E-03	1.49E+01	7.64E+00	1.57E-01
1.57E-03	1.51E+01	7.73E+00	1.58E-01
1.59E-03	1.52E+01	7.82E+00	1.60E-01
1.61E-03	1.54E+01	7.91E+00	1.62E-01

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Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.64E-03	1.56E+01	8.00E+00	1.63E-01
1.69E-03	1.59E+01	8.19E+00	1.67E-01
1.71E-03	1.60E+01	8.28E+00	1.68E-01
1.74E-03	1.62E+01	8.38E+00	1.70E-01
1.76E-03	1.64E+01	8.47E+00	1.72E-01
1.79E-03	1.65E+01	8.57E+00	1.73E-01
1.82E-03	1.67E+01	8.67E+00	1.75E-01
1.84E-03	1.69E+01	8.77E+00	1.77E-01
1.87E-03	1.74E+01	9.10E+00	1.83E-01
2.34E-03	1.98E+01	1.06E+01	2.08E-01
2.41E-03	2.02E+01	1.08E+01	2.12E-01
2.48E-03	2.06E+01	1.11E+01	2.16E-01
2.55E-03	2.10E+01	1.13E+01	2.21E-01
2.63E-03	2.14E+01	1.16E+01	2.25E-01
2.71E-03	2.19E+01	1.18E+01	2.30E-01
2.79E-03	2.23E+01	1.21E+01	2.34E-01
2.87E-03	2.28E+01	1.24E+01	2.39E-01
2.96E-03	2.32E+01	1.27E+01	2.44E-01
3.05E-03	2.37E+01	1.30E+01	2.48E-01
3.14E-03	2.41E+01	1.33E+01	2.53E-01
3.23E-03	2.46E+01	1.36E+01	2.58E-01
3.33E-03	2.51E+01	1.39E+01	2.63E-01
3.43E-03	2.56E+01	1.42E+01	2.69E-01
3.53E-03	2.61E+01	1.46E+01	2.74E-01
3.64E-03	2.66E+01	1.49E+01	2.79E-01
4.22E-03	2.83E+01	1.60E+01	2.96E-01
4.35E-03	2.88E+01	1.63E+01	3.02E-01
4.48E-03	2.93E+01	1.67E+01	3.08E-01
4.61E-03	2.99E+01	1.71E+01	3.14E-01
4.75E-03	3.05E+01	1.75E+01	3.20E-01
4.89E-03	3.11E+01	1.79E+01	3.26E-01
5.04E-03	3.30E+01	1.92E+01	3.46E-01
5.19E-03	3.41E+01	2.00E+01	3.58E-01
5.35E-03	3.49E+01	2.05E+01	3.66E-01
5.51E-03	3.55E+01	2.10E+01	3.73E-01
5.67E-03	3.62E+01	2.14E+01	3.80E-01
5.84E-03	3.69E+01	2.19E+01	3.87E-01
5.93E-03	3.73E+01	2.22E+01	3.91E-01

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
6.02E-03	3.77E+01	2.25E+01	3.95E-01
6.20E-03	3.84E+01	2.30E+01	4.03E-01
6.38E-03	3.92E+01	2.36E+01	4.12E-01
6.57E-03	4.00E+01	2.42E+01	4.20E-01
6.77E-03	4.08E+01	2.48E+01	4.28E-01
6.98E-03	4.16E+01	2.54E+01	4.37E-01
7.18E-03	4.25E+01	2.60E+01	4.45E-01
7.40E-03	4.33E+01	2.66E+01	4.54E-01
7.51E-03	4.37E+01	2.69E+01	4.58E-01
8.33E-03	4.46E+01	2.76E+01	4.68E-01
8.58E-03	4.66E+01	2.91E+01	4.89E-01
8.71E-03	4.74E+01	2.97E+01	4.97E-01
8.84E-03	4.79E+01	3.00E+01	5.02E-01
9.10E-03	4.86E+01	3.06E+01	5.10E-01
9.37E-03	4.95E+01	3.13E+01	5.19E-01
9.66E-03	5.07E+01	3.22E+01	5.32E-01
9.94E-03	5.17E+01	3.30E+01	5.42E-01
1.02E-02	5.27E+01	3.38E+01	5.53E-01
1.06E-02	5.37E+01	3.46E+01	5.63E-01
1.09E-02	5.46E+01	3.53E+01	5.73E-01
1.12E-02	5.58E+01	3.63E+01	5.85E-01
1.15E-02	5.67E+01	3.69E+01	5.94E-01
1.19E-02	5.74E+01	3.75E+01	6.02E-01
1.22E-02	5.85E+01	3.84E+01	6.13E-01
1.26E-02	5.96E+01	3.93E+01	6.25E-01
1.30E-02	6.19E+01	4.12E+01	6.49E-01
1.34E-02	6.32E+01	4.23E+01	6.63E-01
1.38E-02	6.45E+01	4.33E+01	6.76E-01
1.42E-02	6.57E+01	4.44E+01	6.89E-01
1.46E-02	6.64E+01	4.49E+01	6.96E-01
1.50E-02	6.78E+01	4.61E+01	7.11E-01
1.55E-02	6.83E+01	4.66E+01	7.16E-01
1.60E-02	6.96E+01	4.76E+01	7.29E-01
1.64E-02	7.09E+01	4.88E+01	7.43E-01
1.69E-02	7.26E+01	5.02E+01	7.61E-01
1.74E-02	7.40E+01	5.14E+01	7.76E-01
1.80E-02	7.54E+01	5.27E+01	7.90E-01
1.85E-02	7.68E+01	5.39E+01	8.06E-01

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.91E-02	7.83E+01	5.52E+01	8.21E-01
1.96E-02	7.98E+01	5.66E+01	8.37E-01
2.02E-02	8.13E+01	5.79E+01	8.53E-01
2.08E-02	8.29E+01	5.94E+01	8.69E-01
2.14E-02	8.45E+01	6.08E+01	8.86E-01
2.21E-02	8.61E+01	6.23E+01	9.03E-01
2.28E-02	8.78E+01	6.38E+01	9.20E-01
2.34E-02	8.95E+01	6.54E+01	9.38E-01
2.41E-02	9.12E+01	6.70E+01	9.56E-01
2.49E-02	9.29E+01	6.86E+01	9.75E-01
2.56E-02	9.47E+01	7.03E+01	9.93E-01
2.64E-02	9.65E+01	7.20E+01	1.01E+00
2.72E-02	9.84E+01	7.37E+01	1.03E+00
2.80E-02	1.00E+02	7.55E+01	1.05E+00
2.88E-02	1.02E+02	7.74E+01	1.07E+00
2.97E-02	1.04E+02	7.93E+01	1.09E+00
3.06E-02	1.07E+02	8.20E+01	1.12E+00
3.15E-02	1.09E+02	8.38E+01	1.14E+00
3.24E-02	1.11E+02	8.57E+01	1.16E+00
3.34E-02	1.13E+02	8.76E+01	1.18E+00
3.44E-02	1.15E+02	8.96E+01	1.20E+00
3.54E-02	1.16E+02	9.15E+01	1.22E+00
3.65E-02	1.18E+02	9.35E+01	1.24E+00
3.76E-02	1.21E+02	9.61E+01	1.27E+00
3.87E-02	1.23E+02	9.84E+01	1.29E+00
3.99E-02	1.26E+02	1.01E+02	1.32E+00
4.11E-02	1.28E+02	1.03E+02	1.34E+00
4.23E-02	1.31E+02	1.06E+02	1.37E+00
4.36E-02	1.34E+02	1.09E+02	1.40E+00
4.49E-02	1.36E+02	1.12E+02	1.43E+00
4.63E-02	1.39E+02	1.15E+02	1.45E+00
4.76E-02	1.42E+02	1.18E+02	1.48E+00
4.91E-02	1.44E+02	1.21E+02	1.51E+00
5.05E-02	1.47E+02	1.24E+02	1.54E+00
5.21E-02	1.50E+02	1.27E+02	1.57E+00
5.36E-02	1.52E+02	1.30E+02	1.60E+00
5.52E-02	1.55E+02	1.33E+02	1.63E+00
5.69E-02	1.59E+02	1.37E+02	1.66E+00

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
5.86E-02	1.62E+02	1.40E+02	1.69E+00
6.03E-02	1.64E+02	1.43E+02	1.72E+00
6.22E-02	1.67E+02	1.46E+02	1.75E+00
6.40E-02	1.70E+02	1.50E+02	1.79E+00
6.59E-02	1.74E+02	1.54E+02	1.82E+00
6.79E-02	1.77E+02	1.58E+02	1.86E+00
7.00E-02	1.80E+02	1.62E+02	1.89E+00
7.21E-02	1.84E+02	1.66E+02	1.93E+00
7.42E-02	1.87E+02	1.70E+02	1.96E+00
7.64E-02	1.91E+02	1.75E+02	2.00E+00
7.87E-02	1.95E+02	1.79E+02	2.05E+00
8.11E-02	1.99E+02	1.84E+02	2.09E+00
8.35E-02	2.03E+02	1.89E+02	2.13E+00
8.60E-02	2.07E+02	1.93E+02	2.17E+00
8.86E-02	2.11E+02	1.98E+02	2.21E+00
9.13E-02	2.15E+02	2.03E+02	2.25E+00
9.40E-02	2.19E+02	2.08E+02	2.29E+00
9.68E-02	2.23E+02	2.14E+02	2.34E+00
9.97E-02	2.28E+02	2.20E+02	2.39E+00
1.03E-01	2.32E+02	2.25E+02	2.43E+00
1.06E-01	2.36E+02	2.31E+02	2.48E+00
1.09E-01	2.40E+02	2.36E+02	2.52E+00
1.12E-01	2.45E+02	2.42E+02	2.57E+00
1.16E-01	2.50E+02	2.49E+02	2.62E+00
1.19E-01	2.55E+02	2.55E+02	2.67E+00
1.23E-01	2.60E+02	2.62E+02	2.72E+00
1.26E-01	2.65E+02	2.69E+02	2.78E+00
1.30E-01	2.70E+02	2.76E+02	2.83E+00
1.34E-01	2.75E+02	2.83E+02	2.89E+00
1.38E-01	2.81E+02	2.91E+02	2.95E+00
1.42E-01	2.87E+02	2.99E+02	3.00E+00
1.46E-01	2.92E+02	3.06E+02	3.06E+00
1.51E-01	2.97E+02	3.14E+02	3.12E+00
1.55E-01	3.03E+02	3.22E+02	3.18E+00
1.60E-01	3.09E+02	3.30E+02	3.24E+00
1.65E-01	3.15E+02	3.39E+02	3.30E+00
1.70E-01	3.21E+02	3.48E+02	3.37E+00
1.75E-01	3.27E+02	3.57E+02	3.43E+00

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
1.80E-01	3.34E+02	3.67E+02	3.50E+00
1.86E-01	3.40E+02	3.76E+02	3.57E+00
1.91E-01	3.47E+02	3.86E+02	3.64E+00
1.97E-01	3.54E+02	3.96E+02	3.71E+00
2.03E-01	3.60E+02	4.06E+02	3.78E+00
2.09E-01	3.68E+02	4.17E+02	3.85E+00
2.15E-01	3.75E+02	4.28E+02	3.93E+00
2.22E-01	3.82E+02	4.40E+02	4.01E+00
2.28E-01	3.90E+02	4.52E+02	4.09E+00
2.35E-01	3.98E+02	4.64E+02	4.17E+00
2.42E-01	4.05E+02	4.76E+02	4.25E+00
2.49E-01	4.13E+02	4.89E+02	4.33E+00
2.57E-01	4.22E+02	5.02E+02	4.42E+00
2.65E-01	4.30E+02	5.15E+02	4.51E+00
2.72E-01	4.38E+02	5.29E+02	4.60E+00
2.81E-01	4.47E+02	5.42E+02	4.68E+00
2.89E-01	4.55E+02	5.56E+02	4.77E+00
2.98E-01	4.64E+02	5.71E+02	4.87E+00
3.07E-01	4.73E+02	5.86E+02	4.96E+00
3.16E-01	4.83E+02	6.03E+02	5.06E+00
3.25E-01	4.92E+02	6.19E+02	5.16E+00
3.35E-01	5.02E+02	6.35E+02	5.26E+00
3.45E-01	5.13E+02	6.53E+02	5.37E+00
3.56E-01	5.23E+02	6.70E+02	5.48E+00
3.66E-01	5.33E+02	6.88E+02	5.59E+00
3.77E-01	5.44E+02	7.07E+02	5.70E+00
3.89E-01	5.55E+02	7.26E+02	5.81E+00
4.00E-01	5.66E+02	7.46E+02	5.93E+00
4.12E-01	5.77E+02	7.66E+02	6.05E+00
4.25E-01	5.88E+02	7.86E+02	6.17E+00
4.37E-01	6.00E+02	8.08E+02	6.29E+00
4.50E-01	6.12E+02	8.30E+02	6.42E+00
4.64E-01	6.24E+02	8.52E+02	6.54E+00
4.92E-01	6.50E+02	8.99E+02	6.81E+00
5.07E-01	6.63E+02	9.23E+02	6.95E+00
5.22E-01	6.76E+02	9.49E+02	7.09E+00
5.54E-01	7.04E+02	1.00E+03	7.38E+00
5.71E-01	7.18E+02	1.03E+03	7.53E+00

Gestational			
Intake (ng/kg/ day)	Fat (ng/kg)	Body Burden (ng/kg)	Blood (ng/kg)
5.88E-01	7.32E+02	1.06E+03	7.68E+00
6.05E-01	7.47E+02	1.08E+03	7.83E+00
6.23E-01	7.62E+02	1.11E+03	7.99E+00
6.61E-01	7.94E+02	1.18E+03	8.32E+00
6.81E-01	8.10E+02	1.21E+03	8.49E+00
7.02E-01	8.27E+02	1.24E+03	8.67E+00
7.23E-01	8.43E+02	1.28E+03	8.84E+00
7.44E-01	8.61E+02	1.31E+03	9.02E+00
7.67E-01	8.78E+02	1.35E+03	9.21E+00
7.90E-01	8.96E+02	1.38E+03	9.40E+00
8.13E-01	9.15E+02	1.42E+03	9.59E+00
8.38E-01	9.33E+02	1.46E+03	9.78E+00
8.63E-01	9.53E+02	1.50E+03	9.99E+00
9.16E-01	9.93E+02	1.59E+03	1.04E+01
9.43E-01	1.01E+03	1.63E+03	1.06E+01
9.71E-01	1.03E+03	1.68E+03	1.08E+01
1.00E+00	1.06E+03	1.72E+03	1.11E+01
1.06E+00	1.10E+03	1.82E+03	1.15E+01
1.09E+00	1.12E+03	1.87E+03	1.18E+01
1.13E+00	1.15E+03	1.92E+03	1.20E+01
1.16E+00	1.17E+03	1.98E+03	1.23E+01
1.19E+00	1.20E+03	2.03E+03	1.25E+01
1.23E+00	1.22E+03	2.09E+03	1.28E+01
1.27E+00	1.25E+03	2.15E+03	1.31E+01
1.31E+00	1.27E+03	2.21E+03	1.33E+01
1.34E+00	1.30E+03	2.27E+03	1.36E+01
1.38E+00	1.33E+03	2.33E+03	1.39E+01
1.43E+00	1.35E+03	2.40E+03	1.42E+01
1.47E+00	1.38E+03	2.46E+03	1.45E+01
1.51E+00	1.41E+03	2.53E+03	1.48E+01
1.56E+00	1.44E+03	2.60E+03	1.51E+01
1.61E+00	1.47E+03	2.68E+03	1.55E+01
1.65E+00	1.51E+03	2.75E+03	1.58E+01
1.70E+00	1.54E+03	2.83E+03	1.61E+01
1.75E+00	1.57E+03	2.91E+03	1.65E+01
1.81E+00	1.61E+03	2.99E+03	1.68E+01
1.86E+00	1.64E+03	3.08E+03	1.72E+01
1.92E+00	1.68E+03	3.16E+03	1.76E+01

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Gestational			
Intake (ng/kg/ day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
1.97E+00	1.71E+03	3.25E+03	1.79E+01
2.03E+00	1.75E+03	3.34E+03	1.83E+01
2.09E+00	1.79E+03	3.44E+03	1.87E+01
2.16E+00	1.83E+03	3.54E+03	1.91E+01
2.22E+00	1.87E+03	3.64E+03	1.96E+01
2.29E+00	1.91E+03	3.74E+03	2.00E+01
2.36E+00	1.95E+03	3.85E+03	2.04E+01
2.43E+00	1.99E+03	3.95E+03	2.09E+01
2.50E+00	2.04E+03	4.07E+03	2.13E+01
2.58E+00	2.08E+03	4.18E+03	2.18E+01
2.65E+00	2.13E+03	4.30E+03	2.23E+01
2.73E+00	2.17E+03	4.42E+03	2.28E+01
2.82E+00	2.22E+03	4.55E+03	2.33E+01
2.90E+00	2.27E+03	4.68E+03	2.38E+01
2.99E+00	2.32E+03	4.81E+03	2.44E+01
3.08E+00	2.38E+03	4.95E+03	2.49E+01
3.17E+00	2.43E+03	5.09E+03	2.55E+01
3.26E+00	2.48E+03	5.24E+03	2.60E+01
3.36E+00	2.54E+03	5.39E+03	2.66E+01
3.46E+00	2.60E+03	5.54E+03	2.72E+01
3.57E+00	2.66E+03	5.70E+03	2.79E+01
3.67E+00	2.72E+03	5.86E+03	2.85E+01
3.78E+00	2.78E+03	6.03E+03	2.91E+01
3.90E+00	2.84E+03	6.20E+03	2.98E+01
4.01E+00	2.91E+03	6.38E+03	3.05E+01
4.13E+00	2.98E+03	6.56E+03	3.12E+01
4.26E+00	3.04E+03	6.75E+03	3.19E+01
4.39E+00	3.12E+03	6.95E+03	3.27E+01
4.52E+00	3.19E+03	7.15E+03	3.34E+01
4.65E+00	3.26E+03	7.35E+03	3.42E+01
4.79E+00	3.34E+03	7.56E+03	3.50E+01
4.94E+00	3.42E+03	7.78E+03	3.58E+01
5.08E+00	3.50E+03	8.01E+03	3.66E+01
5.24E+00	3.58E+03	8.24E+03	3.75E+01
5.39E+00	3.66E+03	8.47E+03	3.84E+01
5.56E+00	3.75E+03	8.72E+03	3.93E+01
5.72E+00	3.84E+03	8.97E+03	4.02E+01
5.89E+00	3.93E+03	9.23E+03	4.12E+01

Gestational			
Intake (ng/kg/ day)	**Fat (ng/kg)**	**Body Burden (ng/kg)**	**Blood (ng/kg)**
6.07E+00	4.02E+03	9.50E+03	4.22E+01
6.25E+00	4.12E+03	9.77E+03	4.32E+01
6.44E+00	4.22E+03	1.01E+04	4.42E+01
6.63E+00	4.32E+03	1.03E+04	4.53E+01
6.83E+00	4.42E+03	1.06E+04	4.64E+01
7.04E+00	4.53E+03	1.10E+04	4.75E+01
7.25E+00	4.64E+03	1.13E+04	4.86E+01
7.47E+00	4.75E+03	1.16E+04	4.98E+01
7.69E+00	4.87E+03	1.19E+04	5.10E+01
7.92E+00	4.99E+03	1.23E+04	5.23E+01
8.16E+00	5.11E+03	1.26E+04	5.36E+01
8.40E+00	5.24E+03	1.30E+04	5.49E+01
8.66E+00	5.37E+03	1.34E+04	5.62E+01
8.92E+00	5.50E+03	1.38E+04	5.76E+01
9.18E+00	5.63E+03	1.42E+04	5.91E+01
9.46E+00	5.77E+03	1.46E+04	6.05E+01
9.74E+00	5.92E+03	1.50E+04	6.20E+01
1.00E+01	6.05E+03	1.54E+04	6.34E+01
1.00E+01	6.07E+03	1.54E+04	6.36E+01
1.34E+01	7.71E+03	2.04E+04	8.08E+01
1.67E+01	9.35E+03	2.53E+04	9.80E+01
2.00E+01	1.10E+04	3.02E+04	1.15E+02
2.33E+01	1.26E+04	3.52E+04	1.32E+02
2.67E+01	1.42E+04	4.01E+04	1.49E+02
3.00E+01	1.58E+04	4.50E+04	1.66E+02
3.33E+01	1.74E+04	4.98E+04	1.83E+02
3.67E+01	1.90E+04	5.47E+04	2.00E+02
4.00E+01	2.07E+04	5.96E+04	2.17E+02
4.33E+01	2.23E+04	6.44E+04	2.33E+02
4.67E+01	2.39E+04	6.93E+04	2.50E+02
5.00E+01	2.54E+04	7.41E+04	2.67E+02

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APPENDIX D

Epidemiological Kinetic Modeling

NOTICE

THIS DOCUMENT IS AN EXTERNAL REVIEW DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

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APPENDIX D. EPIDEMIOLOGICAL KINETIC MODELING

D.1. BACCARELLI ET AL. (2008) MODELING

D.1.1. Input File for Exposure During Pregnancy

```
CINT = 1   %168 %100            %integration time
  %Exposure scenario
EXP_TIME_ON      = 0        % delay before begin exposure (HOUR)
EXP_TIME_OFF     = 401190    %TIME EXPOSURE STOP (HOUR)
DAY_CYCLE        = 24       %TIME
BCK_TIME_ON      = 401190    %DELAY BEFORE BACKGROUND EXP (HOUR)
BCK_TIME_OFF     = 401190    %TIME OF BACKGROUND EXP STOP (HOUR)
IV_LACK          = 401190
IV_PERIOD        = 401190
  %GESTATION CONTROL
MATTING          = 262800    % BEGINNING MATTING (HOUR)at 30 years old
TIMELIMIT        = 269184    %SIMULATION LIMIT TIME (HOUR)
TRANSTIME_ON     = 264312    % EXCHANGE MOTHER FETUS 1512 HOUR POST
MATTING
  %Exposure dose
MSTOT            = 0.021     % ng of TCDD /kg of BW
MSTOTBCKGR       = 0.  %0.1     % ORAL BACKGROUND EXPOSURE DOSE (nG/KG)
DOSEIV           = 0.  %10
DOSEIVLATE       = 0.  %10

   % TRANFER MOTHER TO FETUS CLEARANCE
CLPLA_FET        = 0.001 % MOTHER TO FETUS TRANFERT CLEARANCE(L/HR)
```

D.1.2. Table of Results for Baccarelli et al. (2008)

Table D-1. Estimated continuous intake corresponding to maternal serum concentration in Figure 2A

Variable	Value	Notes
Infant b-TSH	5 uU/mL	BMR
Maternal lipid adjusted serum	270 ng/kg	From Figure 2A
Intake	0.024 ng/kg-day	From Emond model, pregnancy at 30 years

Table D-2. Estimated maximum intake corresponding to maternal serum concentration in Figure 2A

Variable	Value	Notes
Infant b-TSH	--	--
Maternal lipid adjusted serum	309.5 ng/kg	Maximum from Figure 2A
Intake	0.030 ng/kg-day	From Emond model, pregnancy at 30 years

D.2. MOCARELLI ET AL. (2008) MODELING

D.2.1. Input File for Exposure for Pulse to Measurement 0.5 Years After the Seveso Pulse Dose

```
CINT = 1. %
EXP_TIME_ON  = 54312.      % Delay before begin exposure (HOUR) 6.2 years
EXP_TIME_OFF = 54335.    %324120    % HOUR/YEAR !TIME EXPOSURE STOP
(HOUR) 6.2 years + 23 hours
DAY_CYCLE    = 24.       % TIME
BCK_TIME_ON  = 0.        % DELAY BEFORE BACKGROUND EXP (HOUR)
BCK_TIME_OFF = 613200    % TIME OF BACKGROUND EXP STOP (HOUR)
TIMELIMIT    = 58692.    % half a year (July 1976 until January 1977) past 6.2 years
MSTOTBCKGR   = 3.7E-4     % ORAL BACKGROUND EXPOSURE DOSE (UG/KG)

% oral dose oral dose oral dose
MSTOT       = 232.4   % Seveso, ORAL DAILY EXPOSURE DOSE (NG/KG)
DOSEIV      =  0        % 40  %50 %5 %0.5 %0.3 %0.2 %0.1%0.05%0.3 %NG/KG
% oral dose oral dose oral dose

MEANLIPID    = 731       % 711 %664 %778 %468 %671 %730 %662 %592%615%730%
PAS_INDUC= 1         % NON INDUCTION (0) CONTROLE DE L'INDUCTION

%human variable parameter
MALE = 1.
FEMALE = 0.
Y0 = 0.              % 0 years old at the beginning of the simulation
```

D.2.2. Input File for Exposure from Pulse to the End of the Critical Window 3.8 Years After the Seveso Pulse Dose

```
CINT = 1. %
EXP_TIME_ON  = 54312.      % Delay before begin exposure (HOUR) 6.2 years
EXP_TIME_OFF = 54335.    %324120    % HOUR/YEAR !TIME EXPOSURE STOP
(HOUR) 6.2 years + 23 hours
DAY_CYCLE    = 24.       % TIME
```

```
BCK_TIME_ON  = 0.       % DELAY BEFORE BACKGROUND EXP (HOUR)
BCK_TIME_OFF = 613200.    % TIME OF BACKGROUND EXP STOP (HOUR)
TIMELIMIT    = 87600.     % 10 years
MSTOTBCKGR   = 3.7e-4      % ORAL BACKGROUND EXPOSURE DOSE (UG/KG)

% oral dose oral dose oral dose
MSTOT       = 232.5   % Serveso, ORAL DAILY EXPOSURE DOSE (NG/KG)
DOSEIV      =  0        % 40  %50 %5 %0.5 %0.3 %0.2 %0.1%0.05%0.3 %NG/KG
% oral dose oral dose oral dose

MEANLIPID    = 730       % 711 %664 %778 %468 %671 %730 %662 %592%615%730%
PAS_INDUC= 1         % NON INDUCTION (0) CONTROLE DE L'INDUCTION

%human variable parameter
MALE = 1.
FEMALE = 0.
Y0 = 0.             % 0 years old at the beginning of the simulation
```

D.2.3. Input File for Continuous Exposure for 10 Years

```
CINT = 1. %
EXP_TIME_ON  = 0.      % Delay before begin exposure (HOUR)
EXP_TIME_OFF = 87600.    % HOUR/YEAR !TIME EXPOSURE STOP (HOUR)
DAY_CYCLE    = 24.       % TIME
BCK_TIME_ON  = 0.       %324120     % DELAY BEFORE BACKGROUND EXP (HOUR)
BCK_TIME_OFF = 613200    %324120     % TIME OF BACKGROUND EXP STOP (HOUR)
TIMELIMIT    = 87600.     % 10 years
MSTOTBCKGR   = 0. %3.35E-4      % ORAL BACKGROUND EXPOSURE DOSE (UG/KG)

% oral dose oral dose oral dose
MSTOT       = 3.903   % Seveso, ORAL DAILY EXPOSURE DOSE (NG/KG)
DOSEIV      =  0        % 40  %50 %5 %0.5 %0.3 %0.2 %0.1%0.05%0.3 %NG/KG
% oral dose oral dose oral dose

MEANLIPID    = 730       % 711 %664 %778 %468 %671 %730 %662 %592%615%730%
PAS_INDUC= 1         % NON INDUCTION (0) CONTROLE DE L'INDUCTION

%human variable parameter
MALE = 1.
FEMALE = 0.
Y0 = 0.             % 0 years old at the beginning of the simulation
```

D.2.4. Tables of Results for Mocarelli et al. (2008)

Table D-3. Matching critical window average after pulse to critical window average for continuous intake run

Person modeled, beginning at age 0	Lipid adjusted serum (1976) ng/kg from Figure 3E	Pulse dose, 0.5 year lag time (ng/kg)	Average lipid adjusted serum 3.8 years after incident (ng/kg)	Continuous intake for 10 years (ng/kg-day)
Boy, 1st quartile	68	8.135	57.72	0.008024
Boy, 4th quartile	733	232.5	580.5	0.2128

Table D-4. Matching critical window peak after pulse to peak critical window concentration for continuous intake run

Person modeled, beginning at age 0	Lipid adjusted serum (1976) ng/kg from Figure 3E	Pulse dose, 0.5 year lag time (ng/kg)	Peak lipid adjusted serum after incident (ng/kg)	Continuous intake for 10 years (ng/kg-day)
Boy, 1st quartile	68	8.135	248.0	0.03194
Boy, 4th quartile	733	232.5	6674	3.904

D.3. ALALUUSUA ET AL. (2004) MODELING

D.3.1. Input File for Exposure for Pulse to Measurement 0.5 Years After the Seveso Pulse Dose

```
CINT = 1. %
EXP_TIME_ON  = 21900.    % Delay before begin exposure (HOUR) 2.5 years
EXP_TIME_OFF = 21923.    % 21900+23     % HOUR/YEAR !TIME EXPOSURE STOP
(HOUR) 2.5 years and 23 hours
DAY_CYCLE    = 24.       % TIME
BCK_TIME_ON  = 0.        % DELAY BEFORE BACKGROUND EXP (HOUR)
BCK_TIME_OFF = 613200.   % TIME OF BACKGROUND EXP STOP (HOUR)
TIMELIMIT    = 26280.    % half a year (July 1976 until January 1977) past 2.5 years
MSTOTBCKGR   = 3.7e-4    % ORAL BACKGROUND EXPOSURE DOSE (UG/KG)

% oral dose oral dose oral dose
MSTOT        = 24.22     % Seveso, ORAL DAILY EXPOSURE DOSE (NG/KG)
DOSEIV       =  0        % 40  %50 %5 %0.5 %0.3 %0.2 %0.1%0.05%0.3 %NG/KG
% oral dose oral dose oral dose
```

```
MEANLIPID    = 730       % 711 %664 %778 %468 %671 %730 %662 %592%615%730%
PAS_INDUC= 1         % NON INDUCTION (0) CONTROLE DE L'INDUCTION

%human variable parameter
MALE = 1.
FEMALE = 0.
Y0 = 0.              % 0 years old at the beginning of the simulation
```

D.3.2. Input File for Exposure from Pulse to the End of the Critical Window 2.5 Years After the Seveso Pulse Dose

```
CINT = 1. %
EXP_TIME_ON  = 21900.    % Delay before begin exposure (HOUR) 2.5 years
EXP_TIME_OFF = 21923.    % 324120     % HOUR/YEAR !TIME EXPOSURE STOP
(HOUR) 2.5 years and 23 hours
DAY_CYCLE    = 24.       % TIME
BCK_TIME_ON  = 0.        % 324120     % DELAY BEFORE BACKGROUND EXP (HOUR)
BCK_TIME_OFF = 613200.   % 324120     % TIME OF BACKGROUND EXP STOP (HOUR)
TIMELIMIT    = 43800.    % 5 years
MSTOTBCKGR   = 3.7e-4    % ORAL BACKGROUND EXPOSURE DOSE (UG/KG)

% oral dose oral dose oral dose
MSTOT        = 24.22   % Seveso, ORAL DAILY EXPOSURE DOSE (NG/KG)
DOSEIV       =   0         % 40  %50 %5 %0.5 %0.3 %0.2 %0.1%0.05%0.3 %NG/KG
% oral dose oral dose oral dose

MEANLIPID    = 730       % 711 %664 %778 %468 %671 %730 %662 %592%615%730%
PAS_INDUC= 1         % NON INDUCTION (0) CONTROLE DE L'INDUCTION

%human variable parameter
MALE = 1.
FEMALE = 0.
Y0 = 0.              % 0 years old at the beginning of the simulation
```

D.3.3. Input File for Continuous Exposure for 5 Years

```
CINT = 1. %
EXP_TIME_ON  = 0.        % Delay before begin exposure (HOUR)
EXP_TIME_OFF = 43800.    % 324120     % HOUR/YEAR !TIME EXPOSURE STOP (HOUR)
DAY_CYCLE    = 24.       % TIME
BCK_TIME_ON  = 0.        % 324120     % DELAY BEFORE BACKGROUND EXP (HOUR)
BCK_TIME_OFF = 613200.   % 324120     % TIME OF BACKGROUND EXP STOP (HOUR)
TIMELIMIT    = 43800.    % End of critical window (5 years)
MSTOTBCKGR   = 0.        % ORAL BACKGROUND EXPOSURE DOSE (UG/KG)

% oral dose oral dose oral dose
MSTOT        = 0.03486   % Seveso, ORAL DAILY EXPOSURE DOSE (NG/KG)
```

```
DOSEIV      =  0        % 40  %50 %5 %0.5 %0.3 %0.2 %0.1%0.05%0.3 %NG/KG
% oral dose oral dose oral dose

MEANLIPID   = 730      % 711 %664 %778 %468 %671 %730 %662 %592%615%730%
PAS_INDUC= 1        % NON INDUCTION (0) CONTROLE DE L'INDUCTION

%human variable parameter
MALE = 1.
FEMALE = 0.
Y0 = 0.             % 0 years old at the beginning of the simulation
```

D.3.4. Tables of Results for Alaluusua et al. (2004)

Table D-5. Matching critical window average after pulse to critical window average for continuous intake run

Person modeled, beginning at age 0	Lipid adjusted serum (1976) ng/kg estimated from tertile bins[a]	Pulse dose, 0.5 year lag time (ng/kg)	Average lipid adjusted serum 2.5 years after incident (ng/kg)	Continuous intake for 5 years (ng/kg-day)
Boy, 1st tertile	130	24.22	110.8	0.03486
Boy, 2nd tertile	383	108.9	322.7	0.1578
Boy, 3rd tertile	1830	1041	1538	1.511
Girl, 1st tertile	130	23.03	110.8	0.03211
Girl, 2nd tertile	383	105.3	324.4	0.1481
Girl, 3rd tertile	1830	1015	1546	1.427
Boy and girl, averaged, 1st tertile	130	-	-	0.03349
Boy and girl, averaged, 2nd tertile	383	-	-	0.1530
Boy and girl, averaged, 3rd tertile	1830	-	-	1.469

[a]Mean of tertile bin assuming a lognormal distribution of serum concentrations.

Table D-6. Matching critical window peak after pulse to peak critical window concentration for continuous intake run

Person modeled, beginning at age 0	Lipid adjusted serum (1976) ng/kg estimated from tertile bins	Pulse dose, 0.5 year lag time (ng/kg)	Peak lipid adjusted serum after incident (ng/kg)	Continuous intake for 5 years (ng/kg-day)
Boy, 1st tertile	130	24.22	618.8	0.2113
Boy, 2nd tertile	383	108.9	2700	1.783
Boy, 3rd tertile	1830	1041	24706	31.35
Girl, 1st tertile	130	23.02	588.0	0.1882
Girl, 2nd tertile	383	105.3	2610	1.642
Girl, 3rd tertile	1830	1015	24113	29.52
Boy and girl, averaged, 1st tertile	130	-	-	0.1998
Boy and girl, averaged, 2nd tertile	383	-	-	1.713
Boy and girl, averaged, 3rd tertile	1830	-	-	30.44

[a]Mean of tertile bin assuming a lognormal distribution of serum concentrations.

D.4. ESKANAZI ET AL. (2002) MODELING

D.4.1. Input File for Exposure for Pulse to Measurement 0.5 Years After the Seveso Pulse Dose

```
CINT = 1. %
EXP_TIME_ON  = 58692.      % Delay before begin exposure (HOUR) 6.7 years
EXP_TIME_OFF = 58715.    % HOUR/YEAR !TIME EXPOSURE STOP (HOUR) 6.7 years +
23 hours
DAY_CYCLE    = 24.       % TIME
BCK_TIME_ON  = 0.        %324120    % DELAY BEFORE BACKGROUND EXP (HOUR)
BCK_TIME_OFF = 613200.   %324120    % TIME OF BACKGROUND EXP STOP (HOUR)
TIMELIMIT    = 63072.    % half a year (July 1976 until January 1977) past 6.7 years
MSTOTBCKGR   = 3.7e-4    % ORAL BACKGROUND EXPOSURE DOSE (UG/KG)

% oral dose oral dose oral dose
MSTOT        = 7193   % Seveso, ORAL DAILY EXPOSURE DOSE (NG/KG)
DOSEIV       =  0        % 40  %50 %5 %0.5 %0.3 %0.2 %0.1%0.05%0.3 %NG/KG
% oral dose oral dose oral dose

MEANLIPID    = 730       % 711 %664 %778 %468 %671 %730 %662 %592%615%730%
```

```
PAS_INDUC= 1        % NON INDUCTION (0) CONTROLE DE L'INDUCTION

%human variable parameter
MALE = 0.
FEMALE = 1.
Y0 = 0.             % 0 years old at the beginning of the simulation
```

D.4.2. Input File for Exposure from Pulse to the End of the Critical Window 6.7 Years After the Seveso Pulse Dose

```
CINT = 1. %
EXP_TIME_ON  = 58692.      % Delay before begin exposure (HOUR) 6.7 years
EXP_TIME_OFF = 58715.    %324120    % HOUR/YEAR !TIME EXPOSURE STOP
(HOUR) 6.7 years + 23 hours
DAY_CYCLE    = 24.       % TIME
BCK_TIME_ON  = 0.        %324120    % DELAY BEFORE BACKGROUND EXP (HOUR)
BCK_TIME_OFF = 613200    %324120    % TIME OF BACKGROUND EXP STOP (HOUR)
TIMELIMIT    = 113880.   % 13 years
MSTOTBCKGR   = 3.7e-4    % ORAL BACKGROUND EXPOSURE DOSE (UG/KG)

% oral dose oral dose oral dose
MSTOT        = 7193   % Seveso, ORAL DAILY EXPOSURE DOSE (NG/KG)
DOSEIV       =  0        % 40  %50 %5 %0.5 %0.3 %0.2 %0.1%0.05%0.3 %NG/KG
% oral dose oral dose oral dose

MEANLIPID    = 730       % 711 %664 %778 %468 %671 %730 %662 %592%615%730%
PAS_INDUC= 1        % NON INDUCTION (0) CONTROLE DE L'INDUCTION

%human variable parameter
MALE = 0.
FEMALE = 1.
Y0 = 0.             % 0 years old at the beginning of the simulation
```

D.4.3. Input File for Continuous Exposure for 13 Years

```
CINT = 1. %
EXP_TIME_ON  = 0.       % Delay before begin exposure (HOUR)
EXP_TIME_OFF = 113880.    %324120    % HOUR/YEAR !TIME EXPOSURE STOP
(HOUR) 13 years
DAY_CYCLE    = 24.       % TIME
BCK_TIME_ON  = 0.        %324120    % DELAY BEFORE BACKGROUND EXP (HOUR)
BCK_TIME_OFF = 613200.    %324120    % TIME OF BACKGROUND EXP STOP (HOUR)
TIMELIMIT    = 113880.   % 13 years
MSTOTBCKGR   = 0. %3.35E-4      % ORAL BACKGROUND EXPOSURE DOSE (UG/KG)

% oral dose oral dose oral dose
MSTOT        = 166   % Seveso, ORAL DAILY EXPOSURE DOSE (NG/KG)
```

```
DOSEIV      =  0          % 40  %50 %5 %0.5 %0.3 %0.2 %0.1%0.05%0.3 %NG/KG
% oral dose oral dose oral dose

MEANLIPID   = 730        % 711 %664 %778 %468 %671 %730 %662 %592%615%730%
PAS_INDUC= 1          % NON INDUCTION (0) CONTROLE DE L'INDUCTION

%human variable parameter
MALE = 0.
FEMALE = 1.
Y0 = 0.               % 0 years old at the beginning of the simulation
```

D.4.4. Tables of Results for Eskanazi et al. (2002)

Table D-7. Matching critical window average after pulse to critical window average for continuous intake run

Person modeled, beginning at age 0	Lipid adjusted serum (adjusted to 1976-1977 levels) ng/kg from Figure 1A	Pulse dose, 0.5 year lag time (ng/kg)	Average lipid adjusted serum 6.7 years after incident (ng/kg)	Continuous intake for 13 years (ng/kg-day)
Girl, estrous cycle 28.5 days	166	28.40	114.0	0.01660
Girl, estrous cycle 29 days	693	215.5	455.1	0.1224
Girl, estrous cycle 29.5 days	2020	1008	1295	0.5693
Girl, estrous cycle 30 days	8450	7193	5179	4.054

Table D-8. Matching critical window peak after pulse to peak critical window concentration for continuous intake run

Person modeled, beginning at age 0	Lipid adjusted serum (adjusted to 1976-1977 levels) ng/kg from Figure 1A	Pulse dose, 0.5 year lag time (ng/kg)	Peak lipid adjusted serum after incident (ng/kg)	Continuous intake for 13 years (ng/kg-day)
Girl, estrous cycle 28.5 days	166	28.40	838.2	0.1800
Girl, estrous cycle 29 days	693	215.5	6183	3.148
Girl, estrous cycle 29.5 days	2020	1008	28316	20.86
Girl, estrous cycle 30 days	8450	7193	198240	166.6

D.5. REFERENCES

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APPENDIX E

Noncancer Benchmark Dose Modeling

NOTICE

THIS DOCUMENT IS AN EXTERNAL REVIEW DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

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APPENDIX E. NONCANCER BENCHMARK DOSE MODELING

E.1. BMDS INPUT TABLES

E.1.1. Amin et al. (2000)

Endpoint [c]	Administered Dose (ng/kg-day)		
	0	25 [a]	100
	Internal Dose (ng/kg blood) [b]		
	0	3.38	10.57
	(n = 10)	(n = 10)	(n = 10)
Saccharin consumed, female rats (0.25%) (ml saccharin solution/100 g body weight) [c]	31.67 ± 6.53	24.60 ± 3.79	10.70 ± 1.68
Saccharin consumed, female rats (0.50%) (ml saccharin solution/100 g body weight) [c]	22.40 ± 5.05	11.38 ± 2.42	4.54 ± 1.05
Saccharin preference ratio, female rats (0.25%) (ratio of saccharin solution consumed to total fluid consumed) [d]	82.14 ± 4.22	58.12 ± 10.71	54.87 ± 6.17
Saccharin preference ratio, female rats (0.50%) (ratio of saccharin solution consumed to total fluid consumed) [d]	72.73 ± 7.79	44.48 ± 10.39	33.77 ± 7.79

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Values are the mean ± SE. Data obtained from Figure 2 in Amin et al. 2000.
[d] Values are the ratio ± SE. Data obtained from Figure 3 in Amin et al. 2000.

E.1.2. Bell et al. (2007)

Endpoint	Administered Dose (ng/kg-day)			
	0	2.4 [a]	8	46
	Internal Dose (ng/kg blood) [b]			
	0	2.20	5.14	18.41
	(n = 30)	(n = 30)	(n = 30)	(n = 30)
Proportion of male rat pups that had not undergone balano-preputial separation on PND 49 [c]	1/30 (3%)	5/30 (17%)	6/30 (20%)	15/30 (50%)

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Data obtained from Figure 2 in Bell et al. 2007.

E.1.3. Cantoni et al. (1981)

Endpoint	Administered Dose (ng/kg-day)			
	0	1.43 [a]	14.3	143
	Internal Dose (ng/kg blood) [b]			
	0	1.85	8.84	50.05
	(n = 4)	(n = 4)	(n = 3)	(n = 3)
Urinary coproporphyrins in female rats (µg coproporphyrin methyl ester/24 hr) at 3 months [c]	0.74 ± 0.17	1.81 ± 0.42 [d]	2.73 ± 0.75 [e]	3.00 ± 1.30 [e]
Urinary porphyrins in rats (nmol/24 hr) after 45 weeks [c]	2.27 ± 0.49	5.55 ± 0.85 [d]	7.62 ± 1.79 [d]	196.89 ± 63.14 [e]

[a] LOAEL identifed.
[b] From the Emond PBPK model described in 3.3.
[c] Values are the mean ± SE. Data for urinary coproporphyrins and urinary porphyrins obtained from Figure 1 and Table 1, respectively, in Cantoni et al. 1981.
[d] Statistically significant as compared to control ($p < 0.05$).
[e] Statistically significant as compared to control ($p < 0.01$).

E.1.4. Crofton et al. (2005)

Endpoint	Administered Dose (ng/kg-day)									
	0	0.1	3	10	30 [a]	100 [b]	300	1,000	3,000	10,000
	Internal Dose (ng/kg blood) [c]									
	0	0.02	0.49	1.38	3.46	9.26	23.07	65.65	180.90	583.48
	(n = 14)	(n = 6)	(n = 12)	(n = 6)	(n = 6)	(n = 6)	(n = 6)	(n = 6)	(n = 6)	(n = 4)
Serum T4 in female rats (% control) [d]	100.00 ± 15.44	96.27 ± 14.98	98.57 ± 18.11	99.76 ± 19.04	93.32 ± 12.11	70.94 ± 12.74	62.52 ± 14.75	52.68 ± 22.73	54.66 ± 19.71	49.15 ± 11.15

[a] NOAEL identifed.
[b] LOAEL identifed.
[c] From the Emond PBPK model described in 3.3.
[d] Values are the mean ± SD. Data were obtained from a Crofton et al. supplemental file, available at http://ehp.niehs.nih.gov/docs/2005/8195/supplemental.pdf.

E.1.5. DeCaprio et al. (1986)

Endpoint	Administered Dose (ng/kg-day)				
	0	0.12	0.61 [a]	4.9 [b]	26
	Internal Dose (ng/kg blood) [c]				
	n/a	n/a	n/a	n/a	n/a
	(n = 10)	(n = 10)	(n = 11)	(n = 10)	(n = 4)
Absolute kidney weight (g), males [d]	5.49 ± 0.17	5.14 ± 0.12	4.71 ± 0.12	4.3 ± 0.15 [f]	-
Absolute thymus weight (g), males [d]	0.56 ± 0.050	0.45 ± 0.022	0.44 ± 0.034	0.35 ± 0.167 [g]	-
Body weight (g), males [e]	713 ± 15	682 ± 16	651 ± 19	603 ± 20 [f]	433 ± 38 [h]
Relative brain weight, males [d]	0.54 ± 0.015	0.56 ± 0.016	0.6 ± 0.016	0.65 ± 0.016 [f]	-
Relative liver weight, males [d]	4.54 ± 0.23	4.1 ± 0.14	5.36 ± 0.61	5.63±0.29 [f]	-
Relative thymus weight, males [d]	0.078 ± 0.006	0.066 ± 0.003	0.068 ± 0.004	0.06±0.003 [f]	-
Endpoint	**Administered Dose (ng/kg-day)**				
	0	**0.12**	**0.68**	**4.86**	**31**
	Internal Dose (ng/kg blood) [c]				
	0	**n/a**	**n/a**	**n/a**	**n/a**
	(n = 8)	**(n = 10)**	**(n = 9)**	**(n = 10)**	**(n = 4)**
Body weight (g), females [e]	602 ± 12	583 ± 22	570 ± 22	531 ± 14 [f]	351 ± 49 [h]
Relative liver weight, females [d]	4.3 ± 0.26	4.49 ± 0.35	4.27 ± 0.16	5.54 ± 0.43 [f]	-

[a] NOAEL identified.
[b] LOAEL identified.
[c] Internal dose not calculated using the Emond PBPK (guinea pigs).
[d] Organ weight data in guinea pigs obtained from Table 2 of DeCaprio et al. 1986. Values are the mean ± SE. Relative organs weights were calculated as organ weight (g) / body weight (g) X 100.
[e] Body weight data in guinea pigs obtained from Table 1 of DeCaprio et al. 1986. Values are the mean ± SE.
[f] Statistically significant as compared to control ($p < 0.05$).
[g] Statistically significant as compared to control ($p < 0.01$).
[h] Statistically significant as compared to control ($p < 0.001$).

E.1.6. Franc et al. (2001)

Endpoint	Administered Dose (ng/kg-day)			
	0	10 [a]	30 [b]	100
	Internal Dose (ng/kg blood) [c]			
	0	6.59	14.48	36.43
	(n = 8)	(n = 8)	(n = 8)	(n = 8)
S-D rats, relative liver weight [d]	100.0 ± 5.0	108.1 ± 6.0 [e]	116.8 ± 9.2 [e]	155.3 ± 10.9 [e]
L-E rats, relative liver weight [d]	100.0 ± 3.5	106.3 ± 6.3	116.8 ± 3.2 [e]	122.2 ± 7.0 [e]
S-D rats, relative thymus weight [d]	100.2 ± 29.4	91.2 ± 17.0	51.4 ± 15.4 [e]	22.8 ± 10.6 [e]
L-E rats, relative thymus weight [d]	103.4 ± 19.3	95.4 ± 24.9	38.7 ± 17.0 [e]	35.0 ± 27.6 [e]
H/W rats, relative thymus weight [d]	101.2 ± 12.7	97.5 ± 11.7.0	71.0 ± 8.5 [e]	49.3 ± 15.4 [e]

[a] NOAEL identified.
[b] LOAEL identified.
[c] From the Emond PBPK model described in 3.3.
[d] Values are the mean ± SE. Data obtained from Figure 5 in Franc et al. 2001.
[e] Statistically significant as compared to control ($p < 0.05$).

E.1.7. Hojo et al. (2002)

Endpoint	Administered Dose (ng/kg-day)			
	0	20 [a]	60	180
	Internal Dose (ng/kg blood) [b]			
	0	1.62	4.17	10.70
	(n = 5)	(n = 5)	(n = 6)	(n = 5)
DRL reinforcements/min, rat litters [c]	-0.814 ± 0.45	-0.364 ± 0.82	0.374 ± 0.54	-0.163 ± 0.44
DRL responses/min, rat litters [c]	18.44 ± 7.99	-0.99 ± 10.96	-4.52 ± 7.19	-0.41 ± 15.23

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] DRL = differential reinforcement of low rate. Values are the mean ± SD. Data obtained from Table 5 in Hojo et al. 2002.

E.1.8. Kattainen et al. (2001)

Endpoint	Administered Dose (ng/kg-day)				
	0	30 [a]	100	300	1,000
	Internal Dose (ng/kg blood) [b]				
	0	2.23	6.25	16.08	46.86
	(n = 16)	(n = 17)	(n = 15)	(n = 12)	(n = 19)
3rd molar mesio-distal length in female rat offspring (molar development) (mm) [c]	1.86 ± 0.017	1.58 ± 0.045 [e]	1.6 ± 0.069 [e]	1.5 ± 0.064 [e]	1.35 ± 0.118 [e]
Proportion of female rat offspring without 3rd molar eruption on PND 35 [d]	1/16 (10%)	3/17 (20%)	4/15 (30%)	6/12 (50%) [e]	13/19 (70%) [e]

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Values are the mean ± SE. Data were obtained from Figure 3 in Kattainen et al. 2001.
[d] Data were obtained from Figure 2 in Kattainen et al. 2001.
[e] Statistically significant as compared to control ($p < 0.05$).

E.1.9. Keller et al. (2007, 2008a, b)

Endpoint	Administered Dose (ng/kg-day)			
	0	10 [a]	100	1,000
	Internal Dose (ng/kg blood) [b]			
	0	0.54	4.29	34.06
Frequency of missing 3rd mandibular molars in CBA J mice [c]	0/29 (0%)	2/23 (10%)	6/29 (20%)	30/30 (100%)

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Data obtained from Table 1 in Keller et al. 2007.

E.1.10. Kociba et al. (1978)

Endpoint	Administered Dose (ng/kg-day)			
	0	1 [a]	10 [b]	100
	Internal Dose (ng/kg blood) [c]			
	0	1.55	7.15	38.56
	(n = 5)	(n = 5)	(n = 5)	(n = 5)
Urinary coproporphyrin (µg/48 h), female rats [d]	9.8 ± 1.3	8.6 ± 2	16.4 ± 4.7 [e]	17.4 ± 4 [e]
µg uroporphyrin per mg creatinine, female rats [d]	0.157 ± 0.05	0.143 ± 0.037	0.181 ± 0.053	0.296 ± 0.074 [e]

[a] NOAEL identified.
[b] LOAEL identified.
[c] From the Emond PBPK model described in 3.3.
[d] Values are the mean ± SD. Data obtained from Table 2 in Kociba et al. 1978.
[e] Statistically significant as compared to control ($p < 0.05$).

E.1.11. Latchoumycandane and Mathur (2002)

Endpoint	Administered Dose (ng/kg-day)			
	0	1 [a]	10	100
	Internal Dose (ng/kg blood) [b]			
	0	0.78	4.65	27.27
	(n = 6)	(n = 6)	(n = 6)	(n = 6)
Daily sperm production ($\times 10^6$) in adult male rats (mg) [c]	22.19 ± 2.67	15.67 ± 2.65 [d]	13.65 ± 2.19 [d]	13.1 ± 3.16 [d]

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Values are the mean ± SD. Data obtained from Table 1 in Latchoumycandane and Mathur 2002.
[d] Statistically significant as compared to control ($p < 0.05$).

E.1.12. Li et al. (1997)

Endpoint	Administered Dose (ng/kg-day)									
	0	3 [a]	10 [b]	30	100	300	1,000	3,000	10,000	30,000
	Internal Dose (ng/kg blood) [c]									
	0	0.27	0.80	2.1	5.87	15	43.33	119.94	385.96	1171.90
	(n = 10)	(n =10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)
Serum FSH (ng/ml) in female rats [d]	23.86 ± 9.38	22.16 ± 15.34	85.23 ± 29.83	73.30 ± 15.34	126.14 ± 50.28	132.10 ± 36.65	116.76 ± 16.19	304.26 ± 48.58	346.88 ± 47.73	455.11 ± 90.34

[a] NOAEL identified.
[b] LOAEL identified.
[c] From the Emond PBPK model described in 3.3.
[d] Values are the mean ± SE. Data obtained from Figure 3 in Li et al. 1997.

E.1.13. Li et al. (2006)

Endpoint	Administered Dose (ng/kg-day)			
	0	2 [a]	50	100
	Internal Dose (ng/kg blood) [b]			
	0	0.16	2.84	5.12
	(n = 10)	(n = 10)	(n = 10)	(n = 10)
Serum estradiol/(pg·ml)$^{-1}$ in female mice (1~3d) [c]	10.17 ± 3.85	19.91 ± 6.31	24.72 ± 4.60	18.09 ± 5.57
Serum progesterone (ng·ml)$^{-1}$ in female mice (1~3d) [c]	61.74 ± 3.51	30.56 ± 12.80 [d]	16.93 ± 10.53	11.36 ± 13.83

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Values are the mean ± SE. Data obtained from Figures 3 (estradiol) and 4 (progesterone) in Li et al. 2006.
[d] Statistically significant as compared to control ($p < 0.01$).

E.1.14. Markowski et al. (2001)

Endpoint	Administered Dose (ng/kg-day)			
	0	20 [a]	60	180
	Internal Dose (ng/kg blood) [b]			
	0	1.56	4.03	10.32
	(n = 7)	(n = 4)	(n = 6)	(n = 7)
FR10 earned run opportunities, adult female offspring [c]	13.29 ± 8.65	11.25 ± 5.56	5.75 ± 3.53	7 ± 6.01
FR2 total revolutions, adult female offspring [c]	119.29 ± 69.9	108.5 ± 61	56.5 ± 31.21	68.14 ± 33.23
FR5 earned run opportunities, adult female offspring [c]	26.14 ± 12.28	23.5 ± 7.04	12.8 ± 6.17	13.14 ± 7.14

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Values are the mean ± SD. Data obtained from Table 3 in Markowski et al. 2001.

E.1.15. Miettinen et al. (2006)

Endpoint	Administered Dose (ng/kg-day)				
	0	30 [a]	100	300	1,000
	Internal Dose (ng/kg blood) [b]				
	0	2.22	6.23	16.01	46.64
	(n = 42)	(n = 29)	(n = 15)	(n = 24)	(n = 32)
Cariogenic lesions in rat pups [c]	25/42 (60%)	23/29 (79%) [d]	19/25 (76%)	20/24 (83%) [d]	29/32 (91%) [d]

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Data obtained from Table 2 in Miettinen et al. 2006.
[d] Statistically significant as compared to control ($p < 0.05$).

E.1.16. National Toxicology Program (1982)

Endpoint	Administered Dose (ng/kg-day)			
	0	1.43 [a]	7.14	71.4
	Internal Dose (ng/kg blood) [b]			
	0	0.77	2.27	11.24
	(n = 73)	(n = 49)	(n = 49)	(n = 50)
Numbers of male mice with toxic hepatitis [c]	1/73 (1.4%)	5/49 (10%)	3/49 (6.1%)	44/50 (88%)

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Data obtained from Table 11 in NTP 1982.

E.1.17. National Toxicology Program (2006)

Endpoint [e]	Administered Dose (ng/kg-day)					
	0	2.14 [a]	7.14	15.7	32.9	71.4
	Internal Dose (ng/kg blood) [b]					
	0	2.56	5.69	9.79	16.57	29.70
	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)
Gingival squamous hyperplasia	1/53 (2%)	7/54 (13%)[d]	14/53 (26%)[c]	13/53 (25%)[c]	15/53 (28%)[c]	16/53 (30%)[c]
Liver, hepatocyte hypertrophy	0/53 (0%)	19/54 (40%)[c,]	19/53 (40%)[c]	42/53 (80%)[c]	41/53 (80%)[c]	52/53 (100%)[c]
Heart, cardiomyopathy	10/53 (19%)	12/54 (22%)	22/53[c] (42%)	25/52[c] (48%)	32/53[c] (60%)	36/52[c] (69%)
Liver, eosinophilic focus, multiple	3/53 (6%)	8/54 (15%)	14/53 (26%)	17/53 (32%)	22/53 (42%)	42/53 (79%)
Liver, fatty change, diffuse	0/53 (0%)	2/54 (4%)	12/53[c] (23%)	17/53[c] (32%)	30/53[c] (57%)	48/53[c] (91%)
Liver, necrosis	1/53 (2%)	4/54 (7%)	4/53 (8%)	8/53[d] (15%)	10/53[c] (19%)	17/53[c] (32%)
Liver, pigmentation	4/53 (8%)	9/54 (17%)	34/53[c] (64%)	48/53[c] (91%)	52/53[c] (98%)	53/53[c] (100%)
Liver, toxic hepatopathy	0/53 (0%)	2/54 (4%)	8/53 (15%)	30/53 (57%)	45/50 (85%)	53/53 (100%)
Oval cell hyperplasia	0/53 (0%)	4/54 (10%)[d]	3/53 (10%)	20/53 (40%)[c]	38/53 (70%)[d]	53/53 (100%)[c]
Lung, alveolar to bronchiolar epithelial metaplasia (Alveolar epithelium, metaplasia, bronchiolar)	2/53 (4%)	19/54 [c] (35%)	33/53[c] (62%)	35/52[c] (67%)	45/53[c] (85%)	46/52[c] (89%)

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Statistically significant as compared to control ($p < 0.01$).
[d] Statistically significant as compared to control ($p < 0.05$).
[e] Data are for female rats in 2-year gavage study. Data for all endpoints obtained from Table A5b in NTP 2006.

E.1.18. Ohsako et al. (2001)

Endpoint	Administered Dose (ng/kg-day)				
	0	12.5 [a]	50 [b]	200	800
	Internal Dose (ng/kg blood) [c]				
	0	1.04	3.47	11.36	38.42
	(n = 12)	(n = 10)	(n = 10)	(n = 10)	(n = 12)
Anogenital distance (mm) in male rat offspring, PND120 [d]	28.91 ± 0.90	27.94 ± 0.79	25.17 ± 1.02 [e]	26.01 ± 0.90 [f]	23.80 ± 0.45 [e]

[a] NOAEL for selected endpoint.
[b] LOAEL for selected endpoint.
[c] From the Emond PBPK model described in 3.3.
[d] Values are the mean ± SE. Data obtained from Figure 7 in Ohsako et al. 2001.
[e] Statistically significant as compared to control ($p < 0.01$).
[f] Statistically significant as compared to control ($p < 0.05$).

E.1.19. Shi et al. (2007)

Endpoint	Administered Dose (ng/kg-day)				
	0	0.143 [a]	0.714 [b]	7.14	28.6
	Internal Dose (ng/kg blood) [c]				
	0	0.34	1.07	5.23	13.91
	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)
Serum estradiol – 17β at proestrus 9 in female rats at 9 mo.of age (pg/ml) [d]	102.86 ± 13.10	86.19 ± 6.19	63.33 ± 9.29 [e]	48.1 ± 5.95 [e]	38.57 ± 7.14 [e]

[a] NOAEL identified.
[b] LOAEL identified.
[c] From the Emond PBPK model described in 3.3.
[d] Values are the mean ± SE. Data obtained from Figure 4 in Shi et al. 2007.
[e] Statistically significant as compared to control ($p < 0.05$).

E.1.20. Smialowicz et al. (2008)

Endpoint	Administered Dose (ng/kg-day)				
	0	1.07 [a]	10.7	107	321
	Internal Dose (ng/kg blood) [b]				
	0	0.44	2.46	13.40	31.65
	(n = 15)	(n = 14)	(n = 15)	(n = 15)	(n = 8)
PFC per 10^6 cells in female mice [c]	1491 ± 716	1129 ± 171 [d]	945 ± 516 [d]	677 ± 465 [d]	161 ± 117 [d]
PFC x 10^4 per spleen in female mice [c]	27.8 ± 13.4	21 ± 13.6 [d]	17.6 ± 9.4 [d]	12.6 ± 8.7 [d]	3.0 ± 3.1 [d]

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Values are the mean ± SD. Data obtained from Table 4 in Smialowicz et al. 2008.
[d] Statistically significant as compared to control ($p < 0.05$).

E.1.21. Toth et al. (1979)

Endpoint	Administered Dose (ng/kg-day)			
	0	1 [a]	100	1,000
	Internal Dose (ng/kg blood) [b]			
	0	0.57	14.21	91.21
	(n =38)	(n = 44)	(n = 44)	(n = 43)
Number with amyloidosis plus skin lesions in mice [c]	0/38 (0%)	5/44 (11%)	10/44 (23%)	17/43 (40%)
Number with skin lesions in mice [c]	0/38 (0%)	5/44 (11%)	13/44 (30%)	25/43 (58%)

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Data obtained from Table 2 in Toth et al. 1979.

E.1.22. Van Birgelen et al. (1995)

Endpoint	Administered Dose (ng/kg-day)					
	0	14 [a]	26	47	320	1,024
	Internal Dose (ng/kg blood) [b]					
	0	7.20	11.76	18.09	86.41	250.16
	n = 8	n = 8	n = 8	n = 8	n = 8	n = 8
Hepatic retinol (mg/g liver) in female rats [c]	14.9 ± 3.1	8.4 ± 1.2 [d]	8.2 ± 0.8 [d]	5.1 ± 0.3 [d]	2.2 ± 0.3 [d]	0.6 ± 0.2 [d]
Hepatic retinol palmitate (mg/g liver) in female rats [c]	472 ± 96	94 ± 24 [d]	107 ± 27 [d]	74 ± 14 [d]	22 ± 8 [d]	3 ± 1 [d]
Plasma FT4 (pmol/liter) in female rats [c]	23.4 ± 1.1	24.5 ± 2.0	22.4 ± 1.0	19.3 ± 3.3	16.3 ± 1.5 [d]	10.3 ± 1.7 [d]
Plasma TT4 (nmol/liter) in female rats [c]	40.9 ± 2.4	41.4 ± 1.9	41.4 ± 2.3	32.3 ± 2.6 [d]	33.6 ± 2.2 [d]	25.5 ± 2.7 [d]

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Values are the mean ± SE. Data obtained from Table 3 in Van Birgelen et al. 1995.
[d] Statistically significant as compared to control ($p < 0.05$).

E.1.23. White et al. (1986)

Endpoint	Administered Dose (ng/kg-day)						
	0	10 [a]	50	100	500	1,000	2,000
	Internal Dose (ng/kg blood) [b]						
	0	1.09	4.08	7.14	26.81	48.72	90.56
	(n = 8)	(n = 8)	(n = 8)	(n = 8)	(n = 8)	(n = 8)	(n = 8)
CH50 (U/ml) in female mice [c]	91 ± 5	54 ± 3 [d]	63 ± 4 [d]	56 ± 9 [d]	41 ± 6 [d]	32 ± 6 [d]	17 ± 6 [d]

[a] LOAEL identified.
[b] From the Emond PBPK model described in 3.3.
[c] Values are the mean ± SE. Data obtained from Table 1 in White et al. 1986.
[d] Statistically significant as compared to control ($p < 0.05$).

E.2. ALTERNATE DOSE: WHOLE BLOOD BMDS RESULTS

E.2.1. Amin et al., 2000: 0.25% Saccharin Consumed, Female

E.2.1.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
linear [b]	**1**	**0.551**	**179.214**	**9.147E+00**	**6.094E+00**	
polynomial, 2-degree	1	0.551	179.214	9.147E+00	6.094E+00	
power	1	0.551	179.214	9.147E+00	6.094E+00	power bound hit (power = 1)
power, unrestricted [c]	0	N/A	180.858	8.367E+00	3.419E+00	unrestricted (power = 0.736)

[a] Non-constant variance model selected ($p = 0.0005$)
[b] Best-fitting model, BMDS output presented in this appendix
[c] Alternate model, BMDS output also presented in this appendix

E.2.1.2. *Output for Selected Model: Linear*

Amin et al., 2000: 0.25% Saccharin Consumed, Female

```
 ================================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\Blood\1_Amin_2000_25_SC_Linear_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\1_Amin_2000_25_SC_Linear_1.plt
                                                    Mon Feb 08 10:44:22 2010
 ================================================================================

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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                        lalpha =      5.29482
                           rho =            0
                        beta_0 =      31.5112
                        beta_1 =     -1.97726
```

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-0.99	-0.029	0.044
rho	-0.99	1	0.026	-0.04
beta_0	-0.029	0.026	1	-0.94
beta_1	0.044	-0.04	-0.94	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-2.54215	1.65048	-5.77702	0.692726
rho	2.40985	0.541771	1.34799	3.4717
beta_0	31.2644	4.1929	23.0464	39.4823
beta_1	-1.9414	0.436071	-2.79609	-1.08672

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	31.7	31.3	20.6	17.8	0.0727
3.378	10	24.6	24.7	12	13.4	-0.0264
10.57	10	10.7	10.8	5.33	4.91	-0.0362

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-92.841935	4	193.683870
A2	-85.255316	6	182.510632
A3	-85.429148	5	180.858295
fitted	-85.606998	4	179.213995
R	-98.136607	2	200.273213

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)

```
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              25.7626          4          <.0001
   Test 2              15.1732          2       0.0005072
   Test 3             0.347663          1          0.5554
   Test 4               0.3557          1          0.5509

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

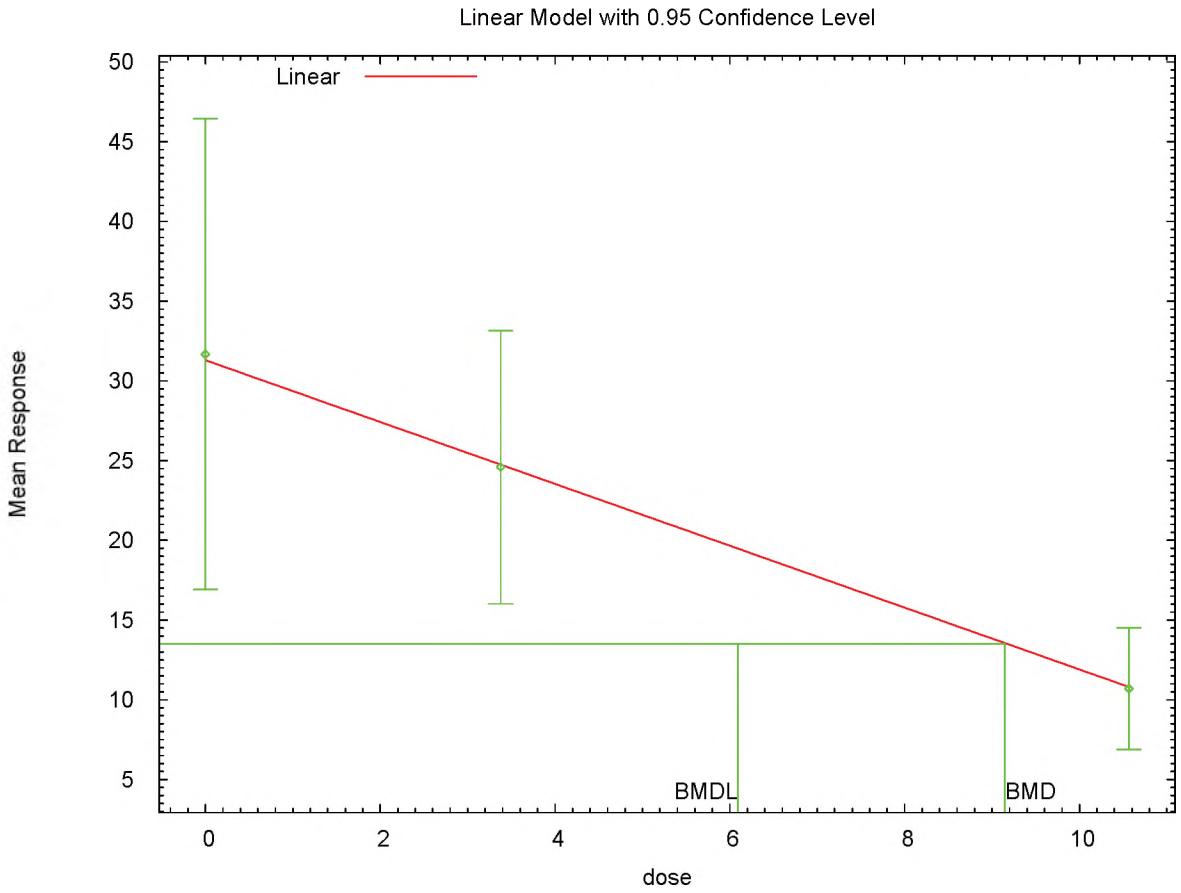
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD =        9.14709


             BMDL =        6.09414
```

E.2.1.3. *Figure for Selected Model: Linear*



E.2.1.4. *Output for Additional Model Presented: Power, Unrestricted*

Amin et al., 2000: 0.25% Saccharin Consumed, Female

```
 ================================================================
          Power Model. (Version: 2.15;  Date: 04/07/2008)
          Input Data File: C:\1\Blood\1_Amin_2000_25_SC_Pwr_U_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\1_Amin_2000_25_SC_Pwr_U_1.plt
                                              Mon Feb 08 10:44:22 2010
 ================================================================

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    The form of the response function is:

    Y[dose] = control + slope * dose^power


    Dependent variable = Mean
    Independent variable = Dose
    The power is not restricted
    The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
```

```
   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         lalpha =      5.29482
                            rho =            0
                        control =      31.6727
                          slope =      -2.2195
                          power =     0.952715


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho      control        slope        power

    lalpha            1        -0.99         0.34        -0.17       -0.061

       rho        -0.99            1        -0.42         0.19        0.068

   control         0.34        -0.42            1        -0.72        -0.56

     slope        -0.17         0.19        -0.72            1         0.97

     power       -0.061        0.068        -0.56         0.97            1


                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha         -2.48291          2.08669           -6.57274             1.60693
            rho          2.38455         0.692047            1.02817             3.74094
        control            32.99          5.40754            22.3914             43.5886
          slope         -3.91099          3.83883            -11.435             3.61299
          power         0.735877         0.350669          0.0485775             1.42318


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    10       31.7           33         20.6         18.7         -0.223
3.378    10       24.6         23.4           12         12.4          0.302
10.57    10       10.7         10.8         5.33         4.94          -0.08

 Warning: Likelihood for fitted model larger than the Likelihood for model A3.


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user
```

```
Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1          -92.841935           4     193.683870
            A2          -85.255316           6     182.510632
            A3          -85.429148           5     180.858295
         fitted         -85.429148           5     180.858295
             R          -98.136607           2     200.273213


                  Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              25.7626          4          <.0001
   Test 2              15.1732          2        0.0005072
   Test 3             0.347663          1           0.5554
   Test 4         -8.2423e-013          0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


               Benchmark Dose Computation

Specified effect =            1

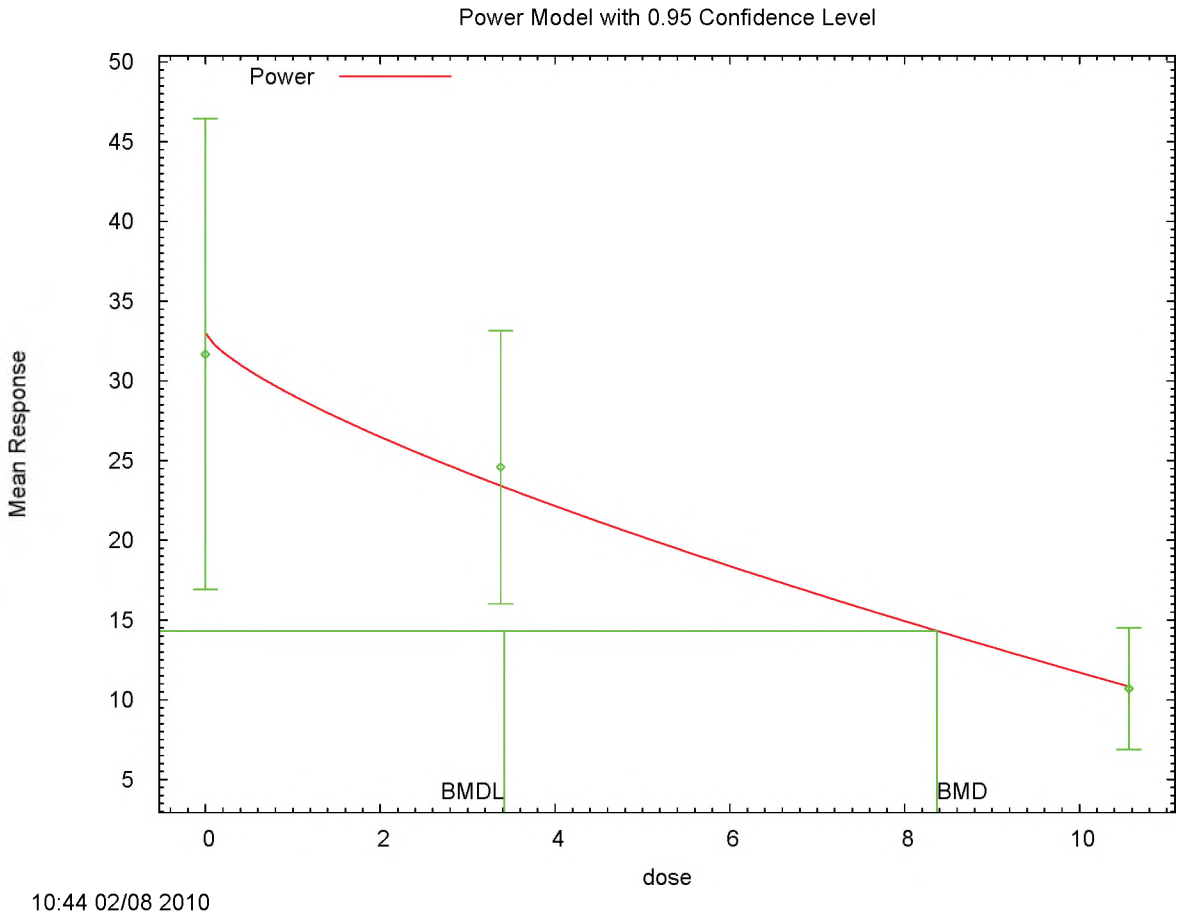
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 8.36678


            BMDL = 3.41906
```

E.2.1.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.2.2. Amin et al., 2000: 0.25% Saccharin Preference Ratio, Female

E.2.2.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
linear [b]	**1**	**0.002**	**227.807**	**1.162E+01**	**5.572E+00**	
polynomial, 2-degree	1	0.002	227.807	1.162E+01	5.572E+00	
power	1	0.002	227.807	1.162E+01	5.572E+00	power bound hit (power = 1)

[a] Non-constant variance model selected ($p = 0.0135$)
[b] Best-fitting model, BMDS output presented in this appendix

E.2.2.2. *Output for Selected Model: Linear*

Amin et al., 2000: 0.25% Saccharin Preference Ratio, Female

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\Blood\2_Amin_2000_25_SP_Linear_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\2_Amin_2000_25_SP_Linear_1.plt
                                                Mon Feb 08 10:44:49 2010
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                        lalpha =      6.34368
                           rho =            0
                        beta_0 =      75.4888
                        beta_1 =     -2.24733


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho       beta_0       beta_1

    lalpha            1           -1         0.22        -0.31

       rho           -1            1        -0.22         0.31

    beta_0         0.22        -0.22            1        -0.77

    beta_1        -0.31         0.31        -0.77            1


                              Parameter Estimates

                                                    95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha          3.00523           9.2122           -15.0503            21.0608
            rho         0.797764          2.21138           -3.53646            5.13199
         beta_0          75.1087          6.74312            61.8924            88.3249
         beta_1         -2.16469          1.00825           -4.14082          -0.188553


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------
```

```
    0    10       82.1         75.1         13.3         25.2          0.884
3.378    10       58.1         67.8         33.9         24.2          -1.27
10.57    10       54.9         52.2         19.5         21.8          0.383


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                       Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1         -108.574798            4     225.149597
              A2         -104.269377            6     220.538754
              A3         -105.147952            5     220.295903
          fitted         -109.903705            4     227.807410
               R         -112.382522            2     228.765045


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              16.2263          4         0.00273
   Test 2              8.61084          2          0.0135
   Test 3              1.75715          1           0.185
   Test 4              9.51151          1        0.002042

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


             Benchmark Dose Computation

Specified effect =            1
```

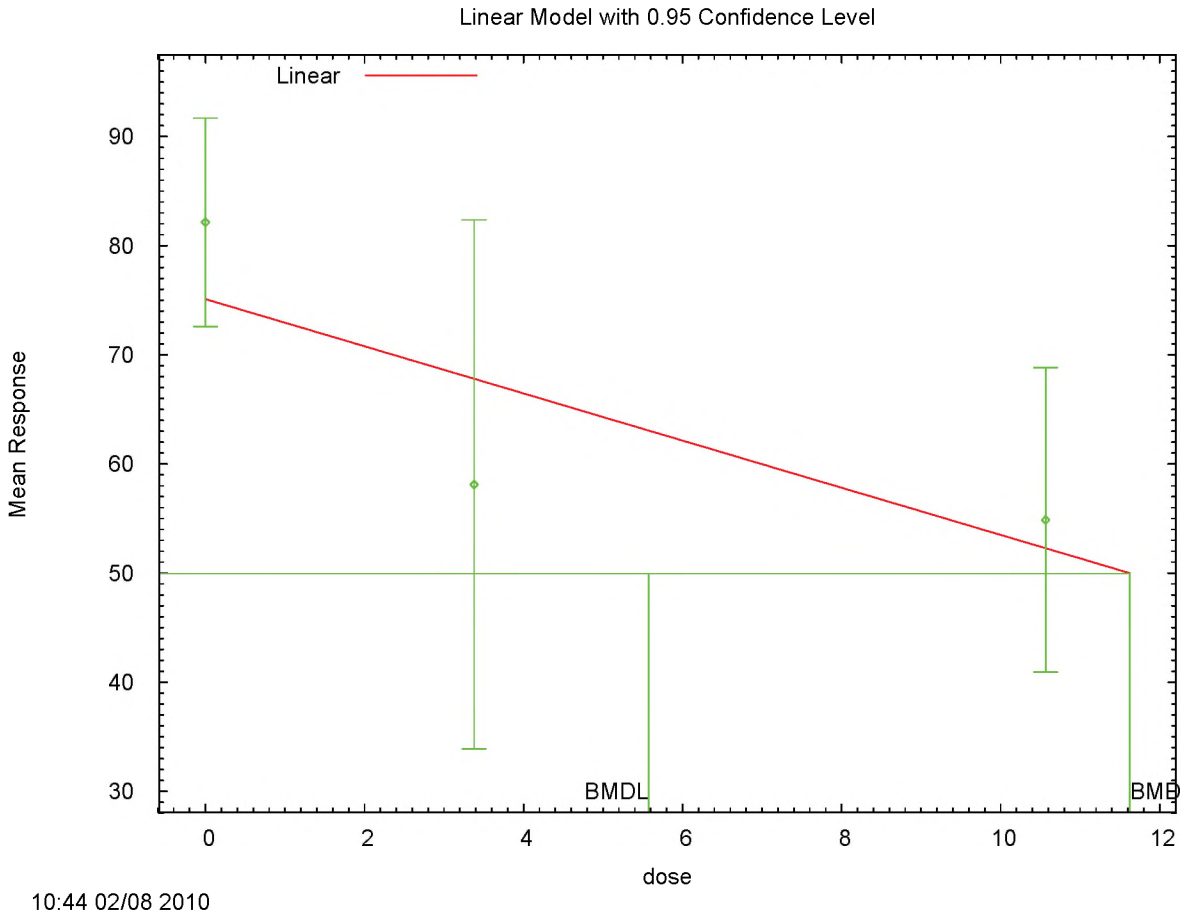
```
Risk Type          =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD =        11.6241


             BMDL =        5.57215
```

E.2.2.3. *Figure for Selected Model: Linear*



E.2.3. Amin et al., 2000: 0.50% Saccharin Consumed, Female

E.2.3.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
linear [b]	**1**	**0.060**	**158.591**	**1.016E+01**	**6.567E+00**	
polynomial, 2-degree	1	0.060	158.591	1.016E+01	6.567E+00	
power	1	0.060	158.591	1.016E+01	6.567E+00	power bound hit (power = 1)
power, unrestricted [c]	0	N/A	157.060	6.567E+00	1.155E+00	unrestricted (power = 0.396)

[a] Non-constant variance model selected (p = <0.0001)
[b] Best-fitting model, BMDS output presented in this appendix
[c] Alternate model, BMDS output also presented in this appendix

E.2.3.2. *Output for Selected Model: Linear*

Amin et al., 2000: 0.50% Saccharin Consumed, Female

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\Blood\3_Amin_2000_50_SC_Linear_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\3_Amin_2000_50_SC_Linear_1.plt
                                                Mon Feb 08 10:45:20 2010
 ================================================================

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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                        lalpha =      4.68512
                           rho =            0
                        beta_0 =      20.0631
                        beta_1 =     -1.57142


          Asymptotic Correlation Matrix of Parameter Estimates
```

	lalpha	rho	beta_0	beta_1
lalpha	1	-0.96	0.019	-0.0016
rho	-0.96	1	-0.031	0.015
beta_0	0.019	-0.031	1	-0.96
beta_1	-0.0016	0.015	-0.96	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-0.982115	0.982262	-2.90731	0.943084
rho	2.11808	0.401166	1.33181	2.90435
beta_0	18.6171	3.1782	12.3879	24.8462
beta_1	-1.33226	0.322037	-1.96344	-0.70108

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	22.4	18.6	16	13.5	0.873
3.378	10	11.4	14.1	7.66	10.1	-0.856
10.57	10	4.54	4.54	3.33	3.04	-0.00339

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-83.696404	4	175.392808
A2	-73.511830	6	159.023660
A3	-73.530233	5	157.060467
fitted	-75.295363	4	158.590726
R	-90.294746	2	184.589492

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	33.5658	4	<.0001
Test 2	20.3691	2	<.0001
Test 3	0.0368066	1	0.8479
Test 4	3.53026	1	0.06026

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation

Specified effect = 1

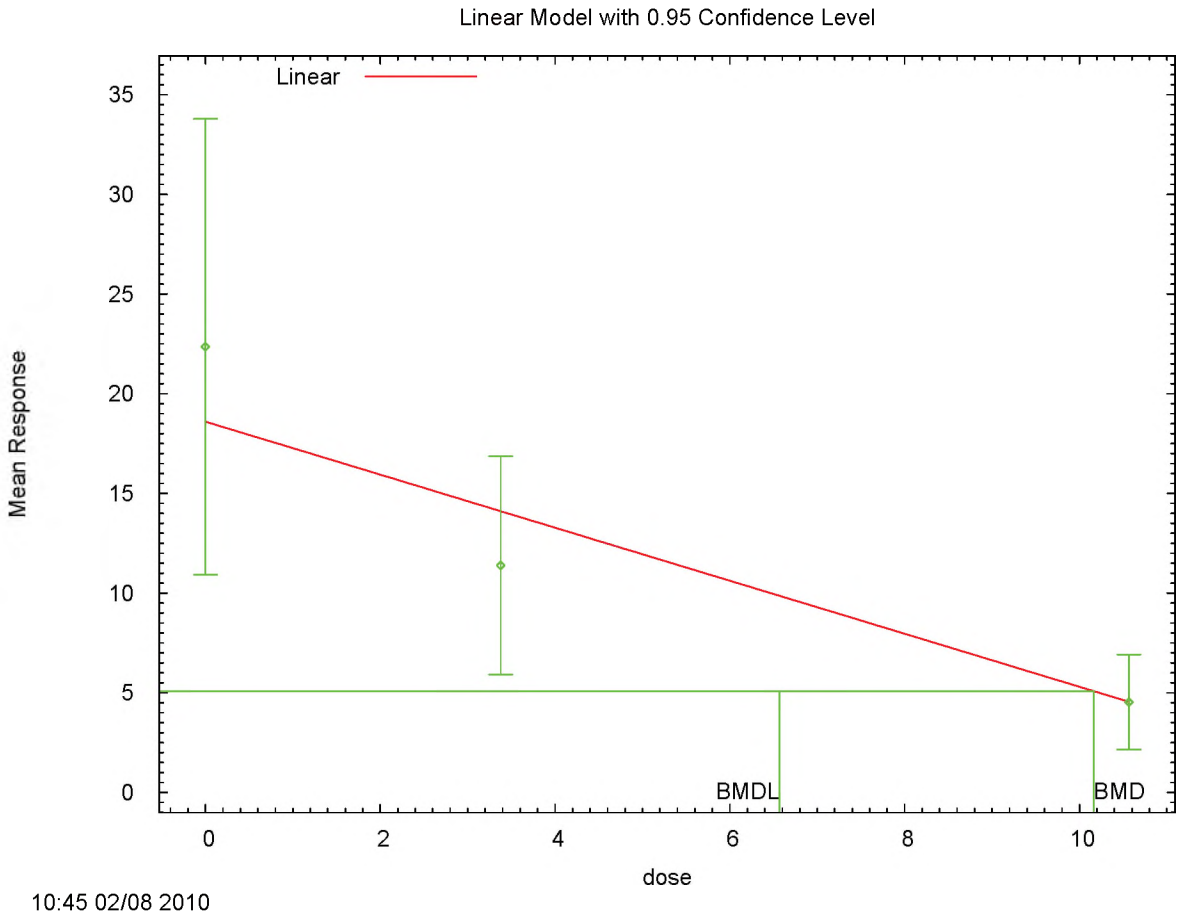
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 10.1633

BMDL = 6.56742

E.2.3.3. *Figure for Selected Model: Linear*



E.2.3.4. *Output for Additional Model Presented: Power, Unrestricted*

Amin et al., 2000: 0.50% Saccharin Consumed, Female

```
================================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\1\Blood\3_Amin_2000_50_SC_Pwr_U_1.(d)
        Gnuplot Plotting File:  C:\1\Blood\3_Amin_2000_50_SC_Pwr_U_1.plt
                                                Mon Feb 08 10:45:20 2010
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  The form of the response function is:

  Y[dose] = control + slope * dose^power


  Dependent variable = Mean
  Independent variable = Dose
  The power is not restricted
  The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
```

```
   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =      4.68512
                            rho =            0
                        control =      22.3564
                          slope =     -6.53901
                          power =     0.425213


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho      control        slope        power

    lalpha            1        -0.96         0.34        -0.31        -0.15

       rho        -0.96            1        -0.47         0.36         0.15

   control         0.34        -0.47            1        -0.81        -0.52

     slope        -0.31         0.36        -0.81            1         0.92

     power        -0.15         0.15        -0.52         0.92            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha        -0.708629            1.298          -3.25267             1.83541
            rho          1.96142         0.529653           0.923323             2.99953
        control          22.6293          4.48416            13.8405             31.4181
          slope         -7.10123          4.04394           -15.0272            0.824743
          power         0.395571         0.168677          0.0649698            0.726173


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    10       22.4         22.6           16           15        -0.0577
3.378    10       11.4         11.1         7.66         7.46          0.105
10.57    10       4.54         4.58         3.33         3.12        -0.0475

Degrees of freedom for Test A3 vs fitted <= 0


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
      Model A3 uses any fixed variance parameters that
      were specified by the user
```

```
Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1          -83.696404           4     175.392808
            A2          -73.511830           6     159.023660
            A3          -73.530233           5     157.060467
         fitted         -73.530233           5     157.060467
             R          -90.294746           2     184.589492


                  Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              33.5658          4          <.0001
   Test 2              20.3691          2          <.0001
   Test 3            0.0368066          1          0.8479
   Test 4                    0          0             NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


               Benchmark Dose Computation

Specified effect =            1

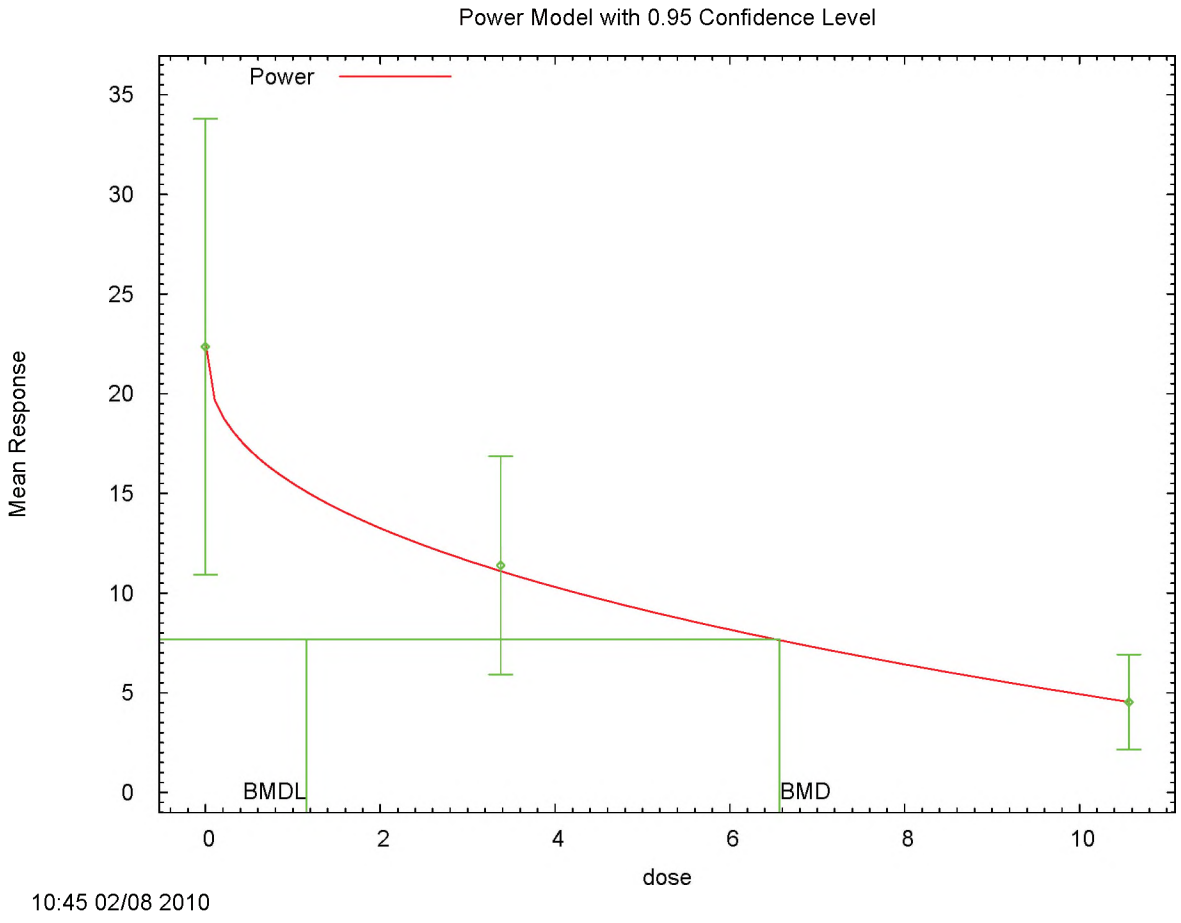
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 6.56719


            BMDL = 1.15476
```

E.2.3.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.2.4. Amin et al., 2000: 0.50% Saccharin Preference Ratio, Female

E.2.4.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
linear [b]	**1**	**0.135**	**234.250**	**8.144E+00**	**5.105E+00**	
polynomial, 2-degree	1	0.135	234.250	8.144E+00	5.105E+00	
power	1	0.135	234.250	8.144E+00	5.105E+00	power bound hit (power = 1)
power, unrestricted [c]	0	N/A	234.020	2.598E+00	1.057E-14	unrestricted (power = 0.282)

[a] Constant variance model selected ($p = 0.5593$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.4.2. *Output for Selected Model: Linear*

Amin et al., 2000: 0.50% Saccharin Preference Ratio, Female

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\Blood\4_Amin_2000_50_SP_LinearCV_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\4_Amin_2000_50_SP_LinearCV_1.plt
                                                  Mon Feb 08 10:45:50 2010
 ================================================================

 -
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                         alpha =      764.602
                           rho =            0   Specified
                        beta_0 =      65.8627
                        beta_1 =     -3.34297
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	2.6e-008	2.1e-009
beta_0	2.6e-008	1	-0.73
beta_1	2.1e-009	-0.73	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	741.255	191.391	366.135	1116.38
beta_0	65.8627	7.22524	51.7015	80.0239
beta_1	-3.34297	1.12815	-5.55412	-1.13183

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	72.7	65.9	24.6	27.2	0.797
3.378	10	44.5	54.6	32.9	27.2	-1.17
10.57	10	33.8	30.5	24.6	27.2	0.375

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-113.009921	4	234.019841
A2	-112.428886	6	236.857773
A3	-113.009921	4	234.019841
fitted	-114.125184	3	234.250368
R	-117.976057	2	239.952114

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)

```
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              11.0943          4         0.02552
   Test 2              1.16207          2          0.5593
   Test 3              1.16207          2          0.5593
   Test 4              2.23053          1          0.1353

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

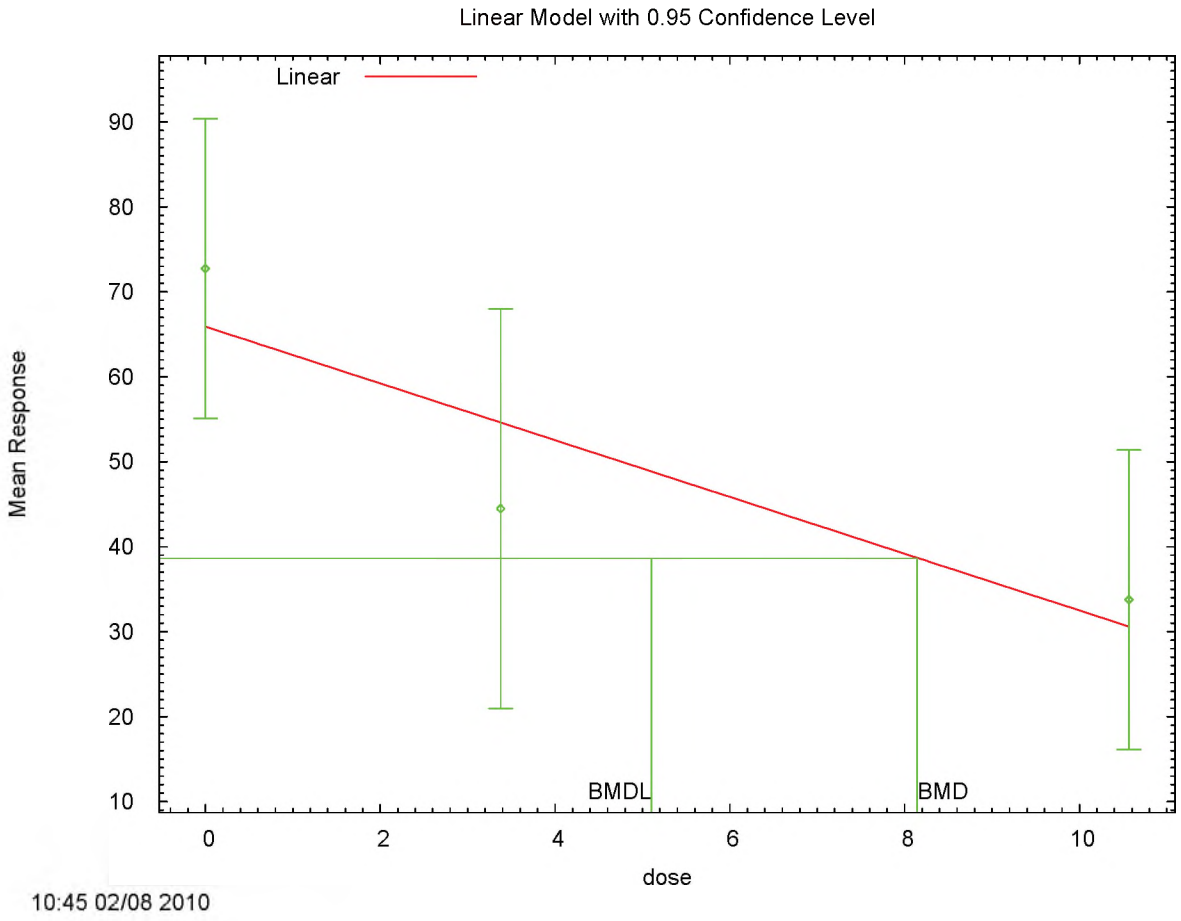
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD =        8.14425


             BMDL =        5.10523
```

E.2.4.3. *Figure for Selected Model: Linear*



E.2.4.4. *Output for Additional Model Presented: Power, Unrestricted*

Amin et al., 2000: 0.50% Saccharin Preference Ratio, Female

```
================================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\1\Blood\4_Amin_2000_50_SP_PwrCV_U_1.(d)
        Gnuplot Plotting File:  C:\1\Blood\4_Amin_2000_50_SP_PwrCV_U_1.plt
                                                 Mon Feb 08 10:45:50 2010
================================================================================

-
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = control + slope * dose^power


  Dependent variable = Mean
  Independent variable = Dose
  rho is set to 0
  The power is not restricted
  A constant variance model is fit
```

```
   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                         alpha =      764.602
                           rho =            0   Specified
                       control =      72.7273
                         slope =     -20.0402
                         power =     0.281985


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha      control        slope        power

    alpha             1    -1.2e-009    -1.2e-009    -2.2e-010

  control    -1.2e-009            1        -0.51        -0.22

    slope    -1.2e-009        -0.51            1         0.92

    power    -2.2e-010        -0.22         0.92            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         alpha          688.142          177.677              339.9             1036.38
       control          72.7273          8.29543             56.4686              88.986
         slope         -20.0402          15.0576            -49.5526             9.47219
         power         0.281985         0.325861            -0.35669            0.920661


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    10       72.7         72.7         24.6         26.2      4.67e-009
3.378    10       44.5         44.5         32.9         26.2      1.52e-008
10.57    10       33.8         33.8         24.6         26.2      1.77e-008

 Warning: Likelihood for fitted model larger than the Likelihood for model A3.


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2
```

```
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -113.009921            4     234.019841
              A2         -112.428886            6     236.857773
              A3         -113.009921            4     234.019841
          fitted         -113.009921            4     234.019841
               R         -117.976057            2     239.952114


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              11.0943          4         0.02552
   Test 2              1.16207          2          0.5593
   Test 3              1.16207          2          0.5593
   Test 4        -2.84217e-014          0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


               Benchmark Dose Computation

Specified effect =             1

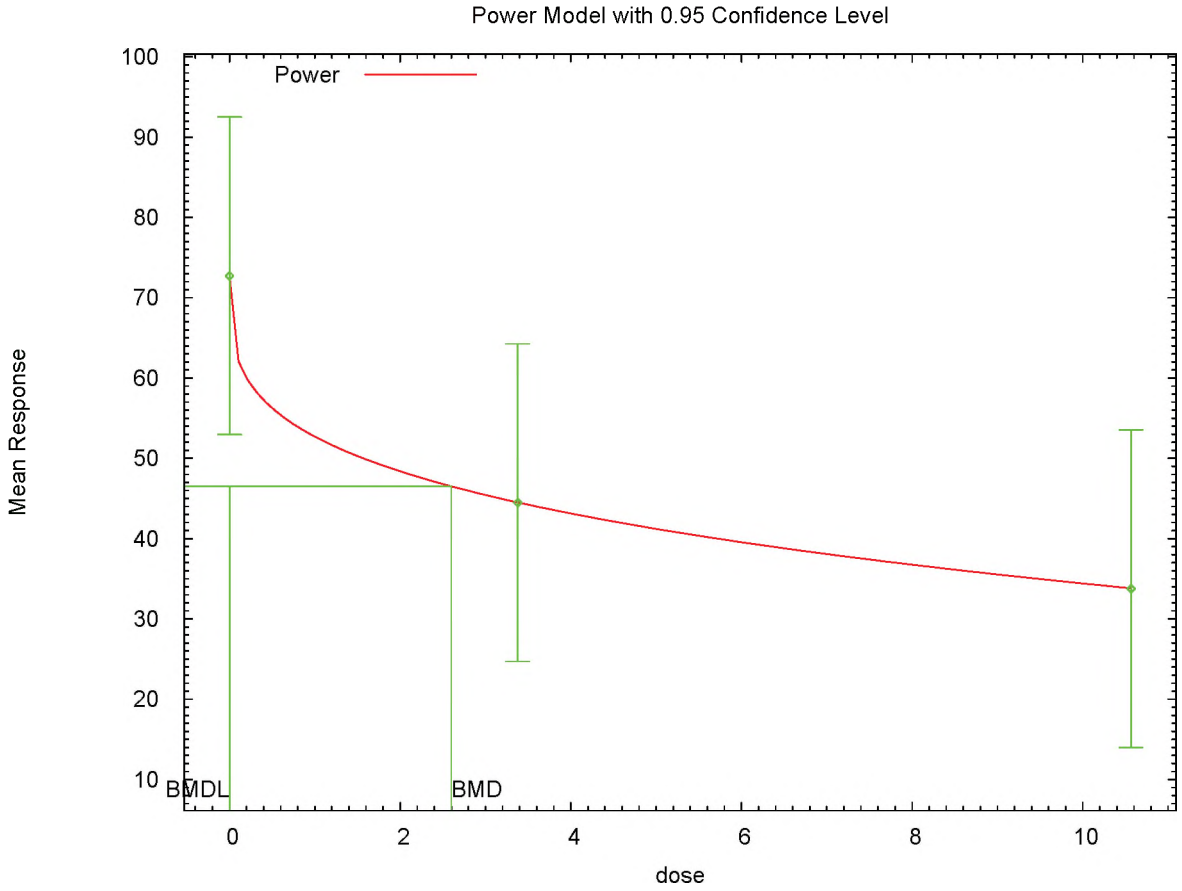
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 2.59831


            BMDL = 1.05661e-014
```

E.2.4.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.2.5. Bell et al., 2007a: Balano-Preputial Separation, Postnatal Day 49

E.2.5.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	2	0.684	112.136	2.867E+00	1.943E+00	power bound hit (power = 1)
logistic	2	0.342	113.915	6.159E+00	4.746E+00	negative intercept (intercept = -2.246)
log-logistic [a]	**2**	**0.777**	**111.908**	**2.246E+00**	**1.394E+00**	**slope bound hit (slope = 1)**
log-probit	2	0.269	114.254	5.322E+00	3.512E+00	slope bound hit (slope = 1)
multistage, 3-degree	2	0.684	112.136	2.867E+00	1.943E+00	final ß = 0
probit	2	0.367	113.713	5.715E+00	4.422E+00	
Weibull	2	0.684	112.136	2.867E+00	1.943E+00	power bound hit (power = 1)
gamma, unrestricted	1	0.566	113.746	1.862E+00	1.829E-01	unrestricted (power = 0.741)
log-logistic, unrestricted [b]	1	0.501	113.871	1.998E+00	2.795E-01	unrestricted (slope = 0.93)
log-probit, unrestricted	1	0.456	113.977	2.038E+00	3.250E-01	unrestricted (slope = 0.54)
Weibull, unrestricted	1	0.551	113.771	1.914E+00	2.346E-01	unrestricted (power = 0.795)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.2.5.2. *Output for Selected Model: Log-Logistic*

Bell et al., 2007a: Balano-Preputial Separation, Postnatal Day 49

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\Blood\5_Bell_2007_BPS_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\5_Bell_2007_BPS_LogLogistic_1.plt
                                                Mon Feb 08 10:46:18 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
```

```
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0333333
                     intercept =     -2.99896
                         slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.49

 intercept        -0.49            1



                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background         0.038005            *               *                   *
      intercept         -3.00658            *               *                   *
          slope                1            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -53.7077         4
   Fitted model          -53.954         2     0.492596      2         0.7817
  Reduced model         -63.9797         1       20.544      3        0.0001309

           AIC:          111.908


                                 Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ---------------------------------------------------------------------
    0.0000     0.0380         1.140     1.000          30        -0.134
    2.2040     0.1326         3.977     5.000          30         0.551
    5.1378     0.2329         6.988     6.000          30        -0.427
   18.4110     0.4965        14.895    15.000          30         0.038

 Chi^2 = 0.50      d.f. = 2        P-value = 0.7769


   Benchmark Dose Computation

Specified effect =           0.1
```

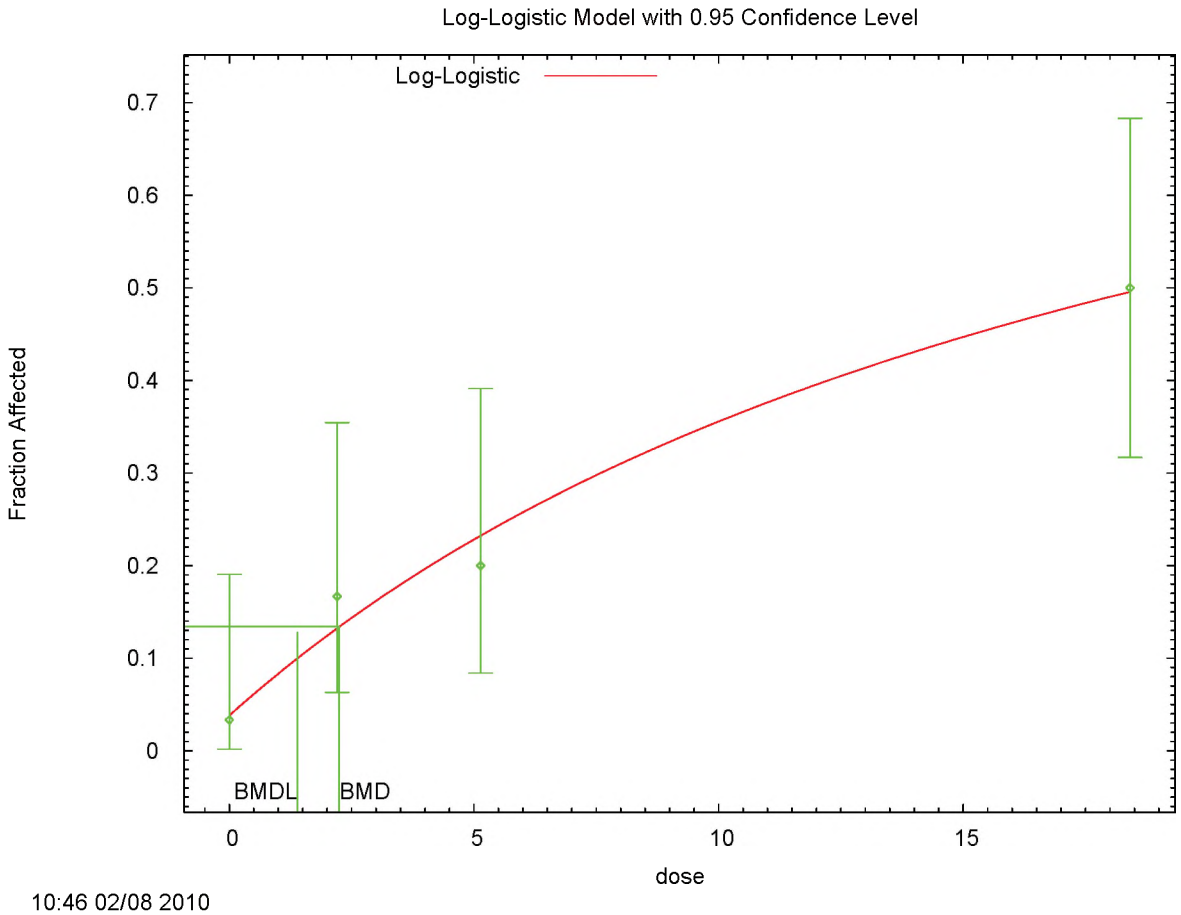
```
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        2.24647

            BMDL =        1.39385
```

E.2.5.3. *Figure for Selected Model: Log-Logistic*



E.2.5.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

Bell et al., 2007a: Balano-Preputial Separation, Postnatal Day 49

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\Blood\5_Bell_2007_BPS_LogLogistic_U_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\5_Bell_2007_BPS_LogLogistic_U_1.plt
                                                Mon Feb 08 10:46:18 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0333333
                     intercept =     -2.68464
                         slope =     0.858398


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.48         0.35

 intercept        -0.48            1        -0.94

     slope         0.35        -0.94            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    background        0.0353402            *                *                   *
     intercept         -2.84051            *                *                   *
         slope         0.929645            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -53.7077         4
```

Fitted model	-53.9354	3	0.455534	1	0.4997
Reduced model	-63.9797	1	20.544	3	0.0001309

AIC: 113.871

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0353	1.060	1.000	30	-0.060
2.2040	0.1400	4.201	5.000	30	0.420
5.1378	0.2389	7.166	6.000	30	-0.499
18.4110	0.4858	14.573	15.000	30	0.156

Chi^2 = 0.45 d.f. = 1 P-value = 0.5005

Benchmark Dose Computation

Specified effect = 0.1

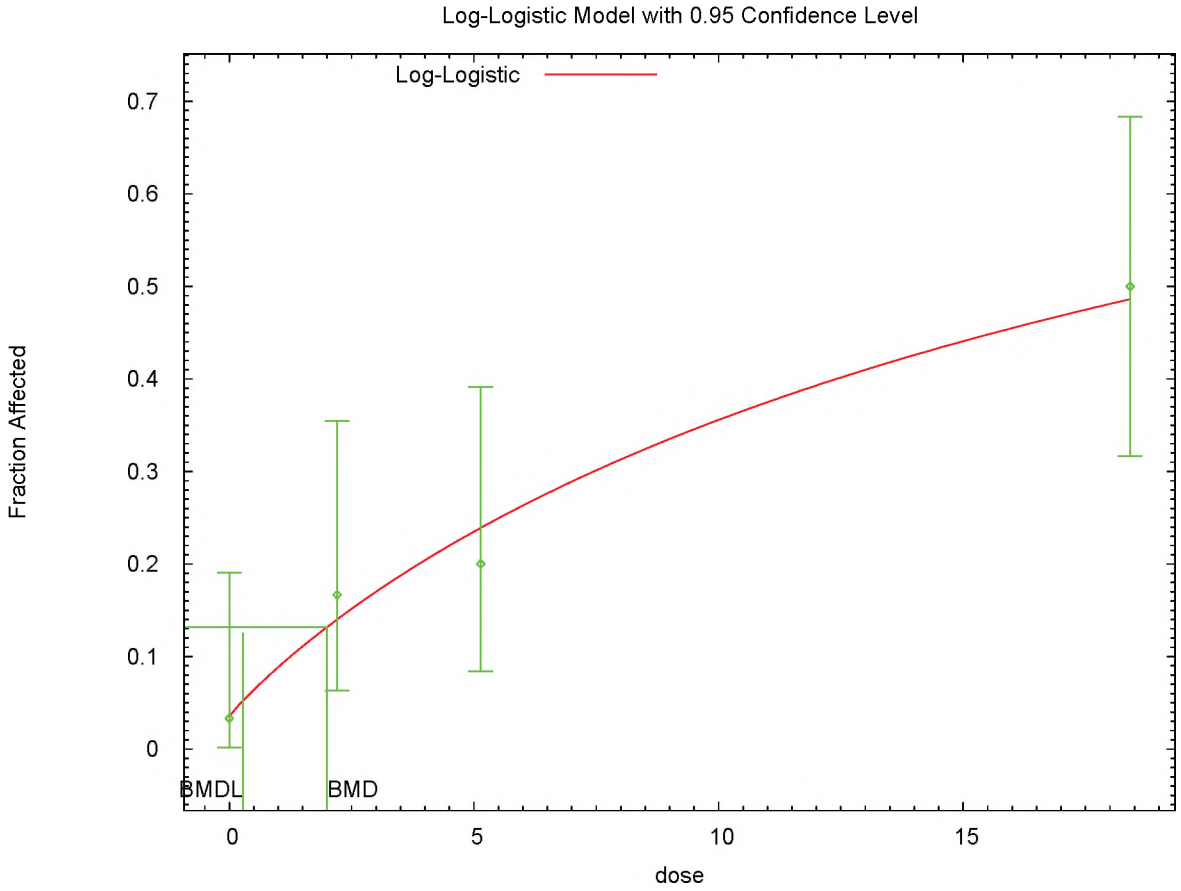
Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.99765

BMDL = 0.279534

E.2.5.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.2.6. Cantoni et al., 1981: Urinary Coproporhyrins, 3 Months

E.2.6.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	0.003	32.882	3.209E+01	1.567E+01	
exponential (M3)	2	0.003	32.882	3.209E+01	1.567E+01	power hit bound (d = 1)
exponential (M4) [b]	**1**	**0.486**	**23.459**	**5.339E-01**	**1.803E-01**	
exponential (M5)	1	0.486	23.459	5.339E-01	1.803E-01	power hit bound (d = 1)
Hill	1	0.788	23.047	4.333E-01	error	n lower bound hit (n = 1)
linear	2	0.005	31.595	1.464E+01	2.753E+00	
polynomial, 3-degree	2	0.005	31.595	1.464E+01	2.753E+00	
power	2	0.005	31.595	1.464E+01	2.753E+00	power bound hit (power = 1)
power, unrestricted [c]	1	0.610	23.235	2.766E-02	2.031E-05	unrestricted (power = 0.304)
Hill, unrestricted	0	N/A	24.974	2.602E-01	error	unrestricted (n = 0.739)

[a] Non-constant variance model selected ($p = 0.0039$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.6.2. *Output for Selected Model: Exponential (M4)*

Cantoni et al., 1981: Urinary Coproporhyrins, 3 Months

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\6_Cantoni_1981_UriCopro_Exp_1.(d)
         Gnuplot Plotting File:
                                                Mon Feb 08 10:46:46 2010
 ================================================================

 Figure1-UrinaryCoproporphyrin_3months
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
```

```
      sign = -1 for decreasing trend.

   Model 2 is nested within Models 3 and 4.
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


              Initial Parameter Values

              Variable          Model 4
              --------          --------
                lnalpha             -1.50063
                    rho              2.60979
                      a             0.704303
                      b            0.0604961
                      c              4.47268
                      d                    1



                Parameter Estimates

               Variable          Model 4
               --------          -------
                lnalpha            -1.75302
                    rho              2.6322
                      a            0.761218
                      b            0.241561
                      c             4.15597
                      d                   1


        Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0      4       0.7414       0.3475
  1.847      4        1.807       0.8341
  8.839      4        2.734        1.506
  50.05      4            3          2.6


              Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0        0.7612       0.2907          -0.1366
  1.847         1.626       0.7892           0.4588
  8.839          2.88        1.674          -0.1743
  50.05         3.164        1.895          -0.1725


Other models for which likelihoods are calculated:
```

```
  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                 Model      Log(likelihood)      DF         AIC
                -------    -----------------    ----   ------------
                    A1       -12.90166           5      35.80333
                    A2       -6.203643           8      28.40729
                    A3       -6.487204           6      24.97441
                     R       -15.73713           2      35.47427
                     4       -6.729737           5      23.45947


Additive constant for all log-likelihoods =      -14.7.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                          Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                        19.07           6            0.004052
  Test 2                         13.4           3            0.003854
  Test 3                       0.5671           2              0.7531
 Test 6a                       0.4851           1              0.4861


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

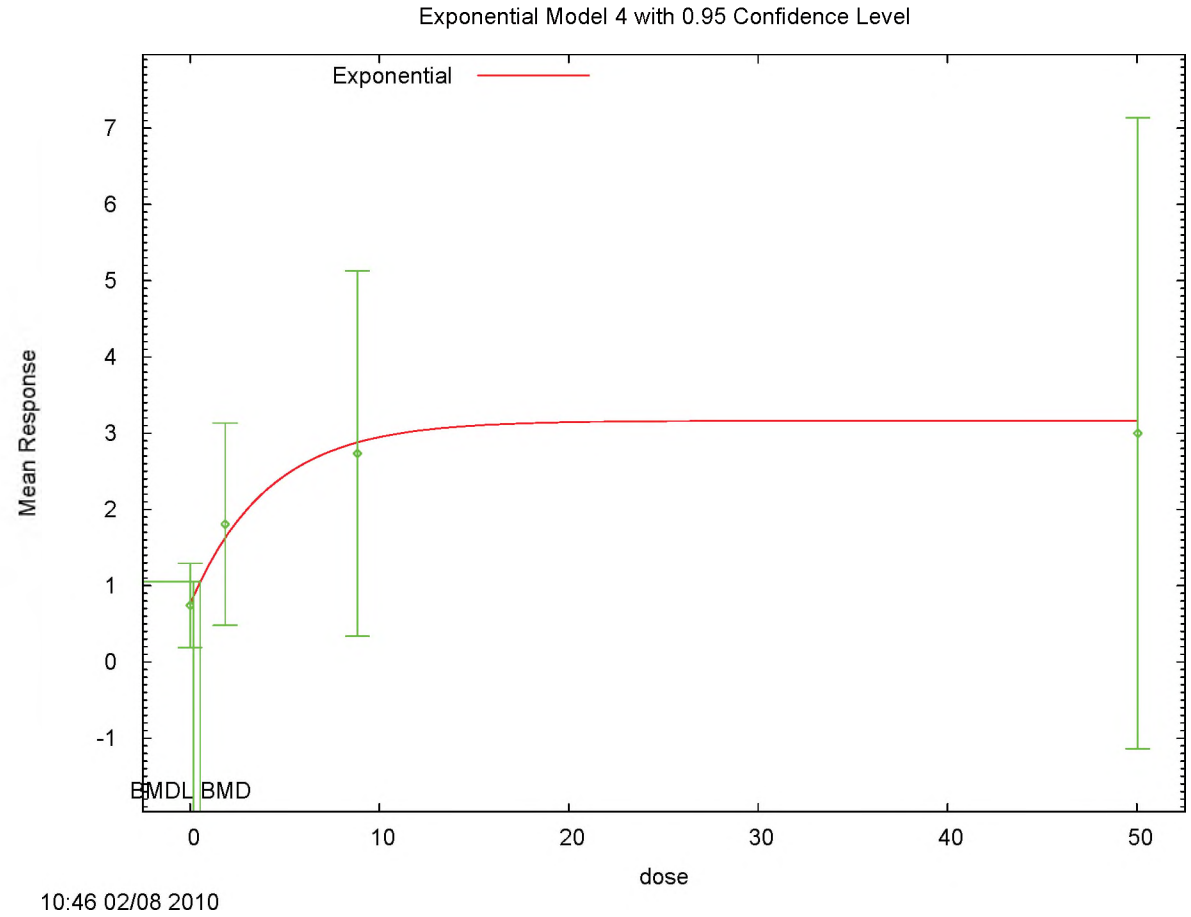
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000
```

```
BMD =      0.533855

BMDL =     0.180293
```

E.2.6.3. *Figure for Selected Model: Exponential (M4)*



E.2.6.4. *Output for Additional Model Presented: Power, Unrestricted*

Cantoni et al., 1981: Urinary Coproporhyrins, 3 Months

```
================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\1\Blood\6_Cantoni_1981_UriCopro_Pwr_U_1.(d)
        Gnuplot Plotting File:  C:\1\Blood\6_Cantoni_1981_UriCopro_Pwr_U_1.plt
                                          Mon Feb 08 10:46:47 2010
================================================================

Figure1-UrinaryCoproporphyrin_3months
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = control + slope * dose^power
```

Dependent variable = Mean
Independent variable = Dose
The power is not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	0.90039
rho =	0
control =	0.741372
slope =	0.93685
power =	0.224904

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.62	-0.53	-0.036	0.024
rho	-0.62	1	0.43	-0.2	-0.16
control	-0.53	0.43	1	-0.28	0.086
slope	-0.036	-0.2	-0.28	1	-0.77
power	0.024	-0.16	0.086	-0.77	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-1.78125	0.617807	-2.99213	-0.570373
rho	2.64332	0.744946	1.18325	4.10338
control	0.75678	0.139979	0.482426	1.03113
slope	0.845767	0.324854	0.209065	1.48247
power	0.304211	0.135053	0.0395119	0.568909

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	4	0.741	0.757	0.348	0.284	-0.109
1.847	4	1.81	1.78	0.834	0.877	0.0705
8.839	4	2.73	2.4	1.51	1.3	0.515
50.05	4	3	3.54	2.6	2.18	-0.493

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

```
Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -12.901663            5      35.803325
             A2           -6.203643            8      28.407287
             A3           -6.487204            6      24.974409
         fitted           -6.617347            5      23.234694
              R          -15.737135            2      35.474269


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1               19.067          6        0.004052
  Test 2               13.396          3        0.003854
  Test 3             0.567122          2          0.7531
  Test 4             0.260285          1          0.6099

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


               Benchmark Dose Computation

Specified effect =             1

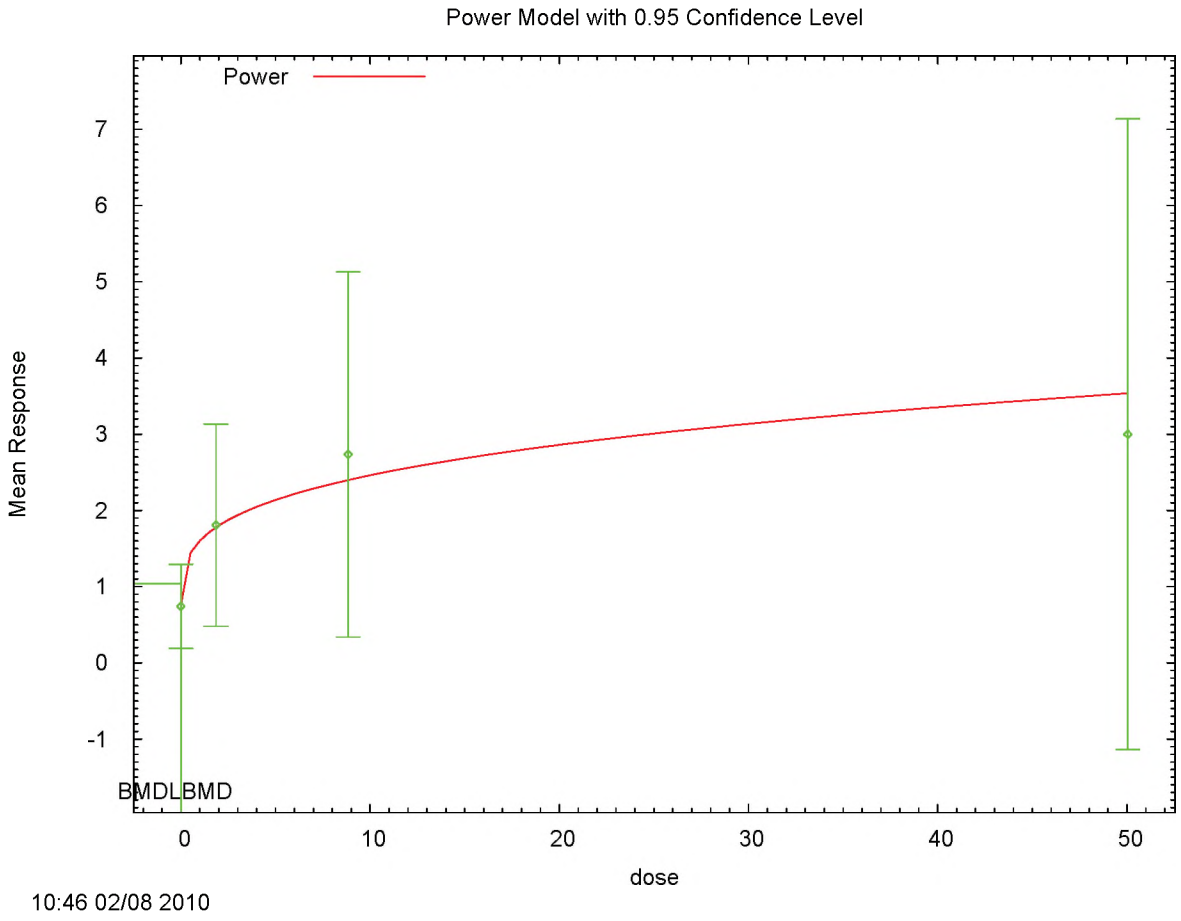
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 0.0276599


             BMDL = 2.03143e-005
```

E.2.6.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.2.7. Cantoni et al., 1981: Urinary Porphyrins

E.2.7.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2) [b]	2	<0.001	55.465	3.760E+00	2.762E+00	
exponential (M3)	2	<0.001	55.465	3.760E+00	2.762E+00	power hit bound (d = 1)
exponential (M4)	1	<0.0001	59.187	2.484E-01	1.448E-01	
exponential (M5)	0	N/A	61.084	2.878E-01	1.461E-01	
Hill	0	N/A	62.199	6.233E+00	3.341E+00	
linear	2	<0.001	57.187	2.484E-01	1.448E-01	
polynomial, 3-degree	1	<0.0001	10.000	error	error	
power	1	<0.0001	59.084	2.878E-01	1.461E-01	

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.7.2. *Output for Selected Model: Exponential (M2)*

Cantoni et al., 1981: Urinary Porphyrins

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\7_Cantoni_1981_UriPor_Exp_1.(d)
         Gnuplot Plotting File:
                                                  Mon Feb 08 10:47:24 2010
 ================================================================

 Table 1, dose converted to ng per kg per day
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
```

```
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


                Initial Parameter Values

                Variable          Model 2
                --------          --------
                  lnalpha          -3.57509
                      rho           2.23456
                        a           3.36453
                        b         0.0819801
                        c                 0
                        d                 1



                  Parameter Estimates

                 Variable          Model 2
                 --------          -------
                  lnalpha          -1.85879
                      rho           1.82273
                        a           3.57896
                        b         0.0803347
                        c                 0
                        d                 1


          Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0      4          2.27          0.49
  1.847      4          5.55          0.85
  8.839      3          7.62          1.79
  50.05      3         196.9         63.14


                Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0         3.579        1.262           -2.074
  1.847         4.152        1.445            1.936
  8.839          7.28         2.41           0.2441
  50.05         199.5        49.25         -0.09069


Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
```

```
  Model  R:        Yij = Mu + e(i)
            Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                   Model      Log(likelihood)      DF         AIC
                  -------    -----------------    ----   ------------
                      A1       -51.42175           5      112.8435
                      A2       -15.31211           8      46.62422
                      A3       -15.66963           6      43.33925
                       R       -68.75058           2      141.5012
                       2       -23.73254           4      55.46509


Additive constant for all log-likelihoods =     -12.87.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does Model 2 fit the data? (A3 vs. 2)


                          Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                        106.9            6           < 0.0001
  Test 2                        72.22            3           < 0.0001
  Test 3                        0.715            2             0.6994
  Test 4                        16.13            2           0.000315


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 4 is less than .1.  Model 2 may not adequately
  describe the data; you may want to consider another model.


Benchmark Dose Computations:

  Specified Effect = 1.000000

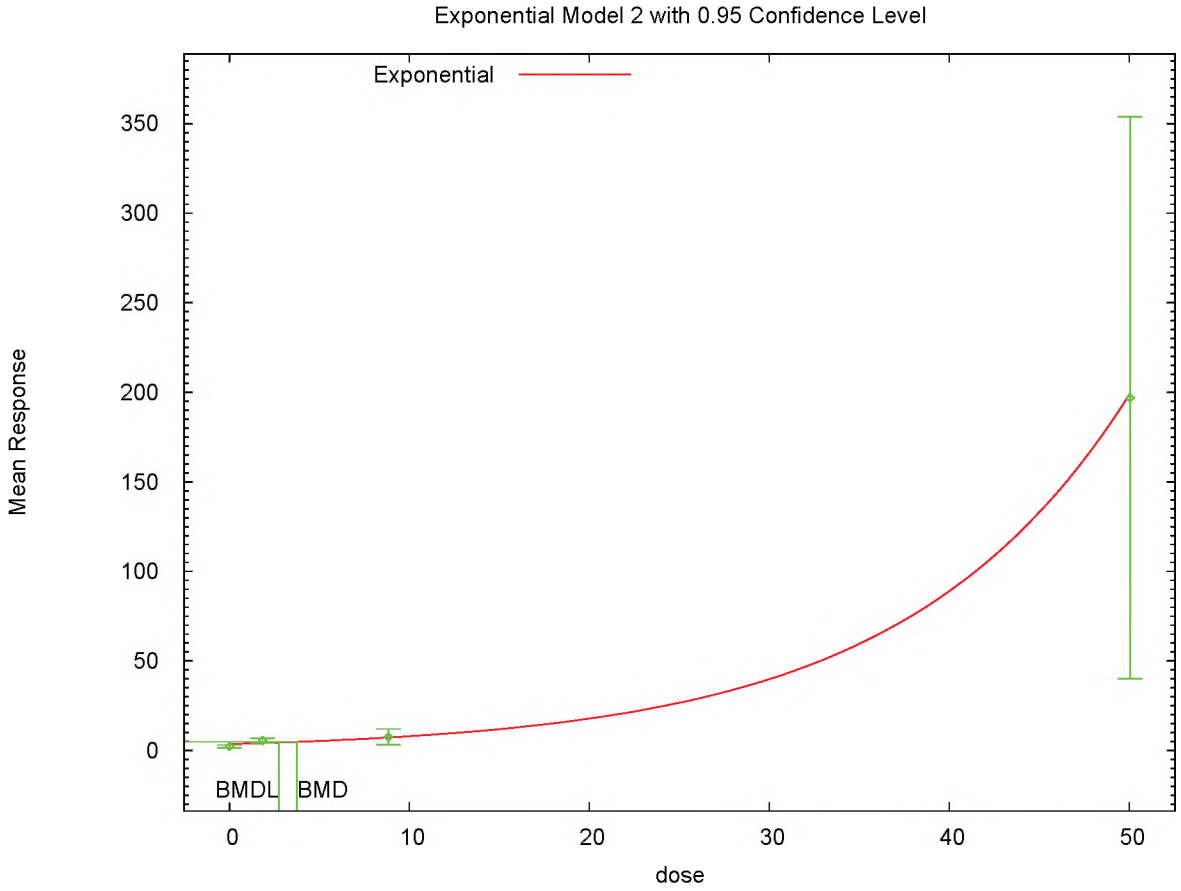
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      3.75968

              BMDL =      2.76247
```

E.2.7.3. *Figure for Selected Model: Exponential (M2)*



E.2.8. Crofton et al., 2005: Serum, T4

E.2.8.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	8	<0.0001	516.356	1.144E+02	6.239E+01	
exponential (M3)	8	<0.0001	516.356	1.144E+02	6.239E+01	power hit bound (d = 1)
exponential (M4) [b]	7	**0.942**	**476.449**	**5.190E+00**	**3.029E+00**	
exponential (M5)	6	0.912	478.234	5.757E+00	3.094E+00	
Hill	6	0.972	477.450	5.724E+00	3.024E+00	
linear	8	<0.0001	522.460	2.406E+02	1.761E+02	
polynomial, 8-degree	8	<0.0001	522.460	2.406E+02	1.761E+02	
power	8	<0.0001	522.460	2.406E+02	1.761E+02	power bound hit (power = 1)
power, unrestricted	7	0.018	491.101	2.449E+00	3.307E-01	unrestricted (power = 0.243)

[a] Constant variance model selected ($p = 0.7647$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.8.2. *Output for Selected Model: Exponential (M4)*

Crofton et al., 2005: Serum, T4

```
 ================================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\8_Crofton_2005_T4_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                    Mon Feb 08 10:48:04 2010
 ================================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 10
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	5.47437
rho(S)	0
a	104.999
b	0.00641895
c	0.445764
d	1

(S) = Specified

Parameter Estimates

Variable	Model 4
lnalpha	5.50623
rho	0
a	100.332
b	0.076678
c	0.523626
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	14	100	15.44
0.0202	6	96.27	14.98
0.4882	12	98.57	18.11
1.384	6	99.76	19.04
3.455	6	93.32	12.11
9.257	6	70.94	12.74
23.07	6	62.52	14.75
65.65	6	52.68	22.73
180.9	6	54.66	19.71
583.5	4	49.15	11.15

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	100.3	15.69	-0.07952
0.0202	100.3	15.69	-0.6231
0.4882	98.58	15.69	-0.000744
1.384	95.52	15.69	0.6614
3.455	89.21	15.69	0.6422

```
   9.257         76.04         15.69          -0.7962
   23.07         60.69         15.69           0.2854
   65.65         52.85         15.69         -0.02621
   180.9         52.54         15.69           0.3319
   583.5         52.54         15.69          -0.4323


Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
                Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                  Model      Log(likelihood)      DF         AIC
                 -------    -----------------    ----   ------------
                     A1       -233.0774           11      488.1549
                     A2       -230.2028           20      500.4056
                     A3       -233.0774           11      488.1549
                      R       -268.4038            2      540.8076
                      4       -234.2243            4      476.4486


Additive constant for all log-likelihoods =     -66.16.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                              Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                          Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                        76.4            18            < 0.0001
  Test 2                       5.749             9              0.7647
  Test 3                       5.749             9              0.7647
 Test 6a                       2.294             7              0.9418


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.
```

```
Benchmark Dose Computations:

  Specified Effect = 1.000000

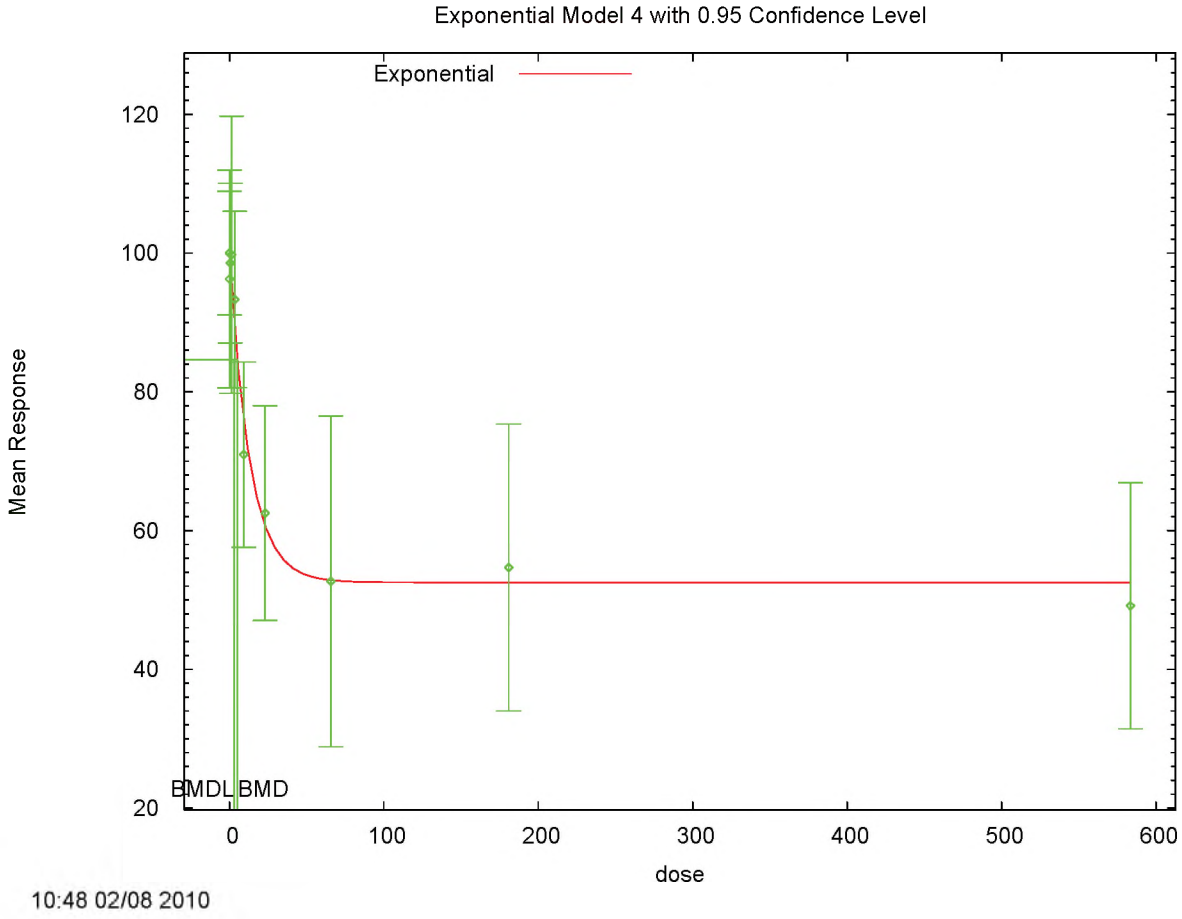
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      5.18983

              BMDL =      3.02894
```

E.2.8.3. *Figure for Selected Model: Exponential (M4)*



E.2.9. Franc et al., 2001: S-D Rats, Relative Liver Weight

E.2.9.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	0.968	234.369	7.800E+00	6.040E+00	
exponential (M3)	1	0.880	236.327	9.201E+00	6.051E+00	
exponential (M4)	1	0.580	236.610	6.365E+00	4.512E+00	
exponential (M5)	0	N/A	238.346	9.474E+00	4.425E+00	
Hill	0	N/A	238.346	9.479E+00	3.004E+00	
linear	2	0.858	234.610	6.365E+00	4.512E+00	
polynomial, 3-degree	1	0.935	236.311	8.946E+00	4.598E+00	
power [b]	1	0.839	236.346	9.474E+00	4.587E+00	

[a] Constant variance model selected ($p = 0.107$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.9.2. *Output for Selected Model: Power*

Franc et al., 2001: S-D Rats, Relative Liver Weight

```
 ======================================================================
          Power Model. (Version: 2.15;  Date: 04/07/2008)
          Input Data File: C:\1\Blood\88_Franc_2001_SD_RelLivWt_PowerCV_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\88_Franc_2001_SD_RelLivWt_PowerCV_1.plt
                                                Thu Apr 15 11:46:32 2010
 ======================================================================

 Figure 5, SD rats, relative liver weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The power is restricted to be greater than or equal to 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	527.447	
rho =	0	Specified
control =	100	
slope =	0.947018	
power =	1.13144	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	control	slope	power
alpha	1	-6.3e-009	5.4e-009	-4.7e-009
control	-6.3e-009	1	-0.74	0.71
slope	5.4e-009	-0.74	1	-1
power	-4.7e-009	0.71	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	462.113	115.528	235.682	688.544
control	100.494	7.31114	86.1645	114.824
slope	0.593276	1.31535	-1.98476	3.17131
power	1.25841	0.597816	0.086712	2.43011

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	100	100	14	21.5	-0.065
6.587	8	108	107	16.9	21.5	0.158
14.48	8	117	118	25.9	21.5	-0.109
36.43	8	155	155	30.9	21.5	0.0157

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-114.152281	5	238.304562
A2	-111.103649	8	238.207299
A3	-114.152281	5	238.304562
fitted	-114.172940	4	236.345880
R	-125.052064	2	254.104127

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	27.8968	6	<.0001
Test 2	6.09726	3	0.107
Test 3	6.09726	3	0.107
Test 4	0.0413179	1	0.8389

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 0.1

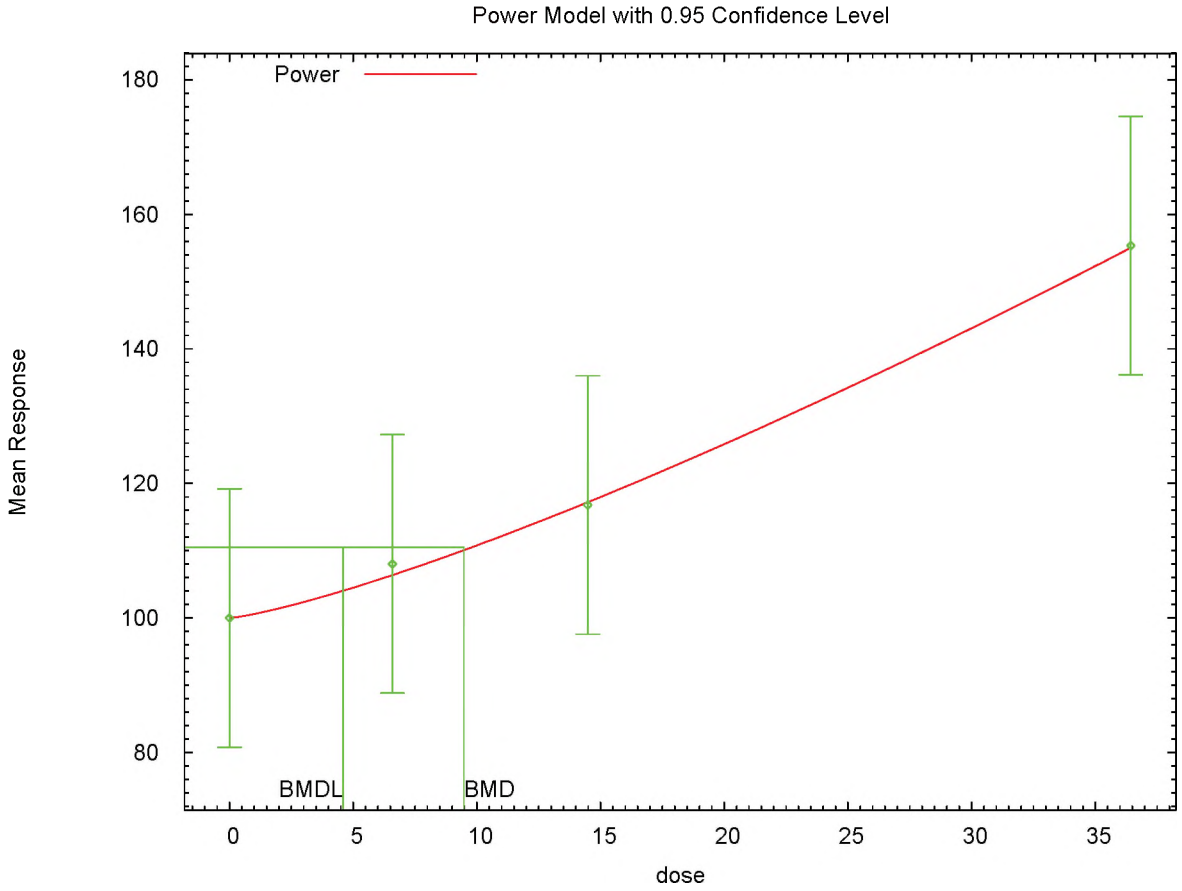
Risk Type = Relative risk

Confidence level = 0.95

BMD = 9.47408

BMDL = 4.5873

E.2.9.3. *Figure for Selected Model: Power*



E.2.10. Franc et al., 2001: L-E Rats, Relative Liver Weight

E.2.10.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	0.441	208.974	1.708E+01	1.098E+01	
exponential (M3)	2	0.441	208.974	1.708E+01	1.098E+01	power hit bound (d = 1)
exponential (M4)	1	0.785	209.408	7.997E+00	2.601E+00	
exponential (M5)	1	0.785	209.408	7.997E+00	2.601E+00	power hit bound (d = 1)
Hill [b]	1	0.829	209.381	7.725E+00	1.225E+00	n lower bound hit (n = 1)
linear	2	0.499	208.725	1.570E+01	9.619E+00	
polynomial, 3-degree	1	<0.0001	10.000	8.604E+00	error	
power	2	0.499	208.725	1.570E+01	9.619E+00	power bound hit (power = 1)
Hill, unrestricted [c]	0	N/A	211.337	7.217E+00	1.147E+00	unrestricted (n = 0.545)
power, unrestricted	1	0.965	209.336	7.193E+00	error	unrestricted (power = 0.524)

[a] Non-constant variance model selected ($p = 0.0632$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.10.2. *Output for Selected Model: Hill*

Franc et al., 2001: L-E Rats, Relative Liver Weight

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\Blood\89_Franc_2001_LE_RelLivWt_Hill_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\89_Franc_2001_LE_RelLivWt_Hill_1.plt
                                                Thu Apr 15 11:48:44 2010
 ================================================================

 Figure 5, L-E rats, relative liver weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
```

```
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                       lalpha =      5.41581
                          rho =            0
                    intercept =          100
                            v =       22.225
                            n =     0.443155
                            k =       18.746


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -n
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 lalpha          rho    intercept            v            k

    lalpha            1           -1        -0.21         0.33         0.18

       rho           -1            1         0.21        -0.33        -0.18

 intercept        -0.21         0.21            1        0.028         0.35

         v         0.33        -0.33        0.028            1         0.91

         k         0.18        -0.18         0.35         0.91            1


                                 Parameter Estimates

                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha         -17.2754          17.3066           -51.1957             16.6449
            rho          4.77884          3.67625           -2.42648             11.9842
      intercept          99.5348          3.61286            92.4538             106.616
              v          36.3963          24.1862           -11.0079             83.8004
              n                1               NA
              k          20.5223          28.2566           -34.8596             75.9042

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     8        100         99.5           10         10.5          0.125
6.584     8        106          108         17.9         12.9         -0.455
14.47     8        117          115         8.97         14.8          0.426
36.41     8        122          123         19.9         17.4        -0.0954


 Model Descriptions for likelihoods calculated
```

```
Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                     Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
             A1         -100.516456           5     211.032912
             A2          -96.870820           8     209.741641
             A3          -99.666984           6     211.333969
         fitted          -99.690373           5     209.380746
              R         -105.717087           2     215.434174


                  Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              17.6925          6        0.007048
  Test 2              7.29127          3         0.06317
  Test 3              5.59233          2         0.06104
  Test 4            0.0467774          1          0.8288

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =           0.1

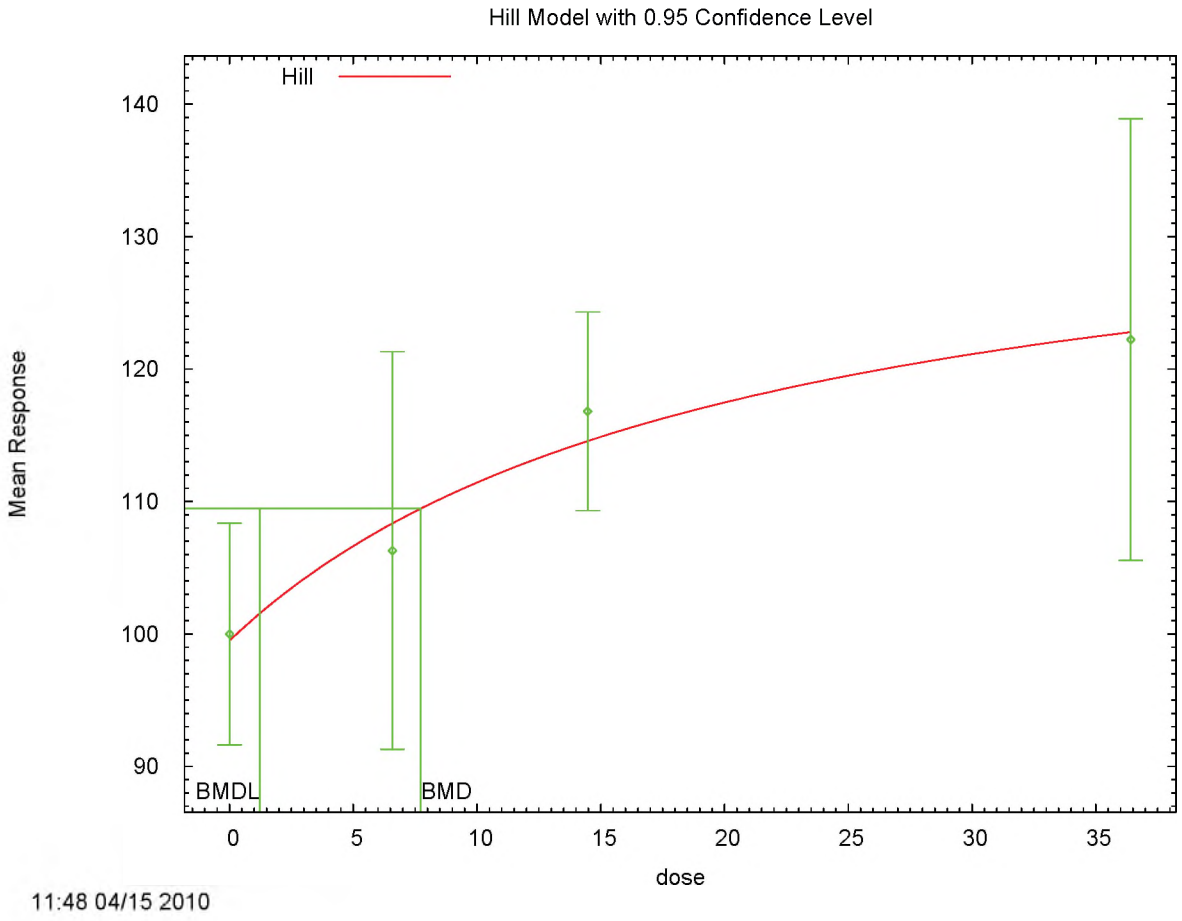
Risk Type        =     Relative risk

Confidence level =           0.95

             BMD =        7.72492

            BMDL =       1.22451
```

E.2.10.3. *Figure for Selected Model: Hill*



E.2.10.4. *Output for Additional Model Presented: Hill, Unrestricted*

Franc et al., 2001: L-E Rats, Relative Liver Weight

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\Blood\89_Franc_2001_LE_RelLivWt_Hill_U_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\89_Franc_2001_LE_RelLivWt_Hill_U_1.plt
                                              Thu Apr 15 11:48:50 2010
 ================================================================

 Figure 5, L-E rats, relative liver weight
------------------------------------------------------------------------

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 4
```

```
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         lalpha =      5.41581
                            rho =            0
                      intercept =          100
                              v =       22.225
                              n =     0.443155
                              k =       18.746


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho    intercept            v            n            k

    lalpha            1           -1        -0.22        -0.14         0.24        -0.15

       rho           -1            1         0.22         0.14        -0.24         0.15

 intercept        -0.22         0.22            1        0.022         0.11        0.013

         v        -0.14         0.14        0.022            1        -0.9            1

         n         0.24        -0.24         0.11         -0.9            1        -0.92

         k        -0.15         0.15        0.013            1        -0.92            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha         -19.2405           18.21          -54.9315             16.4505
            rho          5.19575         3.86861          -2.38657             12.7781
      intercept          99.5348         3.51796           92.6398              106.43
              v          440.285         13708.5          -26427.9             27308.5
              n         0.544741        0.730981         -0.887956             1.97744
              k          7266.27          485402            -944104              958637


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     8        100         99.5           10         10.3          0.128
6.584     8        106          109         17.9           13         -0.589
14.47     8        117          114         8.97         14.6          0.558
36.41     8        122          123         19.9         17.8        -0.0957

Degrees of freedom for Test A3 vs fitted <= 0


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2
```

```
Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -100.516456            5     211.032912
             A2          -96.870820            8     209.741641
             A3          -99.666984            6     211.333969
         fitted          -99.668321            6     211.336641
              R         -105.717087            2     215.434174


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              17.6925          6        0.007048
  Test 2              7.29127          3         0.06317
  Test 3              5.59233          2         0.06104
  Test 4           0.00267242          0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


        Benchmark Dose Computation

Specified effect =           0.1

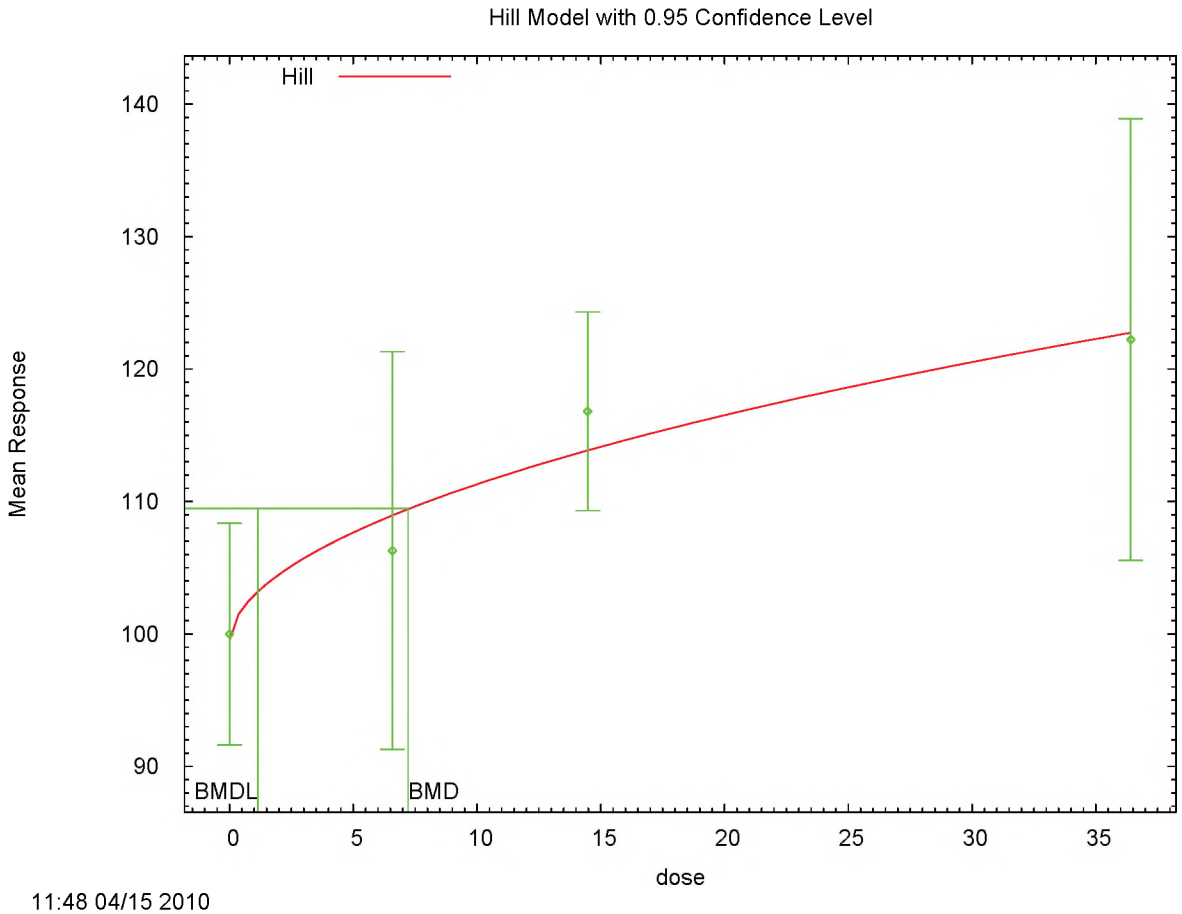
Risk Type        =     Relative risk

Confidence level =           0.95

             BMD =        7.21718

            BMDL =       1.14742
```

E.2.10.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.2.11. Franc et al., 2001: S-D Rats, Relative Thymus Weight

E.2.11.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	0.814	285.107	2.478E+00	1.535E+00	
exponential (M3)	1	0.016	292.452	3.173E+01	1.007E+00	
exponential (M4) [b]	1	0.720	286.825	1.878E+00	9.221E-01	
exponential (M5)	0	N/A	288.696	3.296E+00	9.365E-01	
Hill	0	N/A	288.696	3.625E+00	6.199E-01	
linear	2	0.404	286.508	4.783E+00	3.893E+00	
polynomial, 3-degree [c]	2	0.404	286.508	4.783E+00	3.893E+00	
power	2	0.404	286.508	4.783E+00	3.893E+00	power bound hit (power = 1)
power, unrestricted	1	0.483	287.189	6.795E-01	3.271E-03	unrestricted (power = 0.515)

[a] Non-constant variance model selected ($p = 0.0320$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.11.2. *Output for Selected Model: Exponential (M4)*

Franc et al., 2001: S-D Rats, Relative Thymus Weight

```
 ================================================================
          Exponential Model. (Version: 1.61;  Date: 7/24/2009)
          Input Data File: C:\1\Blood\91_Franc_2001_SD_RelThyWt_Exp_1.(d)
          Gnuplot Plotting File:
                                                  Thu Apr 15 11:51:19 2010
 ================================================================

 Figure 5, SD rats, relative thymus weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
```

```
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


                Initial Parameter Values

                Variable          Model 4
                --------          --------
                  lnalpha             3.35464
                      rho             1.08199
                        a                 105
                        b           0.0569979
                        c            0.108531
                        d                   1



                  Parameter Estimates

                 Variable          Model 4
                 --------          -------
                  lnalpha             2.4312
                      rho            1.28672
                        a            110.959
                        b          0.0663498
                        c           0.146486
                        d                  1


         Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0      8          100          83.2
  6.587      8         91.17         47.97
  14.48      8         51.41         43.48
  36.43      8         22.79         29.98


                Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0           111         69.78          -0.4442
  6.587         77.43         55.36           0.7019
  14.48         52.49         43.11          -0.0709
  36.43          24.7         26.54          -0.2031


Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
```

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                   Model      Log(likelihood)      DF         AIC
                  -------    ------------------    ----   ------------
                      A1       -141.9834            5      293.9669
                      A2       -137.5818            8      291.1637
                      A3       -138.3482            6      288.6964
                       R       -146.9973            2      297.9946
                       4       -138.4123            5      286.8245


Additive constant for all log-likelihoods =     -29.41.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                        18.83            6           0.004459
  Test 2                        8.803            3            0.03203
  Test 3                        1.533            2             0.4647
 Test 6a                       0.1282            1             0.7203


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 0.100000

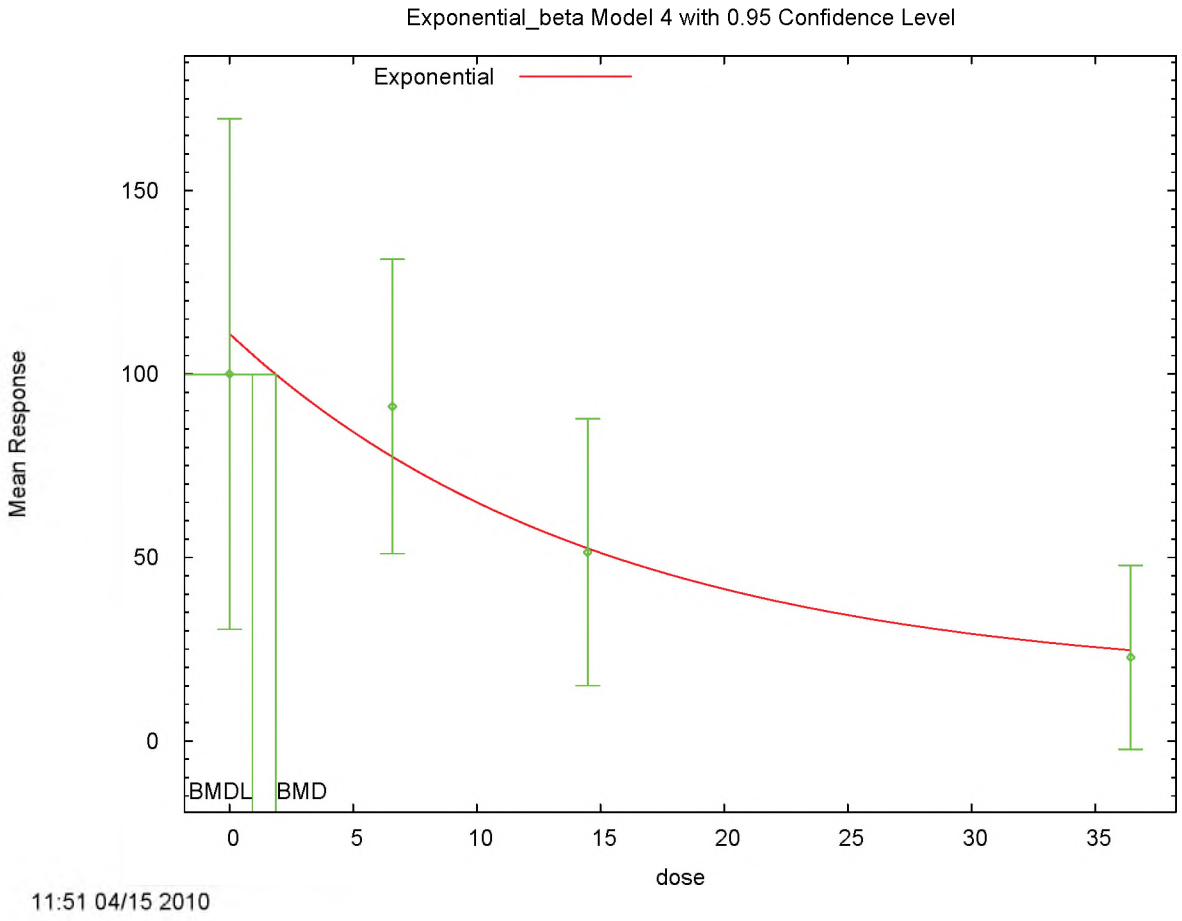
         Risk Type = Relative deviation

  Confidence Level = 0.950000

               BMD =      1.87814

              BMDL =     0.922136
```

E.2.11.3. *Figure for Selected Model: Exponential (M4)*



E.2.11.4. *Output for Additional Model Presented: Polynomial, 3-degree*

Franc et al., 2001: S-D Rats, Relative Thymus Weight

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\Blood\91_Franc_2001_SD_RelThyWt_Poly_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\91_Franc_2001_SD_RelThyWt_Poly_1.plt
                                                 Thu Apr 15 11:51:20 2010
 ================================================================

 Figure 5, SD rats, relative thymus weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   The polynomial coefficients are restricted to be negative
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
```

```
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         lalpha =       8.0075
                            rho =            0
                         beta_0 =          100
                         beta_1 =            0
                         beta_2 =    -0.475283
                         beta_3 =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -beta_2    -beta_3
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 lalpha          rho       beta_0       beta_1

    lalpha            1        -0.99        0.018       0.0095

       rho        -0.99            1       -0.022      -0.0024

    beta_0         0.018       -0.022            1        -0.87

    beta_1        0.0095      -0.0024        -0.87            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha           2.8315          1.71297          -0.525852             6.18885
            rho          1.19884         0.416889           0.381756             2.01593
         beta_0          94.5944          14.6685            65.8446             123.344
         beta_1         -1.97776         0.509904           -2.97715           -0.978362
         beta_2                0               NA
         beta_3                0               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     8        100          94.6         83.2           63          0.243
6.587     8       91.2          81.6           48         57.6          0.471
14.48     8       51.4            66         43.5         50.7         -0.811
36.43     8       22.8          22.5           30         26.7         0.0269


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
```

```
            Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
             Var{e(i)} = Sigma^2


                        Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1         -141.983433            5     293.966865
              A2         -137.581833            8     291.163667
              A3         -138.348184            6     288.696368
          fitted         -139.254163            4     286.508326
               R         -146.997301            2     297.994602


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                      Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              18.8309          6        0.004459
   Test 2               8.8032          3         0.03203
   Test 3               1.5327          2          0.4647
   Test 4              1.81196          2          0.4041

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =           0.1

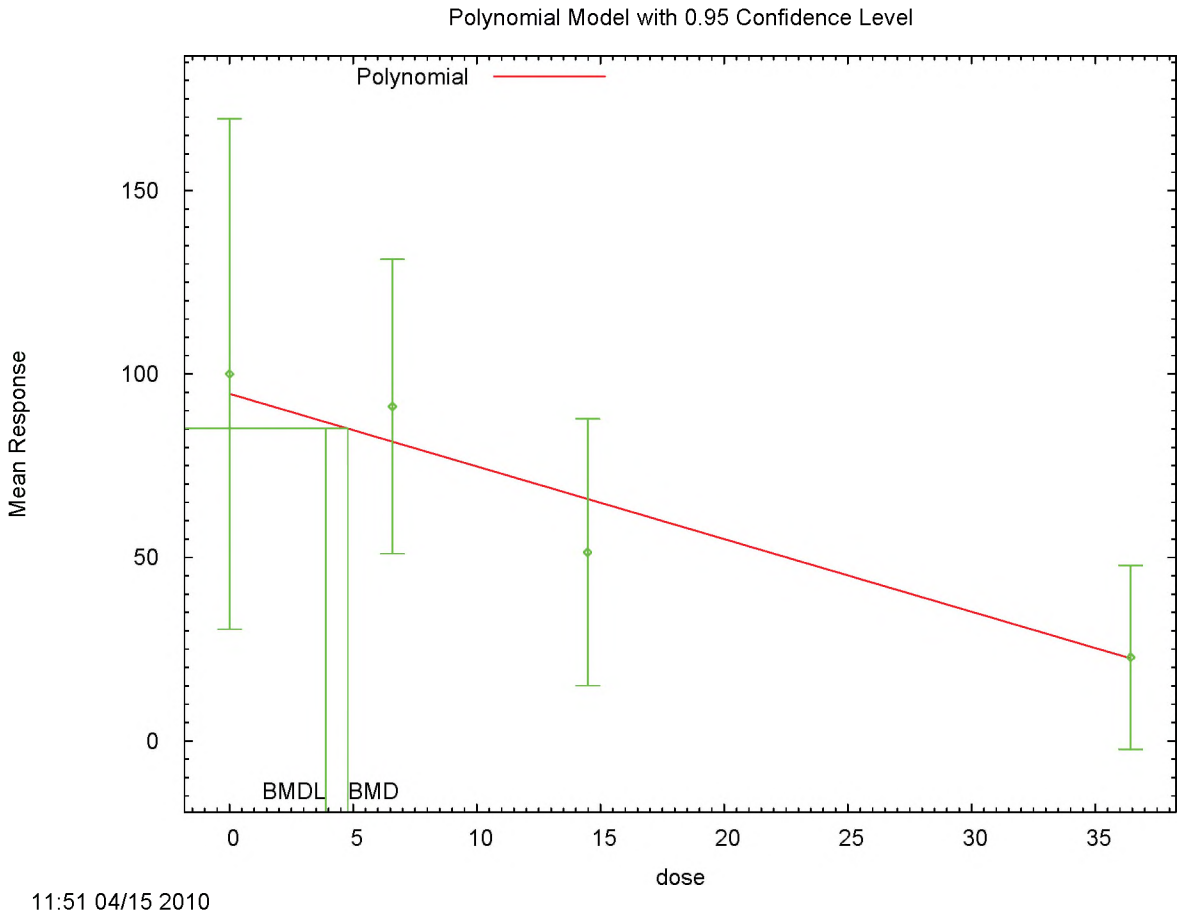
Risk Type        =     Relative risk

Confidence level =          0.95

             BMD =        4.78292


             BMDL =         3.8932
```

E.2.11.5. *Figure for Additional Model Presented: Polynomial, 3-degree*



E.2.12. Franc et al., 2001: L-E Rats, Relative Thymus Weight

E.2.12.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	0.440	301.449	2.726E+00	1.212E+00	
exponential (M3)	2	0.440	301.449	2.726E+00	1.212E+00	power hit bound (d = 1)
exponential (M4) [b]	1	0.227	303.266	2.084E+00	5.926E-01	
exponential (M5)	0	N/A	303.805	7.859E+00	9.801E-01	
Hill	0	N/A	303.805	7.480E+00	7.512E-01	
linear	2	0.304	302.186	5.045E+00	3.349E+00	
polynomial, 3-degree	2	0.304	302.186	5.045E+00	3.349E+00	
power	2	0.304	302.186	5.045E+00	3.349E+00	power bound hit (power = 1)
power, unrestricted	1	0.168	303.710	1.374E+00	9.032E-09	unrestricted (power = 0.601)

[a] Constant variance model selected ($p = 0.5063$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.12.2. *Output for Selected Model: Exponential (M4)*

Franc et al., 2001: L-E Rats, Relative Thymus Weight

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\92_Franc_2001_LE_RelThyWt_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                Thu Apr 15 11:53:37 2010
 ================================================================

 Figure 5, L-E rats, relative thymus weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	8.1814
rho(S)	0
a	105
b	0.0506168
c	0.166582
d	1

(S) = Specified

Parameter Estimates

Variable	Model 4
lnalpha	8.22706
rho	0
a	105.977
b	0.0660042
c	0.221786
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	8	100	54.72
6.584	8	95.41	70.46
14.47	8	38.69	47.97
36.41	8	34.98	77.96

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	106	61.16	-0.2764
6.584	76.91	61.16	0.8555
14.47	55.24	61.16	-0.765
36.41	30.96	61.16	0.186

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                 Model      Log(likelihood)      DF         AIC
                -------    -----------------    ----   ------------
                   A1       -146.9024            5      303.8049
                   A2       -145.7361            8      307.4723
                   A3       -146.9024            5      303.8049
                    R       -150.6049            2      305.2098
                    4       -147.6329            4      303.2658


Additive constant for all log-likelihoods =     -29.41.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                       9.738            6             0.1362
  Test 2                       2.333            3             0.5063
  Test 3                       2.333            3             0.5063
 Test 6a                       1.461            1             0.2268


  The p-value for Test 1 is greater than .05.  There may not be a
  diffence between responses and/or variances among the dose levels
  Modelling the data with a dose/response curve may not be appropriate.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 0.100000

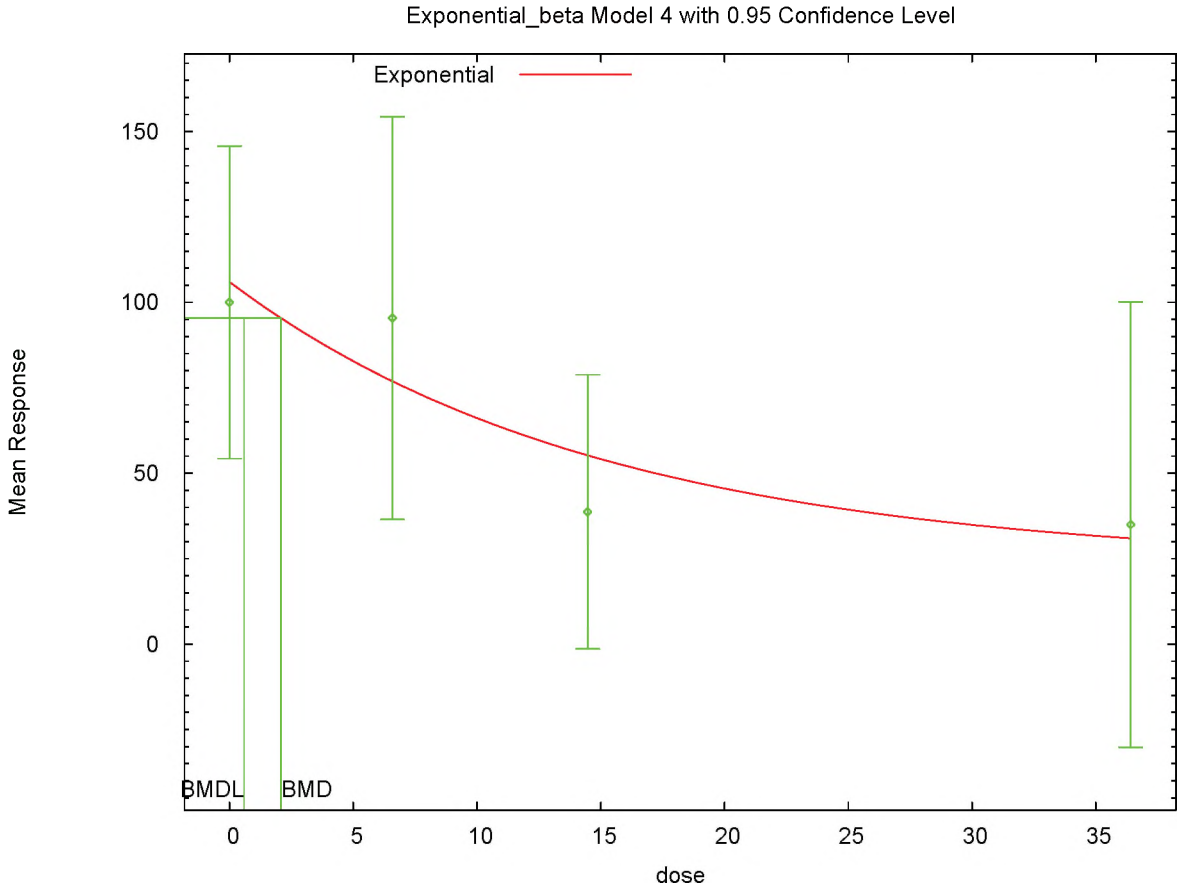
         Risk Type = Relative deviation

  Confidence Level = 0.950000

               BMD =      2.08379

              BMDL =     0.592601
```

E.2.12.3. *Figure for Selected Model: Exponential (M4)*



E.2.13. Franc et al., 2001: H/W Rats, Relative Thymus Weight

E.2.13.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2) [b]	2	0.698	261.646	5.094E+00	3.132E+00	
exponential (M3)	1	0.407	263.616	5.944E+00	3.140E+00	
exponential (M4)	1	0.396	263.646	5.063E+00	1.864E+00	
exponential (M5)	0	N/A	264.927	9.945E+00	2.127E+00	
Hill	0	N/A	264.927	9.638E+00	1.853E+00	
linear	2	0.645	261.804	6.874E+00	5.006E+00	
polynomial, 3-degree	2	0.645	261.804	6.874E+00	5.006E+00	
power	2	0.645	261.804	6.874E+00	5.006E+00	power bound hit (power = 1)
power, unrestricted	1	0.363	263.755	5.487E+00	2.573E-01	unrestricted (power = 0.881)

[a] Constant variance model selected ($p = 0.4331$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.13.2. *Output for Selected Model: Exponential (M2)*

Franc et al., 2001: H/W Rats, Relative Thymus Weight

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\93_Franc_2001_HW_RelThyWt_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                Thu Apr 15 11:55:55 2010
 ================================================================

 Figure 5, H/W rats, relative thymus weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

```
Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact
```

Initial Parameter Values

Variable	Model 2
lnalpha	6.96647
rho(S)	0
a	56.9433
b	0.0204806
c	0
d	1

(S) = Specified

Parameter Estimates

Variable	Model 2
lnalpha	6.98895
rho	0
a	103.047
b	0.0206828
c	0
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	8	100	35.98
6.588	8	97.53	32.98
14.48	8	71.02	23.99
36.44	8	49.29	43.48

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	103	32.93	-0.2617
6.588	89.92	32.93	0.6532
14.48	76.38	32.93	-0.4596
36.44	48.49	32.93	0.06871

```
Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
```

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                   Model      Log(likelihood)      DF         AIC
                  -------    ------------------    ----   ------------
                      A1       -127.4636            5      264.9271
                      A2       -126.0925            8       268.185
                      A3       -127.4636            5      264.9271
                       R        -132.935            2        269.87
                       2       -127.8231            3      261.6463


Additive constant for all log-likelihoods =     -29.41.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                                Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does Model 2 fit the data? (A3 vs. 2)


                            Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                         13.69           6             0.03336
  Test 2                         2.742           3              0.4331
  Test 3                         2.742           3              0.4331
  Test 4                        0.7192           2               0.698


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 4 is greater than .1.  Model 2 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 0.100000

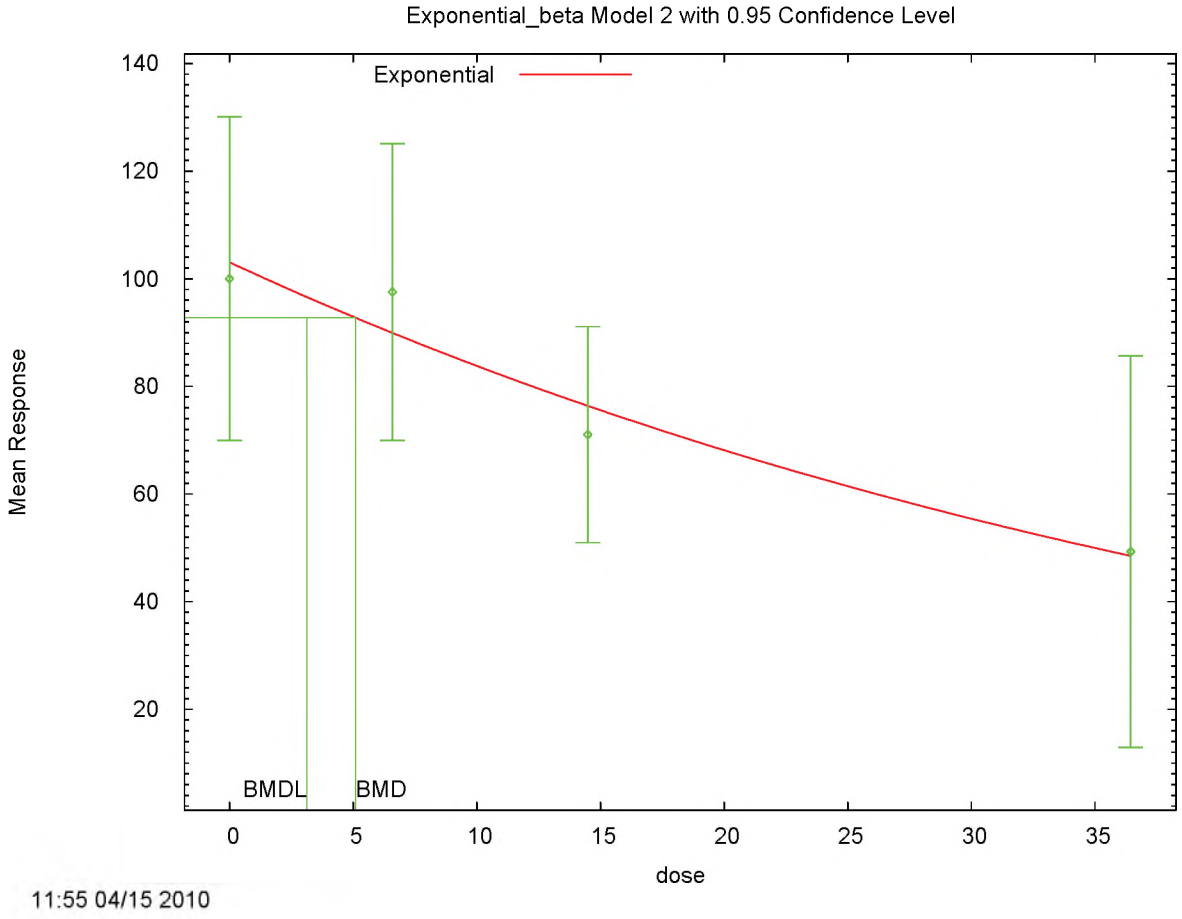
         Risk Type = Relative deviation

  Confidence Level = 0.950000

               BMD =      5.09411

              BMDL =      3.13214
```

E.2.13.3. *Figure for Selected Model: Exponential (M2)*



E.2.14. Hojo et al., 2002: DRL Reinforce Per Minute

E.2.14.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
Hill	1	0.101	4.465	1.667E+00	6.209E-08	n upper bound hit (n = 18)
linear	2	0.009	9.124	1.352E+01	6.020E+00	
polynomial, 3-degree	2	0.009	9.124	1.352E+01	6.020E+00	
power	2	0.009	9.124	1.352E+01	6.020E+00	power bound hit (power = 1)
power, unrestricted	1	0.025	6.780	2.428E-01	1.070E-14	unrestricted (power = 0.103)
exponential (M2)	2	0.007	9.612	1.623E+01	8.673E+00	
exponential (M3)	2	0.007	9.612	1.623E+01	8.673E+00	power hit bound (d = 1)
exponential (M4) [b]	**1**	**0.054**	**5.488**	**1.316E+00**	**2.367E-03**	
exponential (M5)	0	N/A	6.465	1.728E+00	9.452E-03	

[a] Constant variance model selected ($p = 0.4321$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.14.2. *Output for Selected Model: Exponential (M4)*

Hojo et al., 2002: DRL Reinforce Per Minute

```
 ========================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\21_Hojo_2002_DRLrein_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                  Mon Feb 08 10:49:08 2010
 ========================================================================

 Table 5, values adjusted by a constant to allow exponential model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	-1.29672
rho(S)	0
a	0.0817
b	0.15642
c	16.3733
d	1

(S) = Specified

Parameter Estimates

Variable	Model 4
lnalpha	-1.11961
rho	0
a	0.0547452
b	0.708154
c	18.214
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	5	0.086	0.448
1.625	5	0.536	0.821
4.169	6	1.274	0.54
10.7	5	0.737	0.443

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	0.05475	0.5713	0.1223
1.625	0.6989	0.5713	-0.6375
4.169	0.9479	0.5713	1.398
10.7	0.9966	0.5713	-1.016

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                 Model      Log(likelihood)      DF         AIC
                -------    -----------------    ----   ------------
                    A1          3.11555           5        3.7689
                    A2          4.489557          8       7.020886
                    A3           3.11555          5        3.7689
                     R          -2.435087         2       8.870174
                     4           1.255891         4       5.488219


Additive constant for all log-likelihoods =      -19.3.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                      13.85             6             0.03137
  Test 2                      2.748             3              0.4321
  Test 3                      2.748             3              0.4321
 Test 6a                      3.719             1             0.05379


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is less than .1.  Model 4 may not adequately
  describe the data; you may want to consider another model.


Benchmark Dose Computations:

  Specified Effect = 1.000000

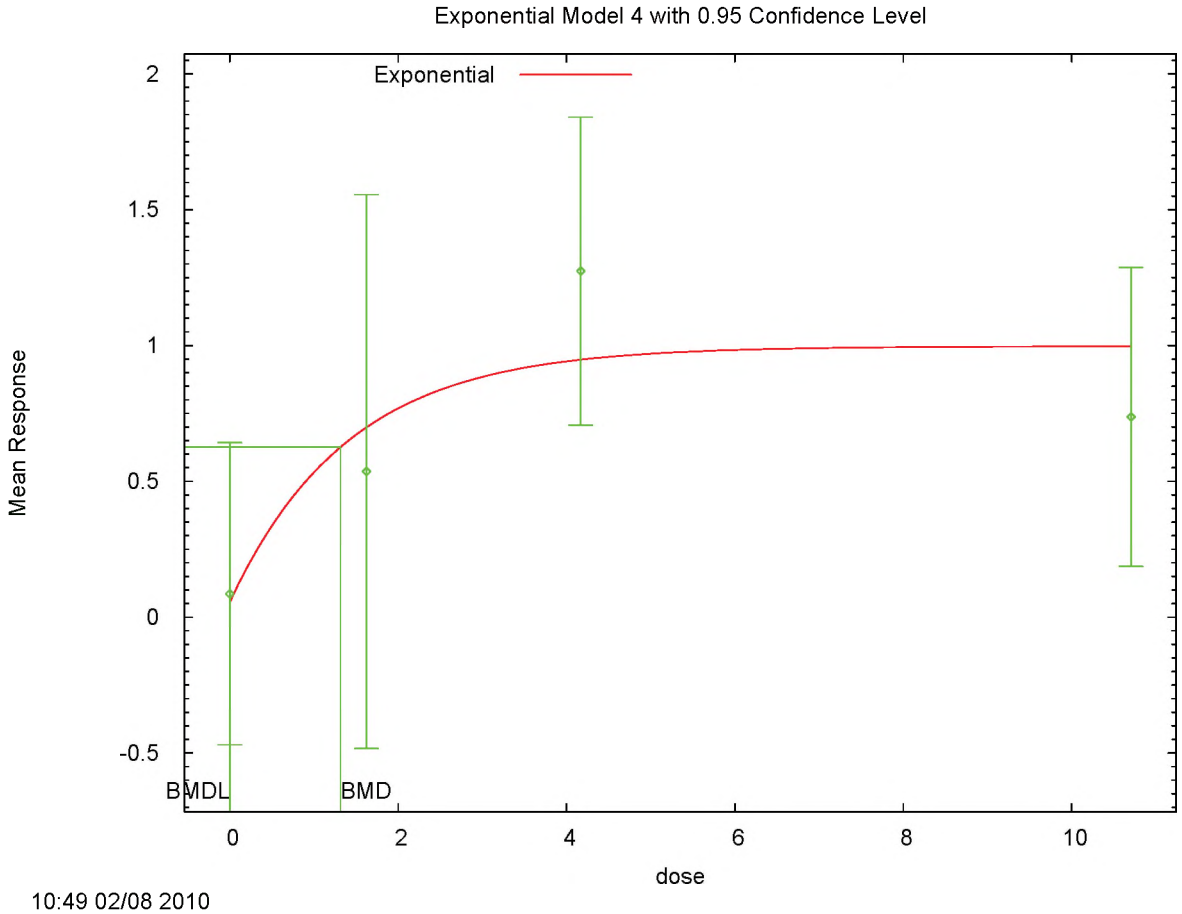
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

              BMD =      1.31616
```

BMDL = 0.00236664

E.2.14.3. *Figure for Selected Model: Exponential (M4)*



10:49 02/08 2010

E.2.15. Hojo et al., 2002: DRL Response Per Minute

E.2.15.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
Hill	0	N/A	126.353	1.373E+00	1.070E-14	
linear	2	0.006	132.243	1.064E+01	5.340E+00	
polynomial, 3-degree	2	0.006	132.243	1.064E+01	5.340E+00	
power	2	0.006	132.243	1.064E+01	5.340E+00	power bound hit (power = 1)
power, unrestricted	2	0.741	122.455	1.070E+03	error	unrestricted (power = 0)
exponential (M2)	2	0.570	122.980	5.027E-01	error	
exponential (M3)	2	0.570	122.980	5.027E-01	error	power hit bound (d = 1)
exponential (M4) [b]	**1**	**0.477**	**124.360**	**3.813E-01**	**1.553E-02**	
exponential (M5)	0	N/A	126.353	8.430E-01	2.221E-02	

[a] Constant variance model selected ($p = 0.3004$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.15.2. *Output for Selected Model: Exponential (M4)*

Hojo et al., 2002: DRL Response Per Minute

```
 ================================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\23_Hojo_2002_DRLresp_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                    Mon Feb 08 10:50:10 2010
 ================================================================================

 Table 5, values adjusted by a constant to allow exponential model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	4.51689
rho(S)	0
a	24.6362
b	0.379327
c	0.0184785
d	1

(S) = Specified

Parameter Estimates

Variable	Model 4
lnalpha	4.54096
rho	0
a	23.4674
b	1.61185
c	0.101317
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	5	23.46	7.986
1.625	5	4.013	10.96
4.169	6	0.478	7.194
10.7	5	4.594	15.23

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	23.47	9.684	-0.001008
1.625	3.915	9.684	0.02265
4.169	2.403	9.684	-0.4869
10.7	2.378	9.684	0.5118

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                   Model      Log(likelihood)      DF         AIC
                  -------    -----------------    ----   ------------
                      A1       -57.92733            5      125.8547
                      A2       -56.09669            8      128.1934
                      A3       -57.92733            5      125.8547
                       R       -64.49611            2      132.9922
                       4        -58.1801            4      124.3602


Additive constant for all log-likelihoods =      -19.3.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                         16.8           6             0.01005
  Test 2                        3.661           3              0.3004
  Test 3                        3.661           3              0.3004
 Test 6a                       0.5056           1              0.4771


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

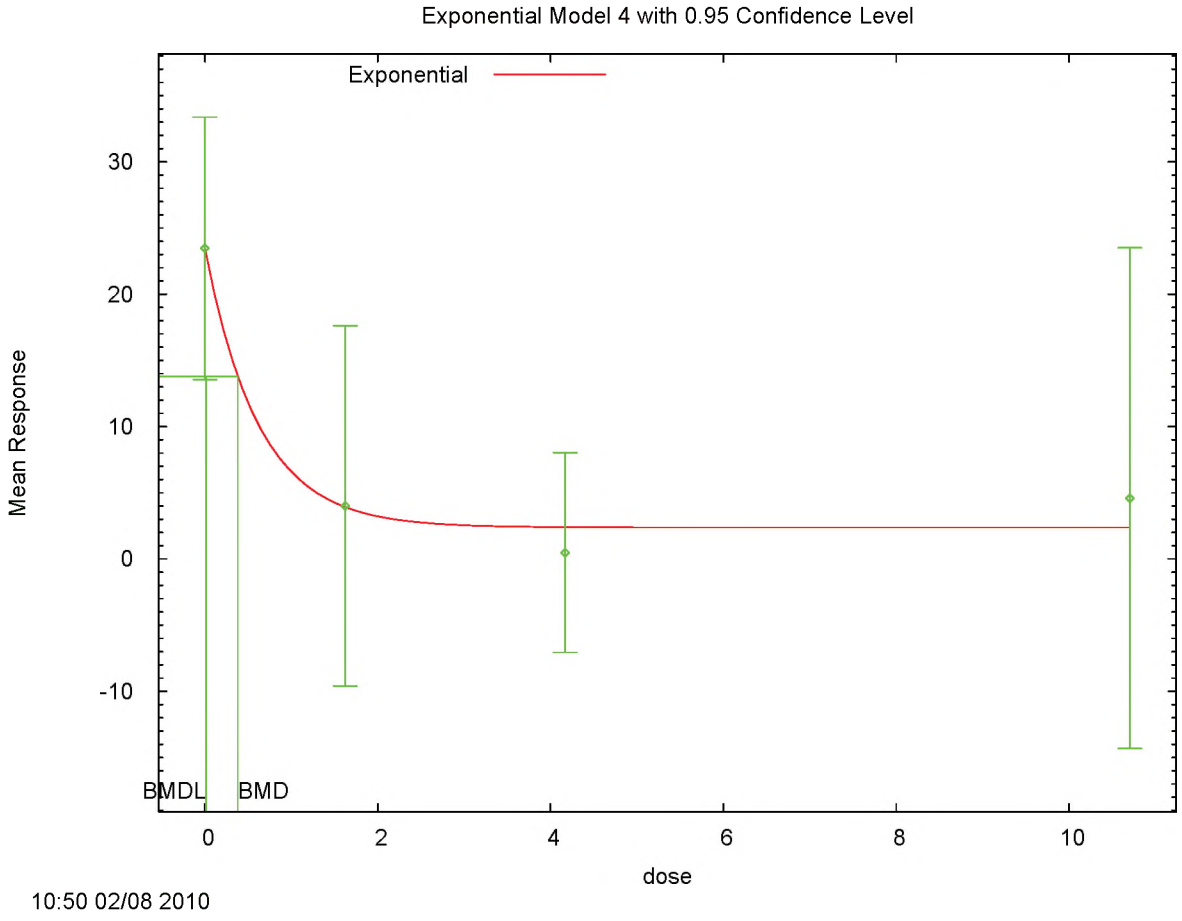
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =     0.381347
```

BMDL = 0.0155267

E.2.15.3. *Figure for Selected Model: Exponential (M4)*



E.2.16. Kattainen et al., 2001: 3rd Molar Eruption, Female

E.2.16.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
logistic	3	0.360	88.508	9.223E+00	6.671E+00	negative intercept (intercept = -1.586)
log-logistic [a]	**3**	**0.982**	**85.227**	**2.399E+00**	**1.328E+00**	**slope bound hit (slope = 1)**
log-probit	3	0.522	87.424	7.346E+00	4.561E+00	slope bound hit (slope = 1)
probit	3	0.379	88.352	8.802E+00	6.549E+00	negative intercept (intercept = -0.975)
multistage, 4-degree	3	0.781	86.155	4.042E+00	2.626E+00	final ß = 0
log-logistic, unrestricted [b]	2	0.949	87.162	1.931E+00	1.840E-01	unrestricted (slope = 0.91)
log-probit, unrestricted	2	0.941	87.181	2.075E+00	2.395E-01	unrestricted (slope = 0.549)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.2.16.2. *Output for Selected Model: Log-Logistic*

Kattainen et al., 2001: 3rd Molar Eruption, Female

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\Blood\24_Katt_2001_Erup_LogLogistic_BMR1.(d)
         Gnuplot Plotting File:  C:\1\Blood\24_Katt_2001_Erup_LogLogistic_BMR1.plt
                                              Mon Feb 08 10:50:39 2010
 ================================================================

 Figure 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model
```

Default Initial Parameter Values
background = 0.0625
intercept = -3.07535
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.53
intercept	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0699339	*	*	*
intercept	-3.07219	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-40.5286	5			
Fitted model	-40.6137	2	0.170195	3	0.9823
Reduced model	-50.7341	1	20.411	4	0.0004142

AIC: 85.2274

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0699	1.119	1.000	16	-0.117
2.2297	0.1570	2.669	3.000	17	0.221
6.2523	0.2788	4.182	4.000	15	-0.105
16.0824	0.4670	5.604	6.000	12	0.229
46.8576	0.7066	13.426	13.000	19	-0.215

Chi^2 = 0.17 d.f. = 3 P-value = 0.9820

Benchmark Dose Computation

Specified effect = 0.1

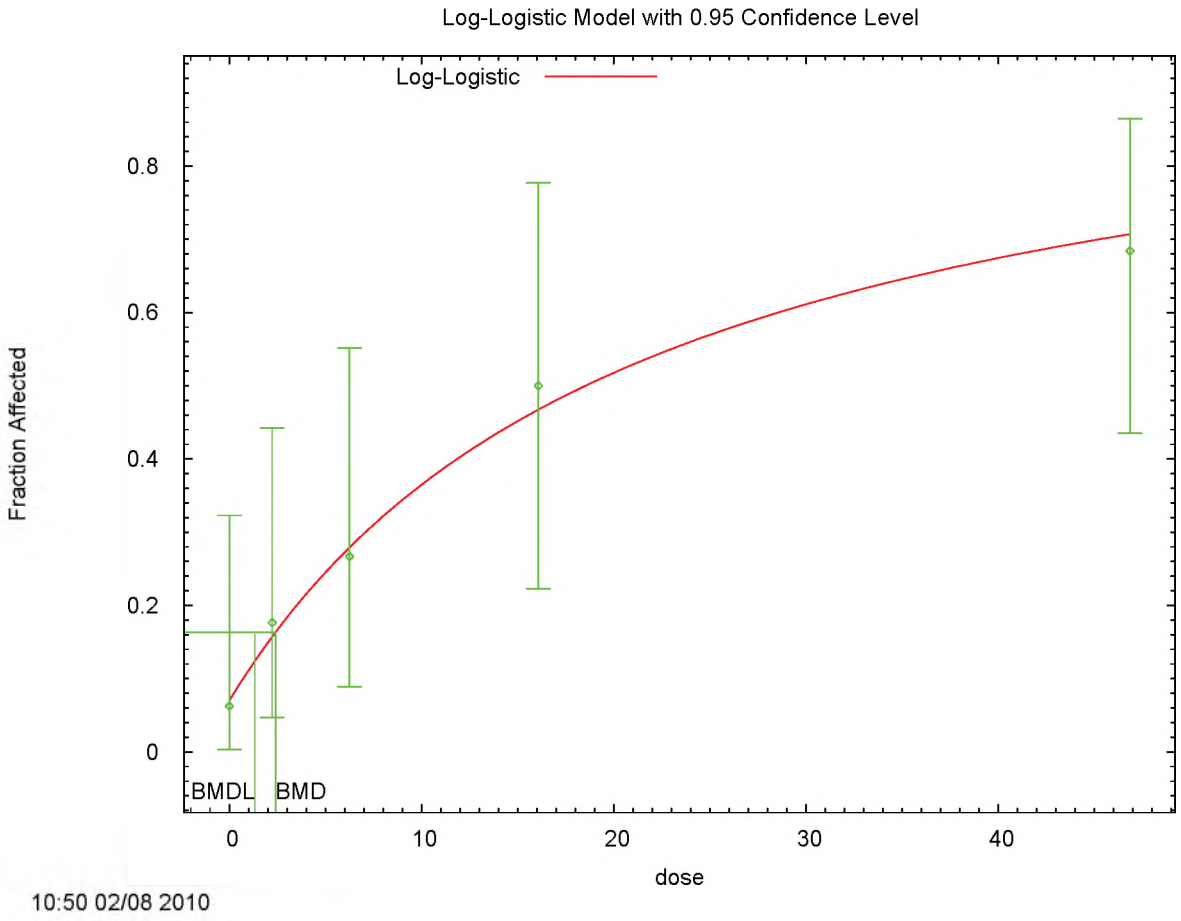
Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.39879

BMDL = 1.32815

E.2.16.3. *Figure for Selected Model: Log-Logistic*



E.2.16.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

Kattainen et al., 2001: 3rd Molar Eruption, Female

```
 ========================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\Blood\24_Katt_2001_Erup_LogLogistic_U_BMR1.(d)
         Gnuplot Plotting File:  C:\1\Blood\24_Katt_2001_Erup_LogLogistic_U_BMR1.plt
                                                Mon Feb 08 10:50:40 2010
 ========================================================================

 Figure 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 5
```

```
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =       0.0625
                     intercept =      -2.7659
                         slope =     0.901885


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.52         0.38

 intercept        -0.52            1        -0.94

     slope         0.38        -0.94            1


                                Parameter Estimates

                                                                95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0630045            *               *                   *
      intercept         -2.79616            *               *                   *
          slope         0.910333            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -40.5286         5
   Fitted model        -40.5811         3      0.105049      2          0.9488
  Reduced model        -50.7341         1        20.411      4        0.0004142

           AIC:         87.1622


                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ---------------------------------------------------------------------------
    0.0000     0.0630         1.008     1.000          16        -0.008
    2.2297     0.1683         2.862     3.000          17         0.090
    6.2523     0.2922         4.383     4.000          15        -0.217
   16.0824     0.4692         5.631     6.000          12         0.214
   46.8576     0.6903        13.116    13.000          19        -0.058

 Chi^2 = 0.10      d.f. = 2        P-value = 0.9491


   Benchmark Dose Computation

Specified effect =            0.1

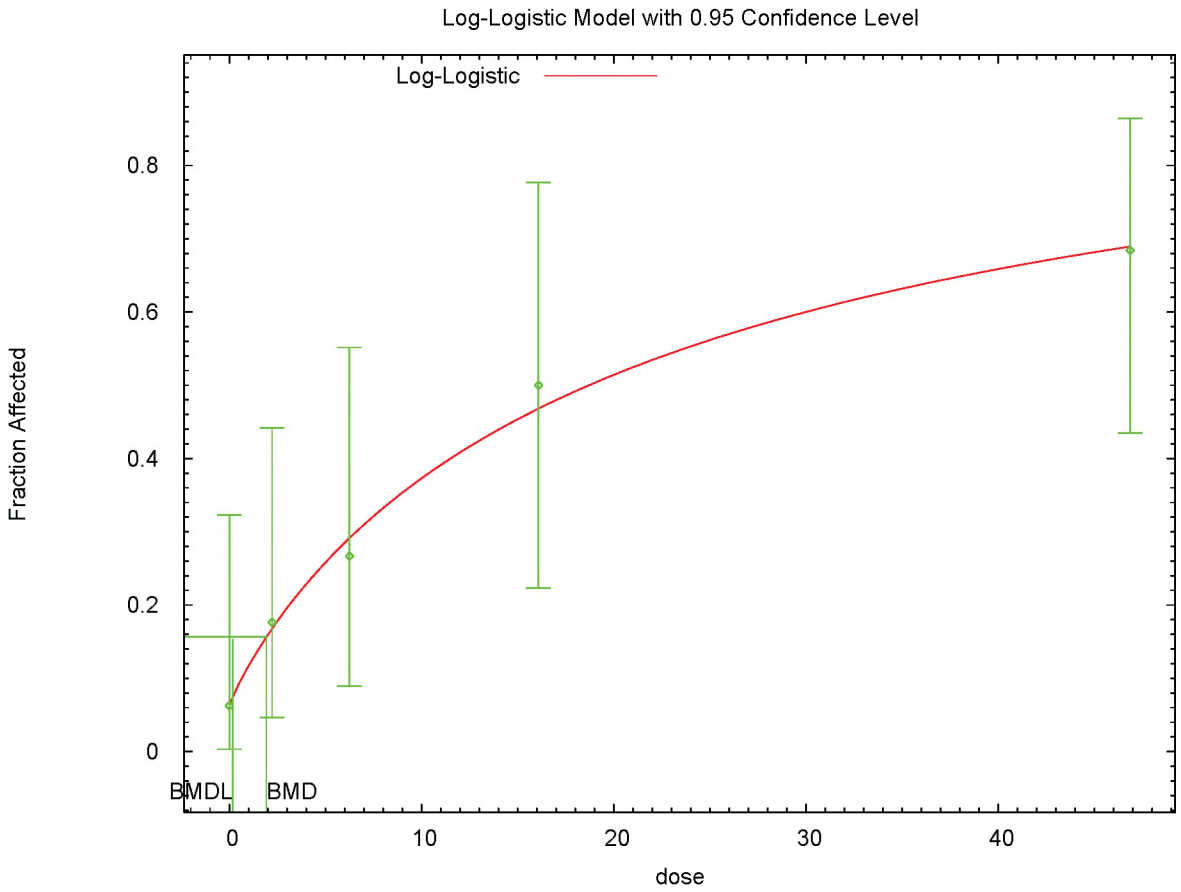
Risk Type        =      Extra risk

Confidence level =           0.95
```

```
BMD =         1.93079

BMDL =        0.18403
```

E.2.16.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.2.17. Kattainen et al., 2001: 3rd Molar Length, Female

E.2.17.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	3	<0.0001	-124.866	1.669E+01	9.933E+00	
exponential (M3)	3	<0.0001	-124.866	1.669E+01	9.933E+00	power hit bound (d = 1)
exponential (M4)	2	0.002	-147.120	4.237E-01	2.530E-01	
exponential (M5)	2	0.002	-147.120	4.237E-01	2.530E-01	power hit bound (d = 1)
Hill [b]	**2**	**0.022**	**-152.239**	**3.132E-01**	**1.679E-01**	**n lower bound hit (n = 1)**
linear	3	<0.0001	-124.024	1.982E+01	1.277E+01	
polynomial, 4-degree	3	<0.0001	-124.024	1.982E+01	1.277E+01	
power	3	<0.0001	-124.024	1.982E+01	1.277E+01	power bound hit (power = 1)
Hill, unrestricted [c]	1	<0.0001	-130.856	1.215E-02	error	unrestricted (n = 13.042)
power, unrestricted	2	0.263	-157.201	1.964E-03	8.002E-06	unrestricted (power = 0.195)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.17.2. *Output for Selected Model: Hill*

Kattainen et al., 2001: 3rd Molar Length, Female

```
 ======================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\Blood\25_Katt_2001_Length_Hill_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\25_Katt_2001_Length_Hill_1.plt
                                                Mon Feb 08 10:51:09 2010
 ======================================================================

 Figure 3 female only
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
```

The variance is to be modeled as Var(i) = exp(lalpha + rho * ln(mean(i)))

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	-2.37155
rho =	0
intercept =	1.85591
v =	-0.507874
n =	0.845932
k =	2.03129

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	intercept	v	k
lalpha	1	-0.98	-0.16	0.84	-0.38
rho	-0.98	1	0.2	-0.79	0.4
intercept	-0.16	0.2	1	-0.3	-0.11
v	0.84	-0.79	-0.3	1	-0.52
k	-0.38	0.4	-0.11	-0.52	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
lalpha	3.31084	1.404	0.559057	6.06262
rho	-14.2657	2.62739	-19.4153	-9.11612
intercept	1.85483	0.0159477	1.82357	1.88609
v	-0.453667	0.0620227	-0.575229	-0.332105
n	1	NA		
k	1.91219	0.624785	0.687636	3.13675

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	16	1.86	1.85	0.0661	0.0639	0.0674
2.23	17	1.58	1.61	0.185	0.175	-0.789
6.252	15	1.6	1.51	0.265	0.28	1.22
16.08	12	1.5	1.45	0.221	0.371	0.51
46.86	19	1.35	1.42	0.515	0.431	-0.716

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1           56.758717           6    -101.517434
              A2           85.856450          10    -151.712901
              A3           84.934314           7    -155.868628
          fitted           81.119648           5    -152.239295
               R           45.373551           2     -86.747101


                  Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              80.9658          8          <.0001
  Test 2              58.1955          4          <.0001
  Test 3              1.84427          3          0.6053
  Test 4              7.62933          2         0.02205

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =            1

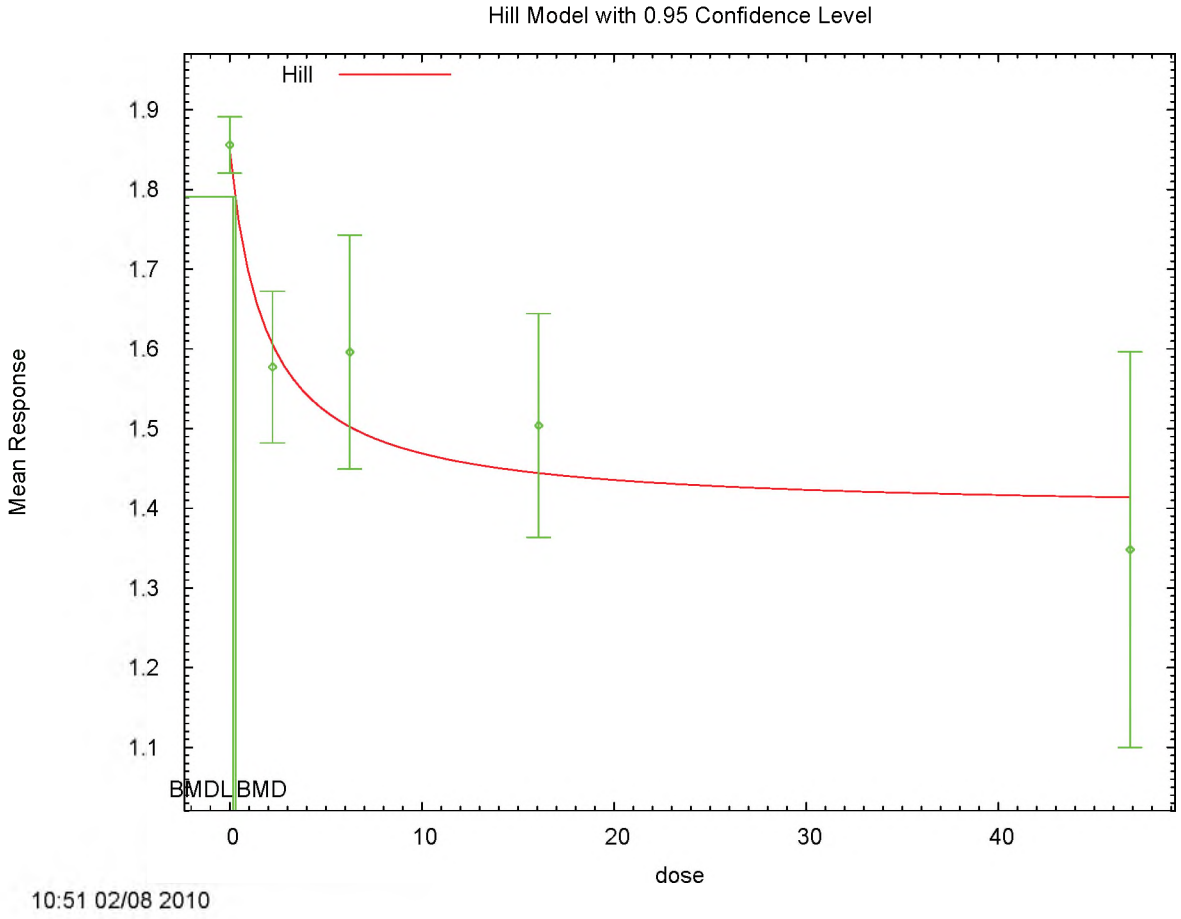
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       0.313211

            BMDL =      0.167922
```

E.2.17.3. *Figure for Selected Model: Hill*



E.2.17.4. *Output for Additional Model Presented: Hill, Unrestricted*

Kattainen et al., 2001: 3rd Molar Length, Female

```
 ================================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\Blood\25_Katt_2001_Length_Hill_U_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\25_Katt_2001_Length_Hill_U_1.plt
                                                  Mon Feb 08 10:51:09 2010
 ================================================================================

 Figure 3 female only
 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))
```

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	-2.37155
rho =	0
intercept =	1.85591
v =	-0.507874
n =	0.845932
k =	2.03129

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	intercept	v	n	k
lalpha	1	-0.98	-0.16	0.84	1.4e-016	3.3e-017
rho	-0.98	1	0.22	-0.77	-2.2e-016	-5.1e-017
intercept	-0.16	0.22	1	-0.35	6e-017	1.4e-017
v	0.84	-0.77	-0.35	1	-2.6e-016	-6.2e-017
n	1.4e-016	-2.2e-016	6e-017	-2.6e-016	1	1
k	3.3e-017	-5.1e-017	1.4e-017	-6.2e-017	1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	4.25154	1.5913	1.13265	7.37044
rho	-15.7639	2.90127	-21.4503	-10.0776
intercept	1.85591	0.0160104	1.82453	1.88729
v	-0.357293	0.0463784	-0.448193	-0.266393
n	13.0417	4.64308e+013	-9.10027e+013	9.10027e+013
k	0.0136512	2.57737e+011	-5.05155e+011	5.05155e+011

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	16	1.86	1.86	0.0661	0.064	2.09e-009
2.23	17	1.58	1.5	0.185	0.345	0.937
6.252	15	1.6	1.5	0.265	0.345	1.09
16.08	12	1.5	1.5	0.221	0.345	0.0534
46.86	19	1.35	1.5	0.515	0.345	-1.9

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

```
Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1           56.758717            6    -101.517434
             A2           85.856450           10    -151.712901
             A3           84.934314            7    -155.868628
         fitted           71.427978            6    -130.855955
              R           45.373551            2     -86.747101


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              80.9658          8          <.0001
  Test 2              58.1955          4          <.0001
  Test 3              1.84427          3          0.6053
  Test 4              27.0127          1          <.0001

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =             1

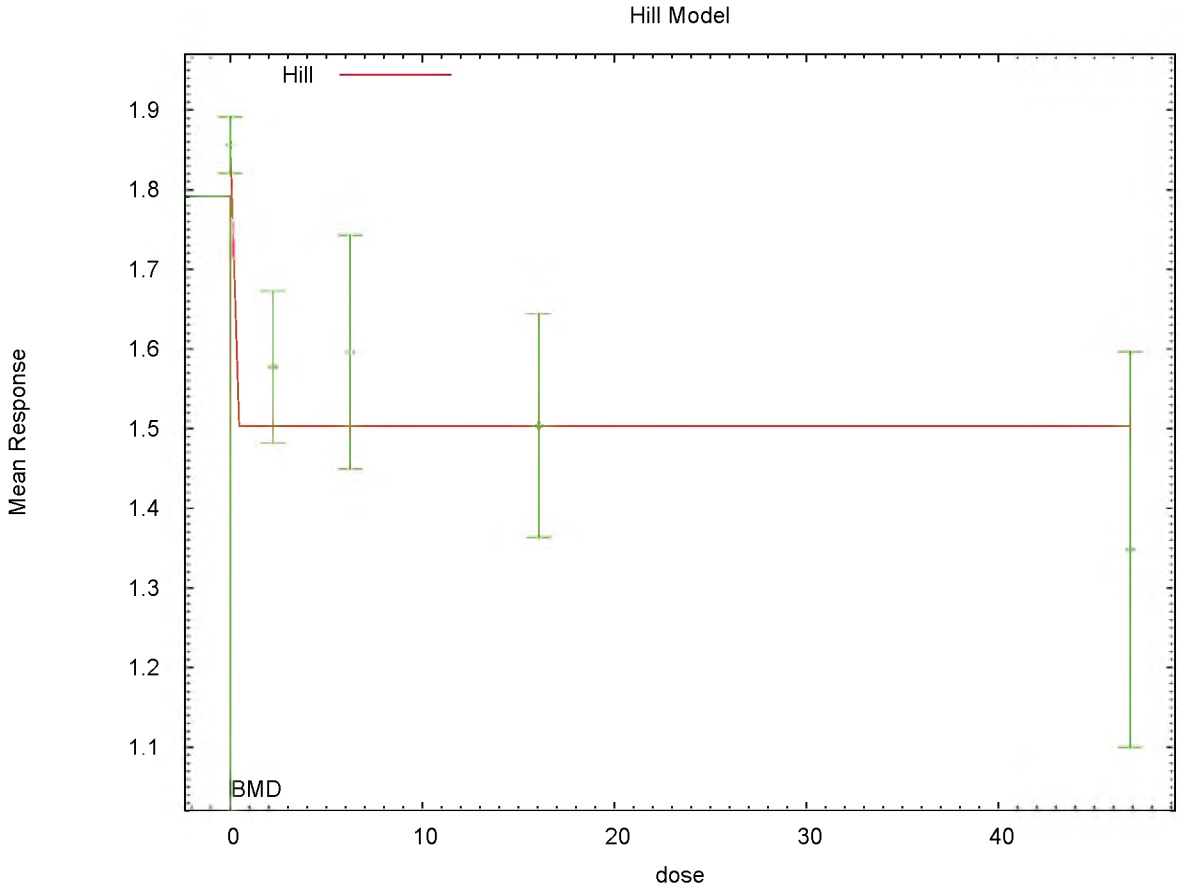
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       0.012148


BMDL computation failed.
```

E.2.17.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.2.18. Keller et al., 2007: Missing Mandibular Molars, CBA J

E.2.18.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	1	0.105	52.510	3.342E+00	8.986E-01	
logistic	2	0.335	49.984	3.069E+00	2.212E+00	negative intercept (intercept = -3.414)
log-logistic	1	0.105	52.524	4.009E+00	2.411E+00	
log-probit	1	0.105	52.524	3.845E+00	2.421E+00	
multistage, 1-degree [a]	**3**	**0.255**	**50.425**	**1.091E+00**	**7.624E-01**	
multistage, 2-degree	1	0.122	51.391	1.916E+00	9.654E-01	
multistage, 3-degree	1	0.150	50.853	1.713E+00	9.584E-01	
probit	2	0.342	49.904	2.927E+00	2.053E+00	negative intercept (intercept = -1.873)
Weibull	1	0.108	52.219	2.744E+00	9.350E-01	

[a] Best-fitting model, BMDS output presented in this appendix

E.2.18.2. *Output for Selected Model: Multistage, 1-Degree*

Keller et al., 2007: Missing Mandibular Molars, CBA J

```
 ================================================================
         Multistage Model. (Version: 3.0;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\26_Keller_2007_Molars_Multi1_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\26_Keller_2007_Molars_Multi1_1.plt
                                              Mon Feb 08 10:51:47 2010
 ================================================================

 Table 1 using mandibular molars only
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
```

```
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                    Background =           0
                       Beta(1) = 3.03988e+018


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                Beta(1)

  Beta(1)            1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background                0            *               *                   *
        Beta(1)         0.096571            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -21.5798         4
   Fitted model         -24.2126         1       5.26564      3          0.1533
  Reduced model          -71.326         1       99.4926      3         <.0001

           AIC:         50.4251


                                 Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          29        0.000
    0.5374     0.0506         1.163     2.000          23        0.796
    4.2881     0.3391         9.833     6.000          29       -1.504
   34.0560     0.9627        28.881    30.000          30        1.078

 Chi^2 = 4.06      d.f. = 3        P-value = 0.2554


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

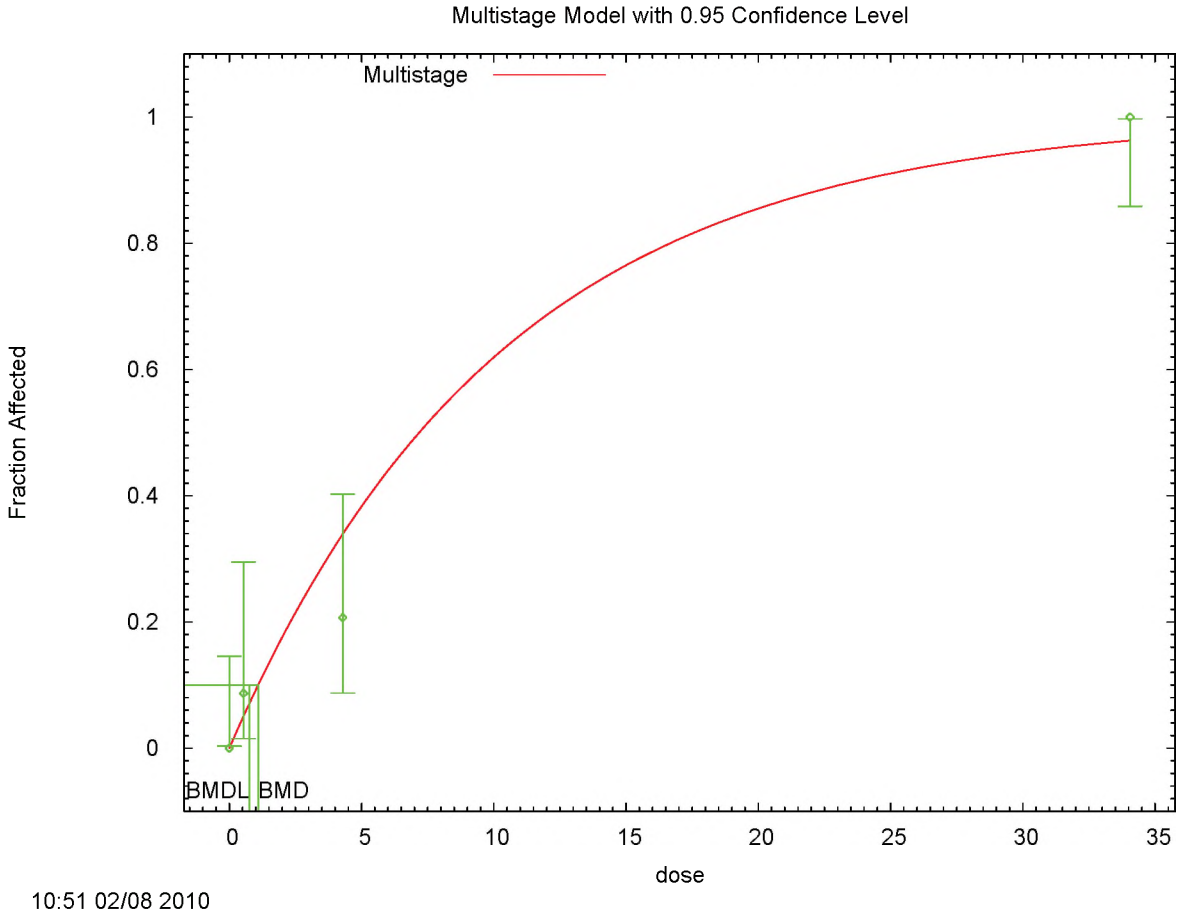
             BMD =       1.09102

            BMDL =      0.762404
```

```
            BMDU =        1.56496

Taken together, (0.762404, 1.56496) is a 90      % two-sided confidence
interval for the BMD
```

E.2.18.3. *Figure for Selected Model: Multistage, 1-Degree*



E.2.19. Kociba et al., 1978: Urinary Coproporphyrin, Females

E.2.19.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	<0.0001	82.975	2.378E+01	1.340E+01	
exponential (M3)	2	<0.0001	82.975	2.378E+01	1.340E+01	power hit bound (d = 1)
exponential (M4) [b]	**1**	**0.006**	**73.823**	**1.566E+00**	**7.180E-01**	
exponential (M5)	0	N/A	69.047	6.225E+00	1.586E+00	
Hill	0	N/A	69.047	5.473E+00	error	
linear	2	<0.001	82.233	1.790E+01	3.862E+00	
polynomial, 3-degree	2	<0.001	82.233	1.790E+01	3.862E+00	
power	2	<0.001	82.233	1.790E+01	3.862E+00	power bound hit (power = 1)
power, unrestricted	1	<0.001	78.691	1.148E+00	8.984E-09	unrestricted (power = 0.416)

[a] Non-constant variance model selected ($p = 0.0298$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.19.2. *Output for Selected Model: Exponential (M4)*

Kociba et al., 1978: Urinary Coproporphyrin, Females

```
 ======================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\29_Kociba_1978_Copro_Exp_1.(d)
         Gnuplot Plotting File:
                                                 Mon Feb 08 10:52:47 2010
 ======================================================================

 Table2-UrinaryCoproporphyrin
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	-5.58269
rho	2.98472
a	8.17
b	0.0692478
c	2.23623
d	1

Parameter Estimates

Variable	Model 4
lnalpha	-4.90852
rho	2.80743
a	8.91071
b	0.15304
c	1.97526
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	5	9.8	1.3
1.547	5	8.6	2
7.155	5	16.4	4.7
38.56	5	17.4	4

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	8.911	1.852	1.074
1.547	10.74	2.407	-1.991
7.155	14.69	3.736	1.021
38.56	17.58	4.805	-0.08246

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

```
  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                 Model      Log(likelihood)      DF         AIC
                -------    -----------------    ----   ------------
                    A1       -31.69739            5      73.39478
                    A2       -27.21541            8      70.43081
                    A3       -28.16434            6      68.32868
                     R       -41.73188            2      87.46376
                     4       -31.91136            5      73.82272


Additive constant for all log-likelihoods =     -18.38.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                              Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                      29.03             6           < 0.0001
  Test 2                      8.964             3            0.02977
  Test 3                      1.898             2             0.3872
 Test 6a                      7.494             1            0.00619


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is less than .1.  Model 4 may not adequately
  describe the data; you may want to consider another model.


Benchmark Dose Computations:

  Specified Effect = 1.000000

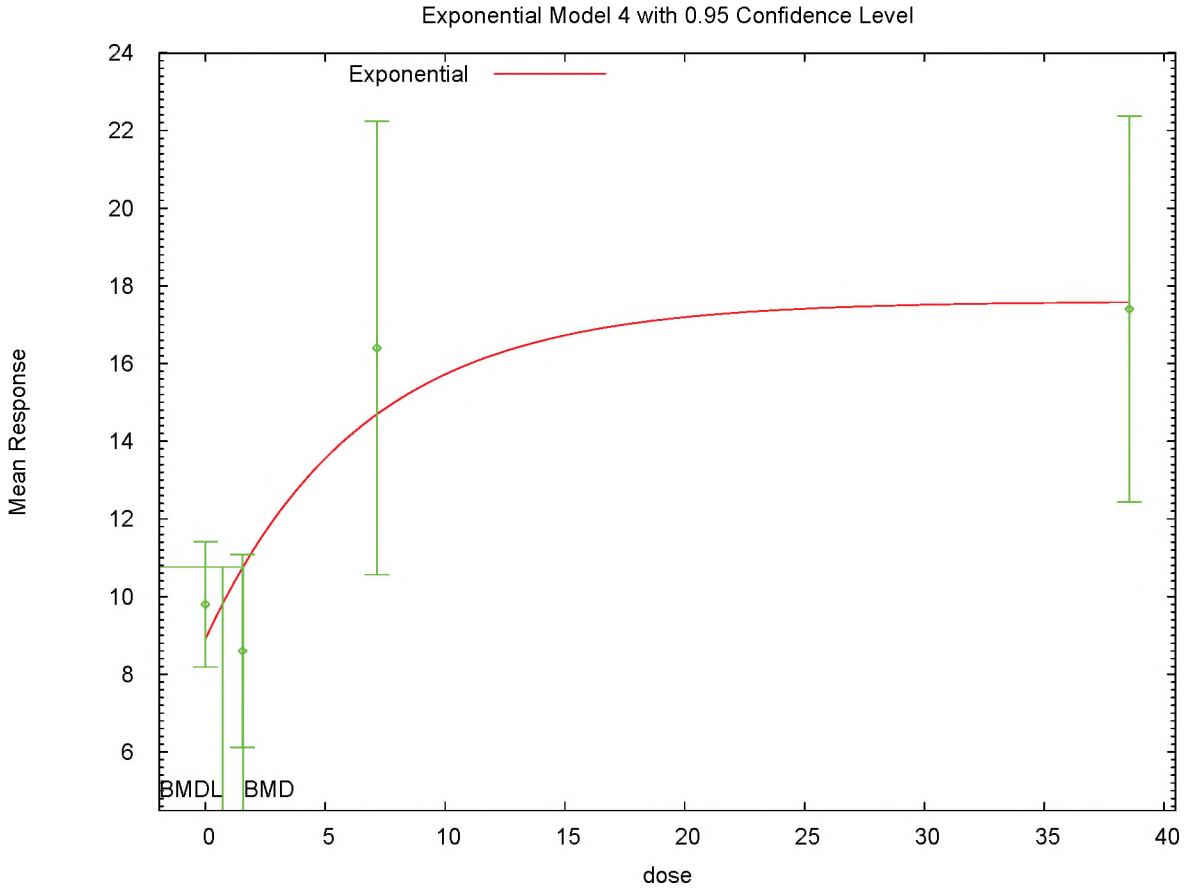
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      1.56562

              BMDL =     0.718033
```

E.2.19.3. *Figure for Selected Model: Exponential (M4)*



E.2.20. Kociba et al., 1978: Uroporphyrin per Creatinine, Female

E.2.20.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	0.755	-93.828	1.641E+01	1.259E+01	
exponential (M3)	2	0.755	-93.828	1.641E+01	1.259E+01	power hit bound (d = 1)
exponential (M4)	1	0.499	-91.935	1.216E+01	3.958E+00	
exponential (M5)	0	N/A	-90.190	7.542E+00	4.128E+00	
Hill	0	N/A	-90.190	7.607E+00	3.966E+00	
linear [b]	**2**	**0.793**	**-93.928**	**1.306E+01**	**9.287E+00**	
polynomial, 3-degree	2	0.793	-93.928	1.306E+01	9.287E+00	
power	1	0.497	-91.928	1.326E+01	9.287E+00	

[a] Constant variance model selected ($p = 0.4919$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.20.2. *Output for Selected Model: Linear*

Kociba et al., 1978: Uroporphyrin per Creatinine, Female

```
 ========================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\Blood\28_Kociba_1978_Uropor_LinearCV_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\28_Kociba_1978_Uropor_LinearCV_1.plt
                                              Mon Feb 08 10:52:17 2010
 ========================================================================

 Table 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	0.0030385	
rho =	0	Specified
beta_0 =	0.149139	
beta_1 =	0.00381789	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	1.9e-009	-2.6e-009
beta_0	1.9e-009	1	-0.6
beta_1	-2.6e-009	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.00248773	0.000786688	0.000945846	0.00402961
beta_0	0.149139	0.0139684	0.121761	0.176517
beta_1	0.00381789	0.000711776	0.00242284	0.00521295

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	0.157	0.149	0.05	0.0499	0.352
1.547	5	0.143	0.155	0.037	0.0499	-0.54
7.155	5	0.181	0.176	0.053	0.0499	0.204
38.56	5	0.296	0.296	0.074	0.0499	-0.0161

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	50.195349	5	-90.390697

```
              A2           51.400051            8     -86.800103
              A3           50.195349            5     -90.390697
          fitted           49.963863            3     -93.927727
               R           41.049755            2     -78.099510


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              20.7006          6        0.002076
   Test 2              2.40941          3          0.4919
   Test 3              2.40941          3          0.4919
   Test 4              0.46297          2          0.7934

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

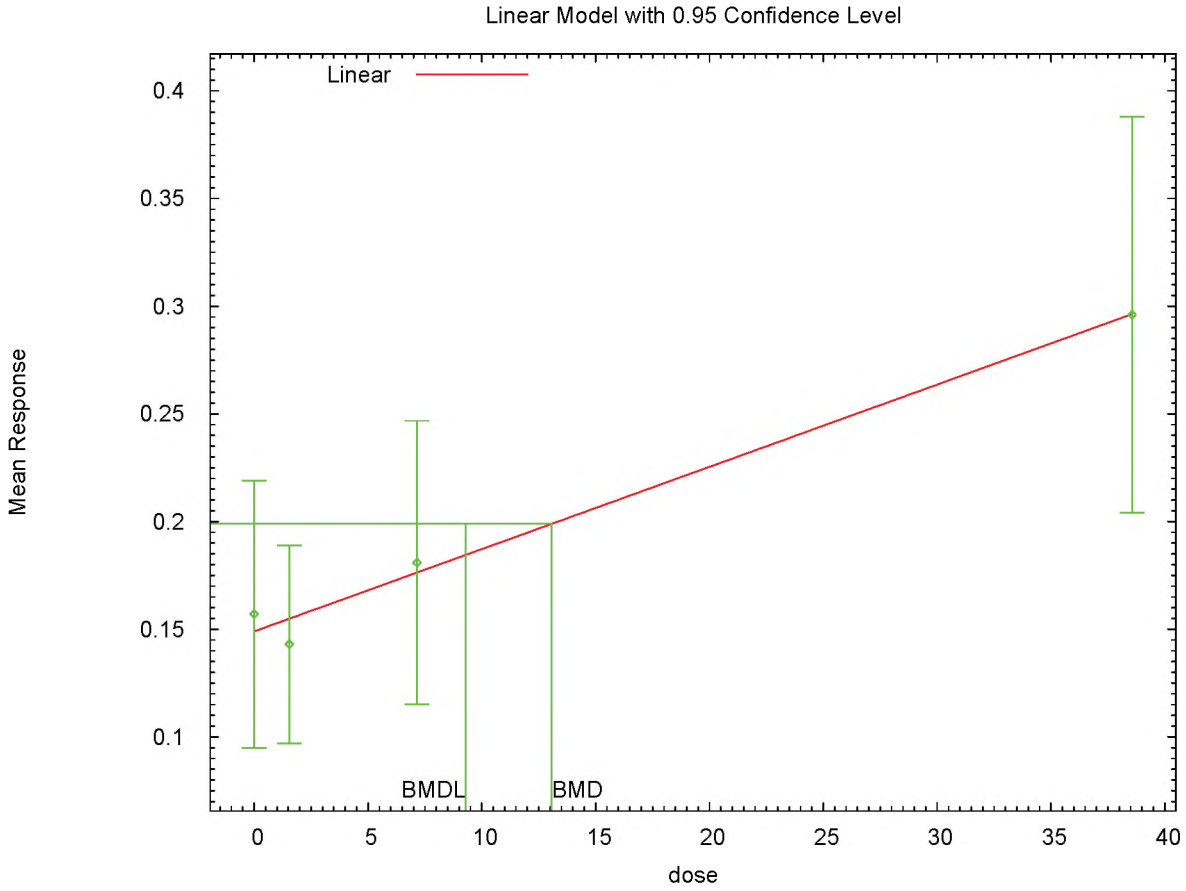
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD =         13.064


             BMDL =        9.28715
```

E.2.20.3. *Figure for Selected Model: Linear*



E.2.21. Latchoumycandane and Mathur, 2002: Sperm Production

E.2.21.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	<0.0001	93.831	1.739E+01	9.432E+00	
exponential (M3)	2	<0.0001	93.831	1.739E+01	9.432E+00	power hit bound (d = 1)
exponential (M4)	1	0.700	75.261	1.912E-01	7.976E-02	
exponential (M5)	0	N/A	77.263	2.925E-01	7.970E-02	
Hill [b]	**1**	**0.962**	**75.115**	**1.171E-01**	**1.324E-02**	**n lower bound hit (n = 1)**
linear	2	<0.0001	94.250	1.995E+01	1.212E+01	
polynomial, 3-degree	2	<0.0001	94.250	1.995E+01	1.212E+01	
power	2	<0.0001	94.250	1.995E+01	1.212E+01	power bound hit (power = 1)
Hill, unrestricted [c]	0	N/A	77.113	9.955E-02	1.228E-09	unrestricted (n = 0.916)
power, unrestricted	1	0.501	75.566	6.921E-06	6.921E-06	unrestricted (power = 0.087)

[a] Constant variance model selected ($p = 0.8506$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.21.2. *Output for Selected Model: Hill*

Latchoumycandane and Mathur, 2002: Sperm Production

```
 ======================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\Blood\30_Latch_2002_Sperm_HillCV_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\30_Latch_2002_Sperm_HillCV_1.plt
                                                 Mon Feb 08 10:53:26 2010
 ======================================================================

 (x10^6) Table 1 without Vitamin E
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
```

```
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                        alpha =      7.23328
                          rho =            0   Specified
                    intercept =        22.19
                            v =        -9.09
                            n =      1.93059
                            k =     0.546864


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -n
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                  alpha    intercept            v            k

      alpha           1    -2.2e-009    -3.7e-008    -5.9e-009

  intercept    -2.2e-009            1        -0.76        -0.23

          v    -3.7e-008        -0.76            1        -0.24

          k    -5.9e-009        -0.23        -0.24            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha           6.0283          1.74022             2.61753             9.43907
       intercept          22.1894          1.00236             20.2248              24.154
               v         -9.16715          1.30966             -11.734            -6.60026
               n                1                NA
               k         0.320198         0.220443          -0.111862            0.752259

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     6       22.2         22.2         2.67         2.46       0.000631
0.7845     6       15.7         15.7         2.65         2.46       -0.00931
4.651      6       13.7         13.6         2.19         2.46         0.0372
27.27      6       13.1         13.1         3.16         2.46        -0.0285


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
```

```
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
       Model A3 uses any fixed variance parameters that
       were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -33.556444            5      77.112888
             A2          -33.158811            8      82.317623
             A3          -33.556444            5      77.112888
          fitted         -33.557588            4      75.115176
              R          -47.392394            2      98.784788


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              28.4672          6          <.0001
   Test 2             0.795266          3          0.8506
   Test 3             0.795266          3          0.8506
   Test 4           0.00228746          1          0.9619

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =             1

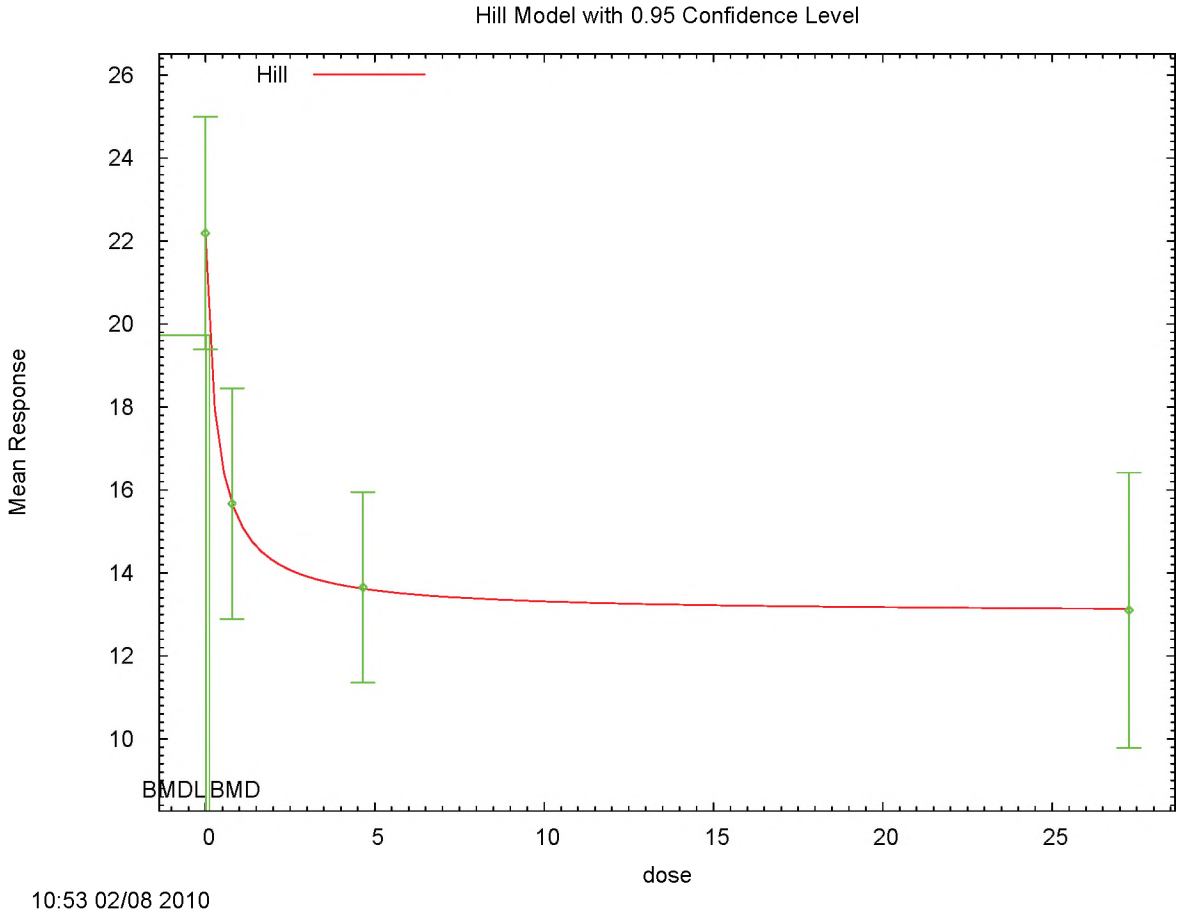
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       0.117131

            BMDL =     0.0132353
```

E.2.21.3. *Figure for Selected Model: Hill*



E.2.21.4. *Output for Additional Model Presented: Hill, Unrestricted*

Latchoumycandane and Mathur, 2002: Sperm Production

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\Blood\30_Latch_2002_Sperm_HillCV_U_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\30_Latch_2002_Sperm_HillCV_U_1.plt
                                              Mon Feb 08 10:53:26 2010
 ================================================================

 (x10^6) Table 1 without Vitamin E
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter is not restricted
   A constant variance model is fit
```

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	7.23328	
rho =	0	Specified
intercept =	22.19	
v =	-9.09	
n =	1.93059	
k =	0.546864	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	n	k
alpha	1	-9.8e-009	1.6e-007	1.6e-007	1.2e-007
intercept	-9.8e-009	1	-0.5	-0.015	-0.13
v	1.6e-007	-0.5	1	0.76	0.56
n	1.6e-007	-0.015	0.76	1	0.86
k	1.2e-007	-0.13	0.56	0.86	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	6.02773	1.74006	2.61728	9.43818
intercept	22.19	1.00231	20.2255	24.1545
v	-9.23667	2.03204	-13.2194	-5.25394
n	0.916265	1.66287	-2.34291	4.17544
k	0.301742	0.440535	-0.561692	1.16518

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	6	22.2	22.2	2.67	2.46	3.4e-008
0.7845	6	15.7	15.7	2.65	2.46	-1.51e-007
4.651	6	13.7	13.6	2.19	2.46	2.62e-007
27.27	6	13.1	13.1	3.16	2.46	-5.45e-007

Degrees of freedom for Test A3 vs fitted <= 0

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -33.556444            5      77.112888
             A2          -33.158811            8      82.317623
             A3          -33.556444            5      77.112888
          fitted         -33.556444            5      77.112888
              R          -47.392394            2      98.784788


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              28.4672          6          <.0001
   Test 2             0.795266          3          0.8506
   Test 3             0.795266          3          0.8506
   Test 4          6.96332e-013         0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


        Benchmark Dose Computation

Specified effect =             1

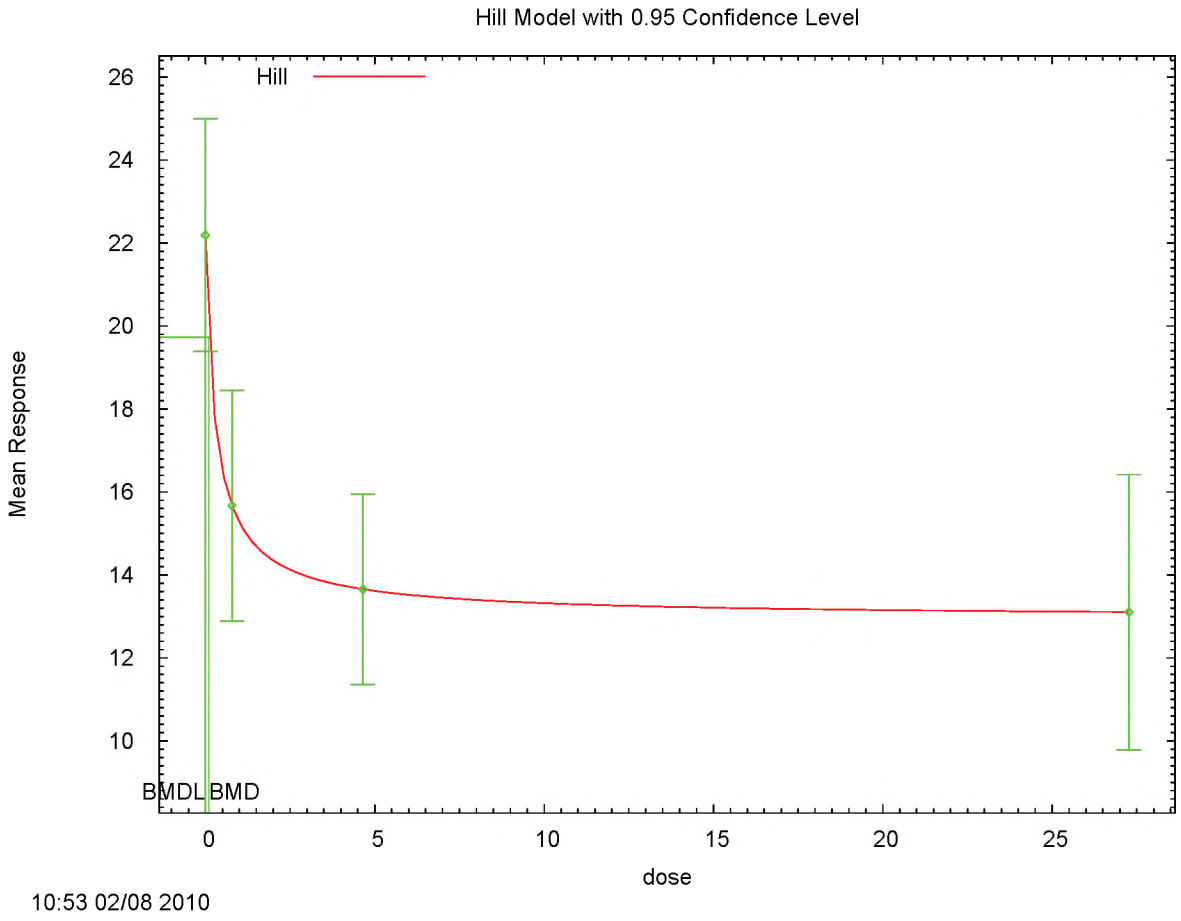
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =      0.0995543

            BMDL =  1.22818e-009
```

E.2.21.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.2.22. Li et al., 1997: FSH

E.2.22.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	8	<0.0001	1095.292	5.222E+02	4.121E+02	
exponential (M3)	8	<0.0001	1095.292	5.222E+02	4.121E+02	power hit bound (d = 1)
exponential (M4)	7	<0.0001	1059.480	3.432E+01	9.930E+00	
exponential (M5)	6	<0.0001	1066.195	1.019E+02	8.583E-01	
Hill	7	<0.0001	1056.459	5.423E+00	error	n lower bound hit (n = 1)
linear	8	<0.0001	1077.695	2.003E+02	1.357E+02	
polynomial, 8-degree	9	<0.0001	1155.670	error	1.916E+02	
power [b]	**8**	**<0.0001**	**1077.695**	**2.003E+02**	**1.357E+02**	**power bound hit (power = 1)**
Hill, unrestricted	6	0.001	1039.481	2.204E-01	error	unrestricted (n = 0.32)
power, unrestricted [c]	7	0.002	1037.474	1.963E-01	2.484E-02	unrestricted (power = 0.305)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.22.2. *Output for Selected Model: Power*

Li et al., 1997: FSH

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\1\Blood\72_Li_1997_FSH_Pwr_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\72_Li_1997_FSH_Pwr_1.plt
                                              Mon Feb 08 13:36:35 2010
 ================================================================

 Figure 3: FSH in female S-D rats 24hr after dosing, 22 day old rats
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   The power is restricted to be greater than or equal to 1
```

The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 10
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	9.8191
rho =	0
control =	22.1591
slope =	52.284
power =	0.294106

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	control	slope
lalpha	1	-0.99	-0.29	-0.033
rho	-0.99	1	0.2	0.033
control	-0.29	0.2	1	-0.36
slope	-0.033	0.033	-0.36	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	3.50054	1.225	1.09958	5.9015
rho	1.27087	0.241869	0.796814	1.74492
control	87.4348	12.9347	62.0833	112.786
slope	0.492306	0.0919718	0.312044	0.672567
power	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	23.9	87.4	29.6	98.6	-2.04
0.266	10	22.2	87.6	48.5	98.7	-2.1
0.7988	10	85.2	87.8	94.3	98.9	-0.0832
2.097	10	73.3	88.5	48.5	99.4	-0.483
5.867	10	126	90.3	159	101	1.12
15	10	132	94.8	116	104	1.14
43.33	10	117	109	51.2	113	0.223
119.9	10	304	146	154	137	3.65
386	10	347	277	151	205	1.07
1172	10	455	664	286	358	-1.85

```
Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -535.687163          11    1093.374327
              A2         -496.367061          20    1032.734122
              A3         -502.709623          12    1029.419246
          fitted         -534.847518           4    1077.695035
               R         -574.835246           2    1153.670492


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              156.936         18          <.0001
  Test 2              78.6402          9          <.0001
  Test 3              12.6851          8          0.1232
  Test 4              64.2758          8          <.0001

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


               Benchmark Dose Computation

Specified effect =             1

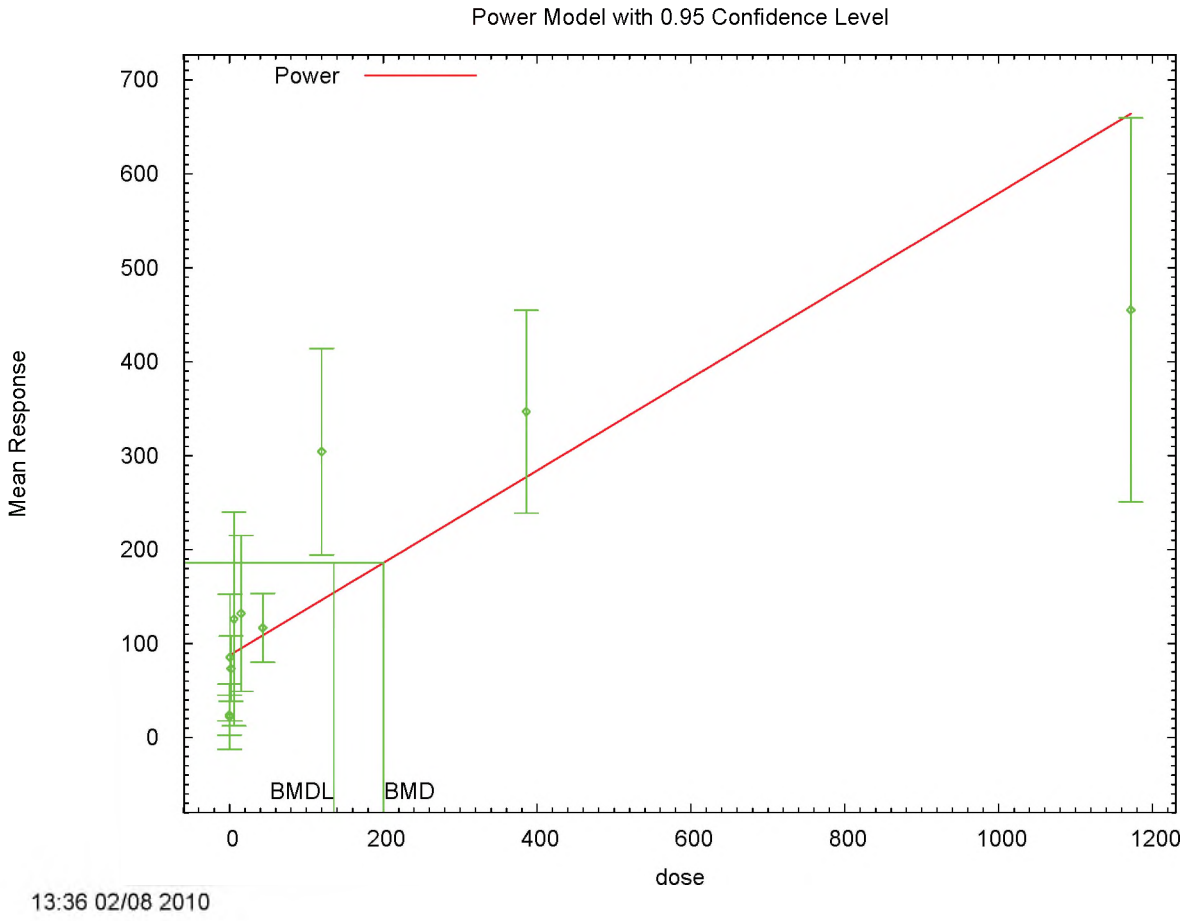
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 200.314
```

```
BMDL = 135.673
```

E.2.22.3. *Figure for Selected Model: Power*



E.2.22.4. *Output for Additional Model Presented: Power, Unrestricted*

Li et al., 1997: FSH

```
================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\1\Blood\72_Li_1997_FSH_Pwr_U_1.(d)
        Gnuplot Plotting File:  C:\1\Blood\72_Li_1997_FSH_Pwr_U_1.plt
                                          Mon Feb 08 13:36:46 2010
================================================================

Figure 3: FSH in female S-D rats 24hr after dosing, 22 day old rats

  The form of the response function is:

  Y[dose] = control + slope * dose^power


  Dependent variable = Mean
```

Independent variable = Dose
The power is not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 10
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	9.8191
rho =	0
control =	22.1591
slope =	52.284
power =	0.294106

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.99	-0.69	-0.06	0.26
rho	-0.99	1	0.65	0.0089	-0.23
control	-0.69	0.65	1	-0.23	0.029
slope	-0.06	0.0089	-0.23	1	-0.85
power	0.26	-0.23	0.029	-0.85	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	3.67487	1.12134	1.47708	5.87265
rho	1.17882	0.221526	0.744632	1.613
control	15.8201	6.87715	2.34113	29.299
slope	52.528	9.46821	33.9706	71.0853
power	0.304867	0.0336805	0.238855	0.37088

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	23.9	15.8	29.6	32	0.795
0.266	10	22.2	50.9	48.5	63.7	-1.43
0.7988	10	85.2	64.9	94.3	73.5	0.876
2.097	10	73.3	81.7	48.5	84.1	-0.314
5.867	10	126	106	159	98.1	0.652
15	10	132	136	116	114	-0.102
43.33	10	117	182	51.2	135	-1.52
119.9	10	304	242	154	160	1.24
386	10	347	339	151	195	0.134
1172	10	455	469	286	236	-0.182

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)

```
            Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
        Model A3 uses any fixed variance parameters that
        were specified by the user

 Model  R:         Yi = Mu + e(i)
             Var{e(i)} = Sigma^2


                       Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1         -535.687163          11    1093.374327
              A2         -496.367061          20    1032.734122
              A3         -502.709623          12    1029.419246
           fitted        -513.737215           5    1037.474431
               R         -574.835246           2    1153.670492


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              156.936         18          <.0001
   Test 2              78.6402          9          <.0001
   Test 3              12.6851          8          0.1232
   Test 4              22.0552          7        0.002485

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


                Benchmark Dose Computation

Specified effect =             1

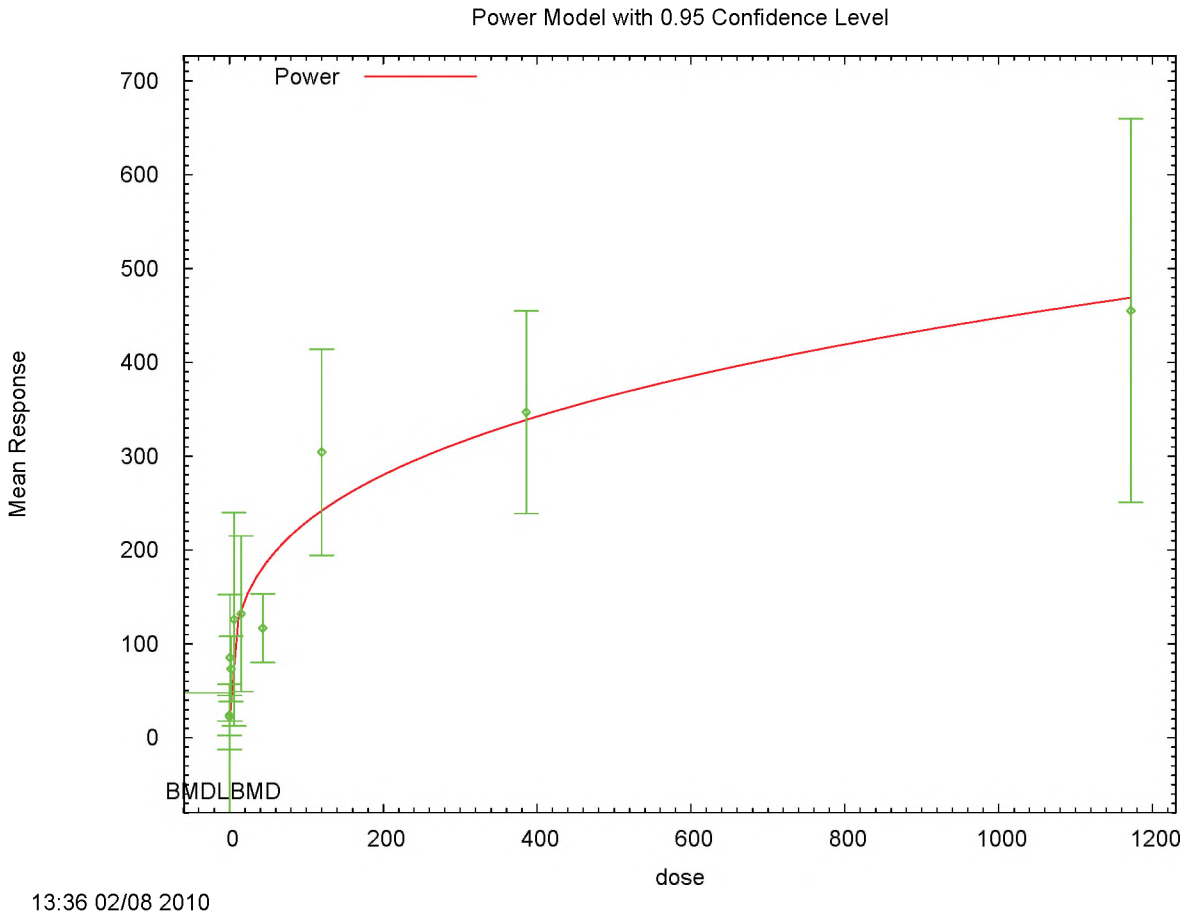
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD = 0.196278


             BMDL = 0.0248364
```

E.2.22.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.2.23. Li et al., 2006: Estradiol, 3-Day

E.2.23.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	0.156	269.027	1.416E+01	5.544E+00	
exponential (M3)	2	0.156	269.027	1.416E+01	5.544E+00	power hit bound (d = 1)
exponential (M4)	1	0.341	268.212	error	error	
exponential (M5)	0	N/A	270.212	error	error	
Hill	0	N/A	270.212	error	error	
linear [b]	**2**	**0.162**	**268.952**	**1.606E+01**	**5.379E+00**	
polynomial, 3-degree	2	0.162	268.952	1.606E+01	5.379E+00	
power	2	0.162	268.952	1.606E+01	5.379E+00	power bound hit (power = 1)
Hill, unrestricted	0	N/A	270.265	9.273E+12	9.273E+12	unrestricted (n = 0.03)
power, unrestricted	1	0.328	268.265	9.455E+10	error	unrestricted (power = 0.015)

[a] Constant variance model selected ($p = 0.4372$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.23.2. *Output for Selected Model: Linear*

Li et al., 2006: Estradiol, 3-Day

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\Blood\31_Li_2006_Estra_LinearCV_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\31_Li_2006_Estra_LinearCV_1.plt
                                          Mon Feb 08 10:54:00 2010
 ================================================================

 Figure 3, 3-day estradiol
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit
```

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	267.211	
rho =	0	Specified
beta_0 =	16.1705	
beta_1 =	1.0106	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	2.1e-012	5e-014
beta_0	2.1e-012	1	-0.69
beta_1	5e-014	-0.69	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	263.435	58.9057	147.981	378.888
beta_0	16.1705	3.55949	9.19407	23.147
beta_1	1.0106	1.2148	-1.37037	3.39156

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	10.2	16.2	12.2	16.2	-1.17
0.1588	10	19.9	16.3	20	16.2	0.697
2.839	10	24.7	19	14.6	16.2	1.11
5.124	10	18.1	21.3	17.6	16.2	-0.635

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-129.653527	5	269.307054
A2	-128.294657	8	272.589314
A3	-129.653527	5	269.307054
fitted	-131.476097	3	268.952193
R	-131.819169	2	267.638338

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	7.04902	6	0.3163
Test 2	2.71774	3	0.4372
Test 3	2.71774	3	0.4372
Test 4	3.64514	2	0.1616

The p-value for Test 1 is greater than .05. There may not be a
diffence between responses and/or variances among the dose levels
Modelling the data with a dose/response curve may not be appropriate

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

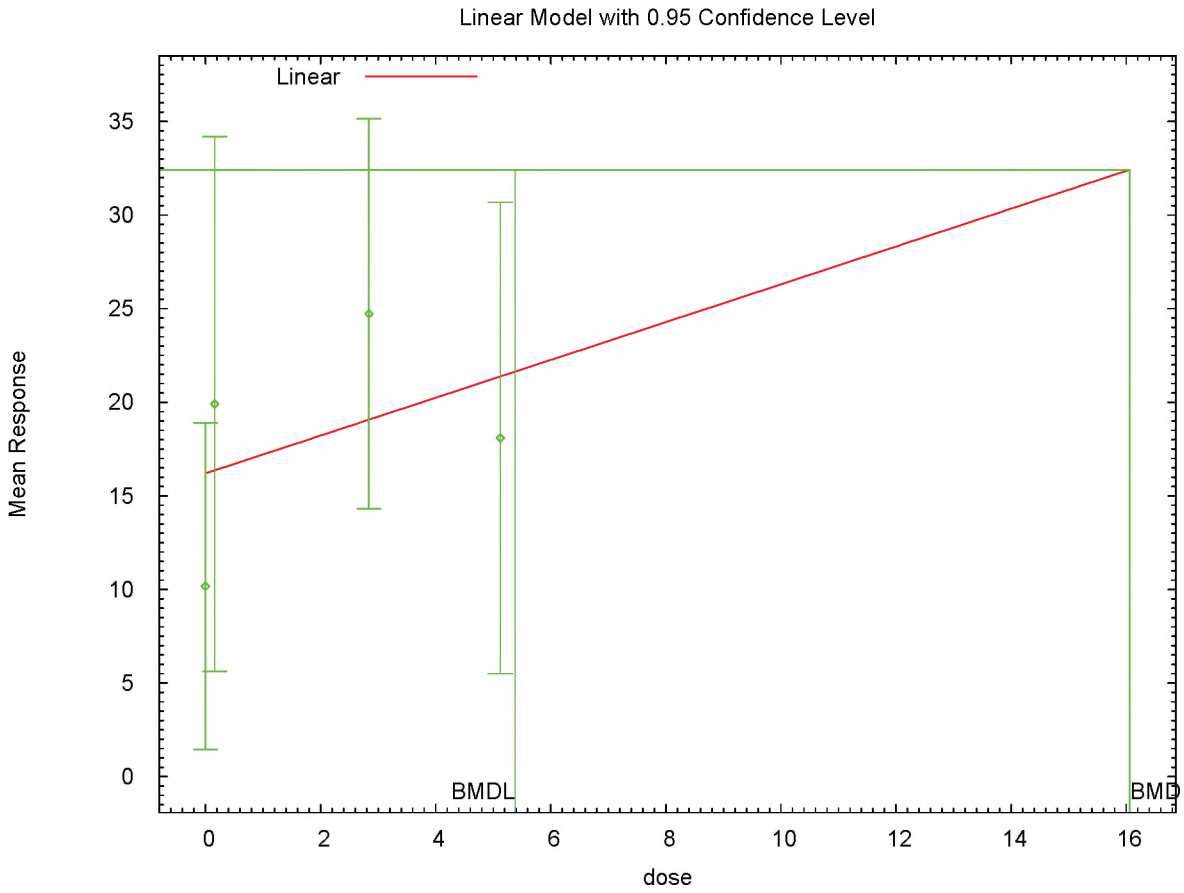
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 16.0605

BMDL = 5.37895

E.2.23.3. *Figure for Selected Model: Linear*



E.2.24. Li et al., 2006: Progesterone, 3-Day

E.2.24.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	<0.001	329.928	2.619E+00	error	
exponential (M3)	2	0.001	328.101	1.340E-01	error	power hit bound (d = 1)
exponential (M4)	1	0.384	315.734	1.074E-02	6.633E-03	
exponential (M5)	0	N/A	317.734	4.301E-02	4.272E-03	
Hill [b]	**1**	**0.386**	**315.728**	**9.461E-04**	**8.006E-11**	**n lower bound hit (n = 1)**
linear	2	<0.001	330.729	3.891E+00	2.626E+00	
polynomial, 3-degree	2	<0.001	330.729	3.891E+00	2.626E+00	
power	2	<0.001	330.729	3.891E+00	2.626E+00	power bound hit (power = 1)
power, unrestricted	1	0.404	315.673	2.812E-59	2.812E-59	unrestricted (power = 0.01)

[a] Non-constant variance model selected ($p = 0.0013$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.24.2. *Output for Selected Model: Hill*

Li et al., 2006: Progesterone, 3-Day

```
 ======================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\Blood\32_Li_2006_Progest_Hill_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\32_Li_2006_Progest_Hill_1.plt
                                                 Wed Feb 10 10:57:14 2010
 ======================================================================

 Figure 4, 3-day progesterone
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
```

```
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                        lalpha =      7.08699
                           rho =            0
                     intercept =      61.7404
                             v =     -50.3835
                             n =      1.47286
                             k =     0.128302


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -n
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 lalpha          rho    intercept            v            k

    lalpha            1        -0.99       -0.093         0.82         0.22

       rho        -0.99            1         0.12        -0.79         -0.2

 intercept       -0.093         0.12            1        -0.43        0.014

         v         0.82        -0.79        -0.43            1        0.035

         k         0.22         -0.2        0.014        0.035            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha          14.0902          3.36095             7.50284             20.6775
            rho         -2.27438         0.861553              -3.963            -0.585772
      intercept          61.7488           3.3373             55.2078             68.2898
              v         -42.1007          7.70852            -57.2091            -26.9922
              n                1               NA
              k       0.00282851         0.020619           -0.037584           0.0432411

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    10       61.7         61.7         11.1         10.6       -0.00251
0.1588    10       30.6         20.4         40.5         37.2          0.865
2.839     10       16.9         19.7         33.3         38.7         -0.225
5.124     10       11.4         19.7         43.7         38.8         -0.678


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
```

```
            Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
             Var{e(i)} = Sigma^2


                        Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1         -159.632675            5     329.265349
              A2         -151.812765            8     319.625529
              A3         -152.488175            6     316.976349
           fitted        -152.863841            5     315.727683
               R         -165.698875            2     335.397750


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                      Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              27.7722          6       0.0001037
   Test 2              15.6398          3        0.001344
   Test 3              1.35082          2          0.5089
   Test 4             0.751333          1          0.3861

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =            1

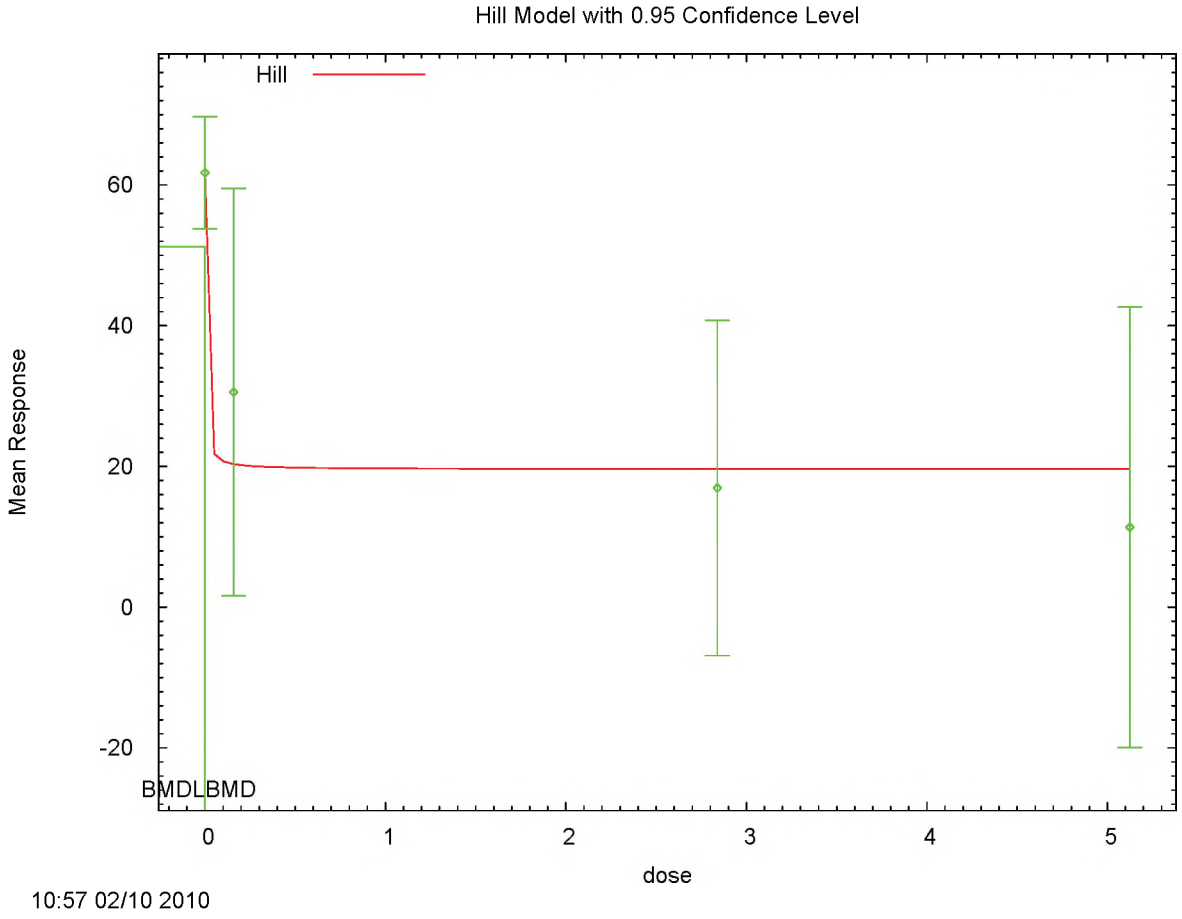
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =    0.000946102

            BMDL =  8.00639e-011
```

E.2.24.3. *Figure for Selected Model: Hill*



E.2.25. Markowski et al., 2001: FR10 Run Opportunities

E.2.25.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2) [b]	**2**	**0.304**	**117.150**	**8.570E+00**	**2.887E+00**	
exponential (M3)	2	0.304	117.150	8.570E+00	2.887E+00	power hit bound (d = 1)
exponential (M4)	1	0.371	117.570	3.452E+00	1.299E-02	
exponential (M5)	0	N/A	118.918	2.315E+00	1.391E-02	
Hill	0	N/A	118.918	1.801E+00	1.274E-09	
linear	2	0.226	117.744	1.106E+01	5.741E+00	
polynomial, 3-degree	2	0.226	117.744	1.106E+01	5.741E+00	
power	2	0.226	117.744	1.106E+01	5.741E+00	power bound hit (power = 1)
power, unrestricted	1	0.239	118.158	5.768E+00	1.032E-14	unrestricted (power = 0.276)

[a] Constant variance model selected ($p = 0.1719$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.25.2. *Output for Selected Model: Exponential (M2)*

Markowski et al., 2001: FR10 Run Opportunities

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\33_Mark_2001_FR10opp_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                 Mon Feb 08 10:55:13 2010
 ================================================================

 Table 3
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

```
Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact
```

Initial Parameter Values

Variable	Model 2
lnalpha	3.5321
rho(S)	0
a	6.77975
b	0.0581937
c	0
d	1

(S) = Specified

Parameter Estimates

Variable	Model 2
lnalpha	3.63127
rho	0
a	12.2901
b	0.0808832
c	0
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	7	13.29	8.65
1.557	4	11.25	5.56
4.03	6	5.75	3.53
10.32	7	7	6.01

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	12.29	6.145	0.4305
1.557	10.84	6.145	0.1347
4.03	8.871	6.145	-1.244
10.32	5.335	6.145	0.717

```
Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
```

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                  Model      Log(likelihood)      DF         AIC
                 -------    -----------------    ----   ------------
                     A1       -54.38526           5      118.7705
                     A2       -51.88568           8      119.7714
                     A3       -54.38526           5      118.7705
                      R       -57.45429           2      118.9086
                      2       -55.57522           3      117.1504


Additive constant for all log-likelihoods =     -22.05.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does Model 2 fit the data? (A3 vs. 2)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                       11.14             6            0.08423
  Test 2                       4.999             3             0.1719
  Test 3                       4.999             3             0.1719
  Test 4                        2.38             2             0.3042


  The p-value for Test 1 is greater than .05.  There may not be a
  diffence between responses and/or variances among the dose levels
  Modelling the data with a dose/response curve may not be appropriate.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 4 is greater than .1.  Model 2 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

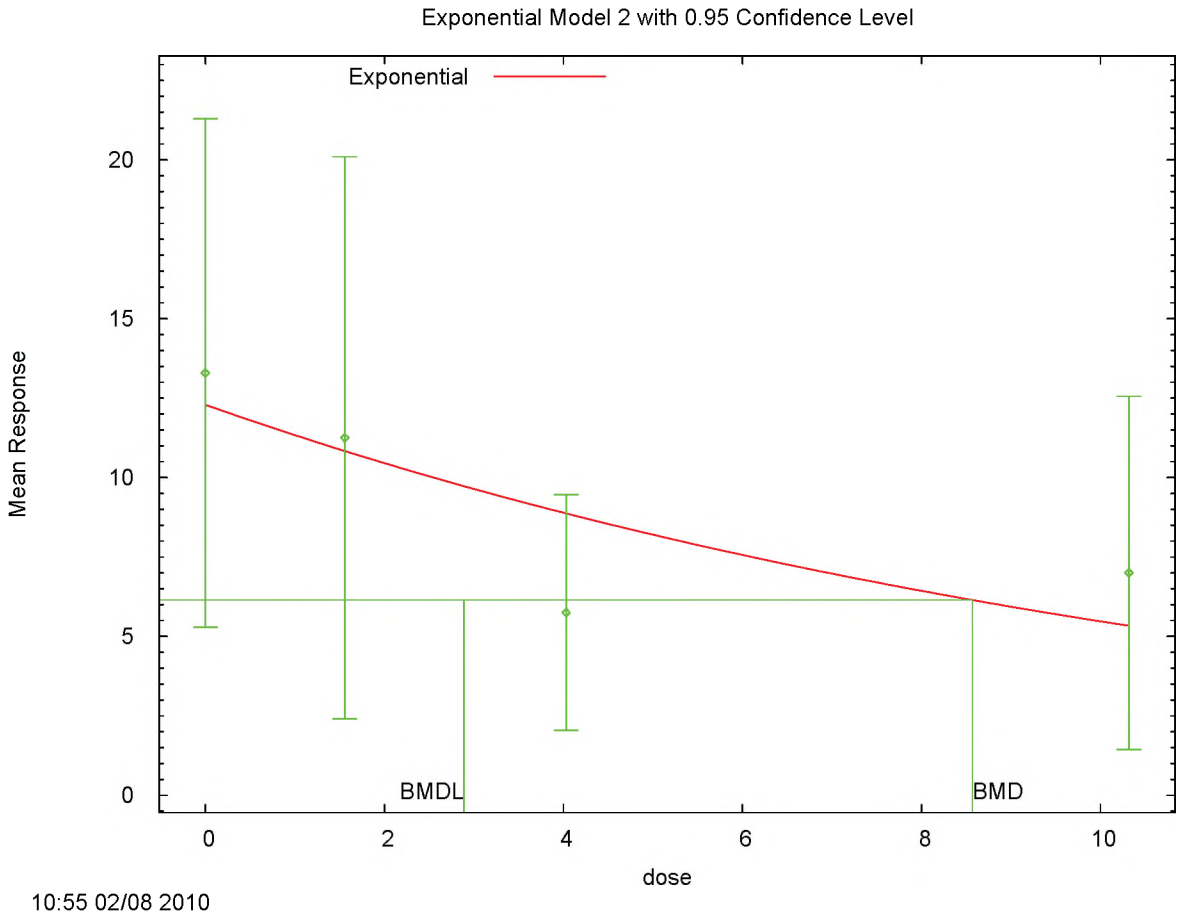
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      8.56961

              BMDL =      2.88708
```

E.2.25.3. *Figure for Selected Model: Exponential (M2)*



E.2.26. Markowski et al., 2001: FR2 Revolutions

E.2.26.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	0.236	217.219	8.486E+00	3.232E+00	
exponential (M3)	2	0.236	217.219	8.486E+00	3.232E+00	power hit bound (d = 1)
exponential (M4)	1	0.263	217.583	3.413E+00	1.766E-02	
exponential (M5)	0	N/A	218.532	2.415E+00	9.313E-01	
Hill [b]	**1**	**0.654**	**216.532**	**1.840E+00**	**5.992E-01**	**n upper bound hit (n = 18)**
linear	2	0.180	217.764	1.058E+01	5.602E+00	
polynomial, 3-degree	2	0.180	217.764	1.058E+01	5.602E+00	
power	2	0.180	217.764	1.058E+01	5.602E+00	power bound hit (power = 1)
power, unrestricted [c]	1	0.161	218.294	5.739E+00	1.032E-14	unrestricted (power = 0.318)

[a] Constant variance model selected ($p = 0.1092$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.26.2. *Output for Selected Model: Hill*

Markowski et al., 2001: FR2 Revolutions

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\Blood\34_Mark_2001_FR2rev_HillCV_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\34_Mark_2001_FR2rev_HillCV_1.plt
                                          Mon Feb 08 10:55:47 2010
 ================================================================

 Table 3
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit
```

```
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =      2598.74
                            rho =            0   Specified
                      intercept =       119.29
                              v =       -62.79
                              n =      2.13752
                              k =      2.53662


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -n
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                    alpha    intercept            v            k

      alpha             1     1.2e-008       1e-009     3.5e-008

  intercept     1.2e-008            1        -0.81        -0.52

          v       1e-009        -0.81            1         0.37

          k     3.5e-008        -0.52         0.37            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha          2183.85          630.425             948.245             3419.46
       intercept           119.29          17.6629             84.6713             153.909
               v         -56.5223          21.9082            -99.4615            -13.5831
               n               18               NA
               k          1.68653         0.295154             1.10804             2.26502

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     7        119          119         69.9         46.7     -2.41e-007
1.557     4        109          108           61         46.7      2.29e-007
 4.03     6       56.5         62.8         31.2         46.7         -0.329
10.32     7       68.1         62.8         33.2         46.7          0.304


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
```

```
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -104.165520            5     218.331040
             A2         -101.140174            8     218.280349
             A3         -104.165520            5     218.331040
          fitted        -104.266162            4     216.532324
              R         -107.599268            2     219.198536


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              12.9182          6         0.04435
   Test 2              6.05069          3          0.1092
   Test 3              6.05069          3          0.1092
   Test 4             0.201284          1          0.6537

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =            1

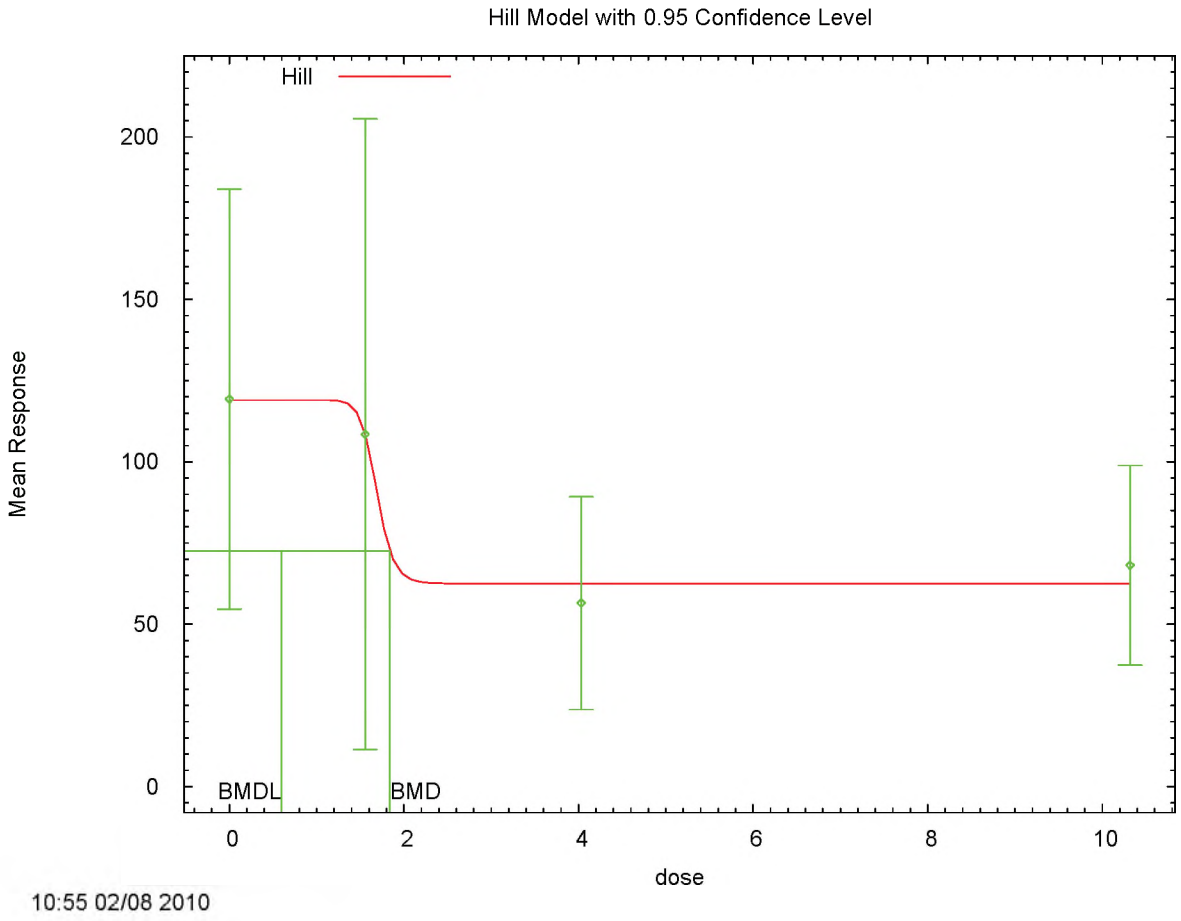
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       1.83952

            BMDL =      0.599228
```

E.2.26.3. *Figure for Selected Model: Hill*



E.2.26.4. *Output for Additional Model Presented: Power, Unrestricted*

Markowski et al., 2001: FR2 Revolutions

```
 ================================================================
          Power Model. (Version: 2.15;  Date: 04/07/2008)
          Input Data File: C:\1\Blood\34_Mark_2001_FR2rev_PowerCV_U_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\34_Mark_2001_FR2rev_PowerCV_U_1.plt
                                                Mon Feb 08 10:55:49 2010
 ================================================================

 Table 3
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the response function is:

    Y[dose] = control + slope * dose^power


    Dependent variable = Mean
    Independent variable = Dose
    rho is set to 0
    The power is not restricted
    A constant variance model is fit
```

```
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                         alpha =      2598.74
                           rho =            0   Specified
                       control =       119.29
                         slope =     -10.3599
                         power =     0.824761


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha      control        slope        power

     alpha            1      -3e-010     6.9e-010     9.9e-010

   control      -3e-010            1        -0.63        -0.28

     slope     6.9e-010        -0.63            1         0.87

     power     9.9e-010        -0.28         0.87            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha          2350.22          678.449            1020.48             3679.95
        control          120.082          18.0782            84.6491             155.514
          slope         -27.8164          24.2447           -75.3352             19.7023
          power         0.317923         0.350841          -0.369713             1.00556


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     7        119          120         69.9         48.5        -0.0432
1.557     4        109         88.1           61         48.5          0.843
 4.03     6       56.5         76.8         31.2         48.5          -1.02
10.32     7       68.1         61.7         33.2         48.5          0.353


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
```

were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-104.165520	5	218.331040
A2	-101.140174	8	218.280349
A3	-104.165520	5	218.331040
fitted	-105.147159	4	218.294317
R	-107.599268	2	219.198536

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	12.9182	6	0.04435
Test 2	6.05069	3	0.1092
Test 3	6.05069	3	0.1092
Test 4	1.96328	1	0.1612

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

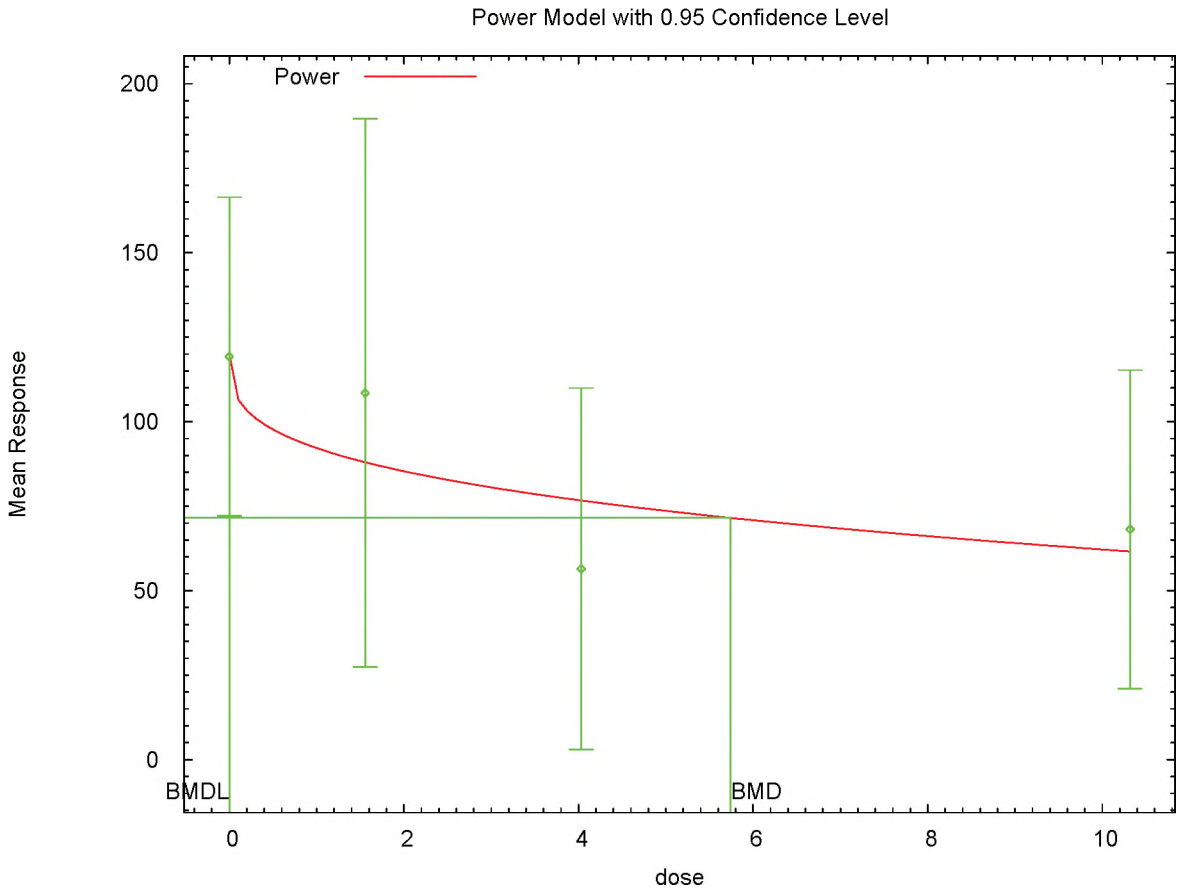
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 5.73906

BMDL = 1.03181e-014

E.2.26.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.2.27. Markowski et al., 2001: FR5 Run Opportunities

E.2.27.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	2	0.205	133.193	5.078E+00	2.439E+00	
exponential (M3)	2	0.205	133.193	5.078E+00	2.439E+00	power hit bound (d = 1)
exponential (M4)	1	0.254	133.328	2.160E+00	6.854E-01	
exponential (M5)	0	N/A	134.032	2.124E+00	9.667E-01	
Hill [b]	**1**	**0.939**	**132.032**	**1.723E+00**	**9.085E-01**	**n upper bound hit (n = 18)**
linear	2	0.122	134.229	7.234E+00	4.430E+00	
polynomial, 3-degree	2	0.122	134.229	7.234E+00	4.430E+00	
power	2	0.122	134.229	7.234E+00	4.430E+00	power bound hit (power = 1)
power, unrestricted [c]	1	0.134	134.268	2.666E+00	1.032E-14	unrestricted (power = 0.392)

[a] Constant variance model selected ($p = 0.2262$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.27.2. *Output for Selected Model: Hill*

Markowski et al., 2001: FR5 Run Opportunities

```
 ================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\Blood\35_Mark_2001_FR5opp_HillCV_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\35_Mark_2001_FR5opp_HillCV_1.plt
                                                Mon Feb 08 10:56:24 2010
 ================================================================

 Table 3
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit
```

```
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =      77.4849
                            rho =            0   Specified
                      intercept =        26.14
                              v =       -13.34
                              n =      2.77257
                              k =      2.48811


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -n
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                    alpha    intercept            v            k

      alpha             1    -3.2e-009     1.9e-008     6.2e-008

  intercept    -3.2e-009            1        -0.81        -0.51

          v     1.9e-008        -0.81            1         0.36

          k     6.2e-008        -0.51         0.36            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha          64.5863          18.6445             28.0438             101.129
       intercept            26.14          3.03753             20.1865             32.0935
               v         -13.1569           3.7676            -20.5413            -5.77257
               n               18               NA
               k          1.68073         0.208677             1.27173             2.08973

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     7       26.1         26.1         12.3         8.04      -1.9e-008
1.557     4       23.5         23.5         7.04         8.04     -1.94e-007
 4.03     6       12.8           13         6.17         8.04        -0.0558
10.32     7       13.1           13         7.14         8.04         0.0517


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
```

```
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                        Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1          -62.013133            5     134.026266
              A2          -59.839035            8     135.678070
              A3          -62.013133            5     134.026266
           fitted         -62.016025            4     132.032049
               R          -67.530040            2     139.060081


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                      Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1               15.382          6         0.01748
   Test 2               4.3482          3          0.2262
   Test 3               4.3482          3          0.2262
   Test 4           0.00578335          1          0.9394

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =             1

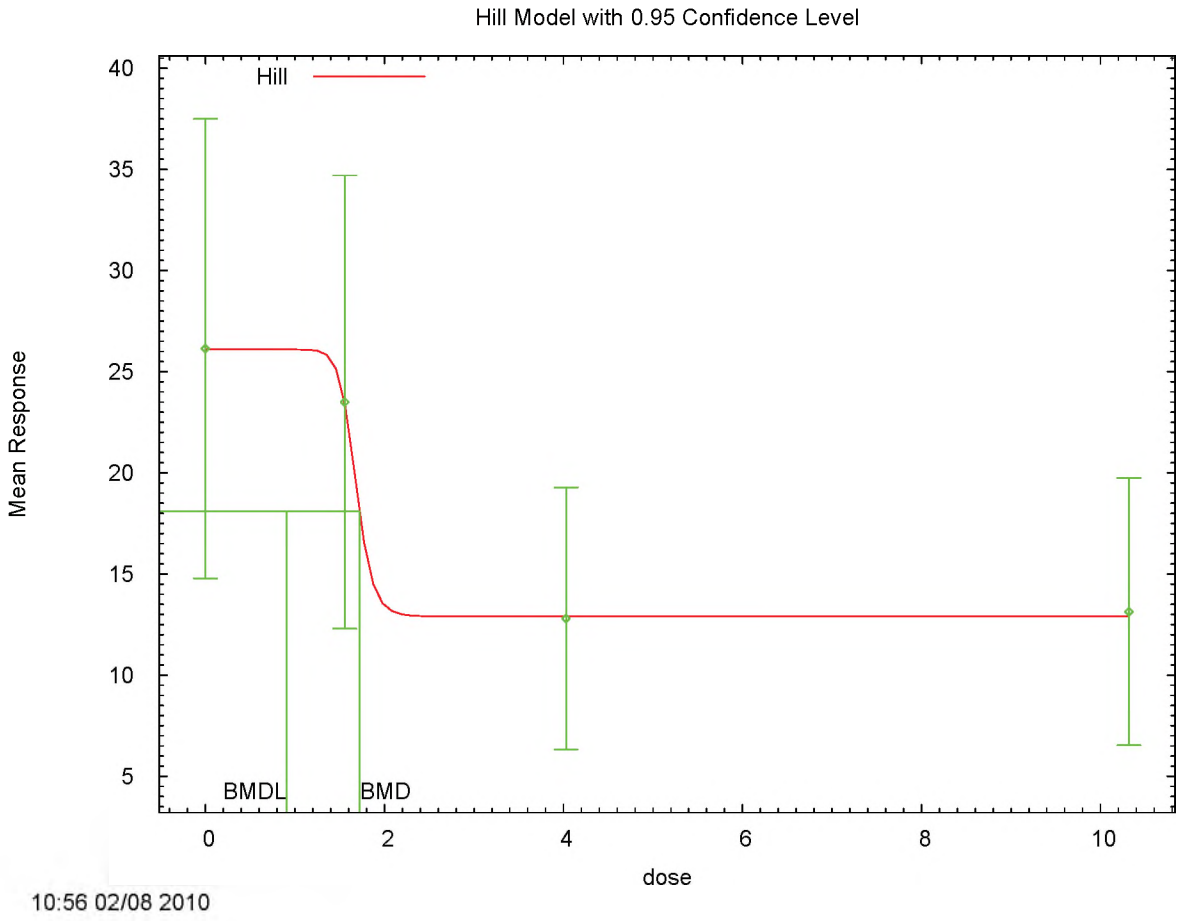
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       1.72335

            BMDL =      0.908491
```

E.2.27.3. *Figure for Selected Model: Hill*



E.2.27.4. *Output for Additional Model Presented: Power, Unrestricted*

Markowski et al., 2001: FR5 Run Opportunities

```
 ================================================================
          Power Model. (Version: 2.15;  Date: 04/07/2008)
          Input Data File: C:\1\Blood\35_Mark_2001_FR5opp_PwrCV_U_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\35_Mark_2001_FR5opp_PwrCV_U_1.plt
                                                Mon Feb 08 10:56:24 2010
 ================================================================

 Table 3
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The power is not restricted
   A constant variance model is fit
```

```
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                Default Initial Parameter Values
                        alpha =      77.4849
                          rho =            0   Specified
                      control =        26.14
                        slope =      -2.3827
                        power =     0.844532


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha      control        slope        power

     alpha            1    -9.3e-009     1.4e-008     9.3e-009

   control    -9.3e-009            1        -0.64        -0.34

     slope     1.4e-008        -0.64            1          0.9

     power     9.3e-009        -0.34          0.9            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha          70.8926          20.4649            30.7821             111.003
        control          26.3582          3.12902            20.2254             32.4909
          slope         -5.73309          4.02937           -13.6305             2.16433
          power         0.391903         0.281862          -0.160536            0.944342


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     7       26.1         26.4         12.3         8.42        -0.0686
1.557     4       23.5         19.5         7.04         8.42          0.941
 4.03     6       12.8         16.5         6.17         8.42          -1.06
10.32     7       13.1           12         7.14         8.42          0.343


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
```

were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-62.013133	5	134.026266
A2	-59.839035	8	135.678070
A3	-62.013133	5	134.026266
fitted	-63.134001	4	134.268002
R	-67.530040	2	139.060081

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	15.382	6	0.01748
Test 2	4.3482	3	0.2262
Test 3	4.3482	3	0.2262
Test 4	2.24174	1	0.1343

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

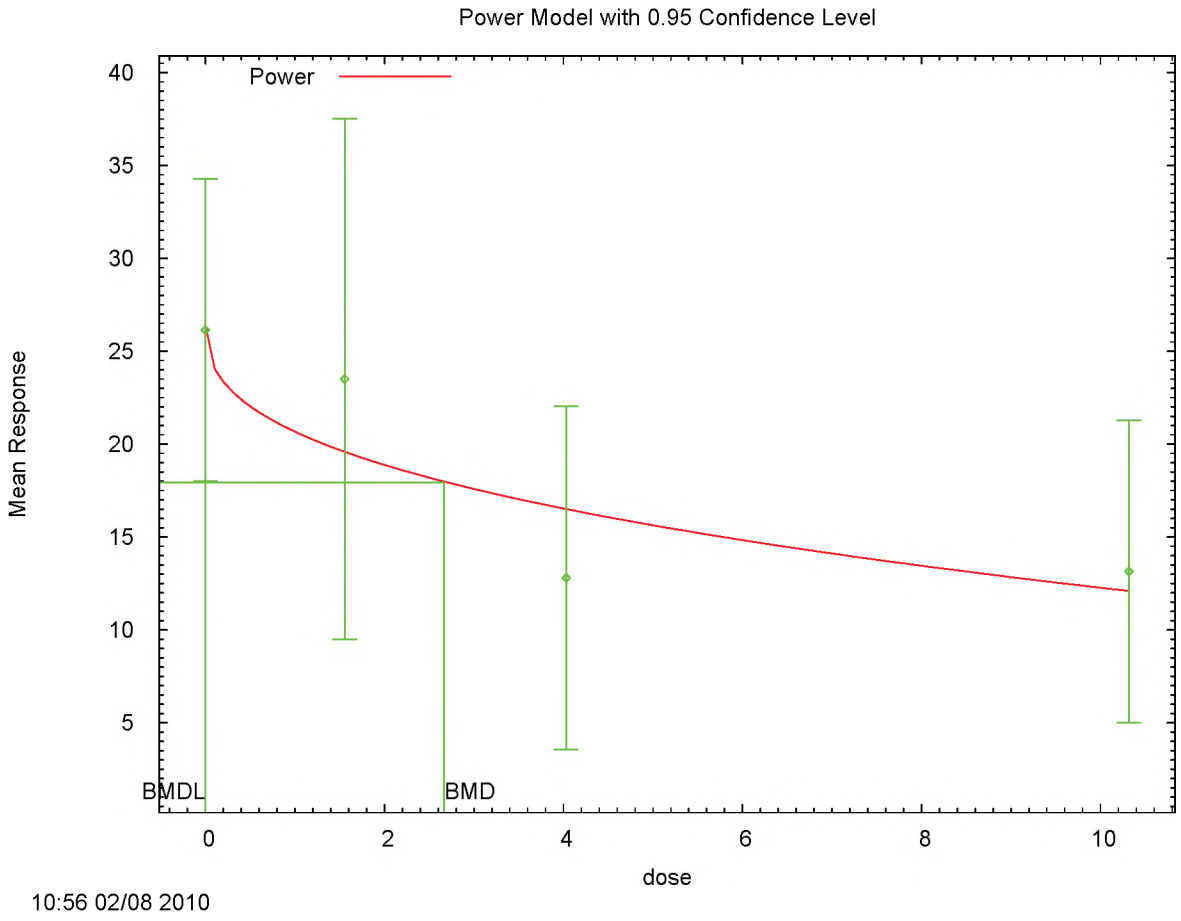
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 2.66625

BMDL = 1.03181e-014

E.2.27.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.2.28. Miettinen et al., 2006: Cariogenic Lesions, Pups

E.2.28.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	3	0.410	162.280	3.401E+00	1.889E+00	power bound hit (power = 1)
logistic	3	0.371	162.518	4.108E+00	2.450E+00	
log-logistic [a]	**3**	**0.602**	**161.292**	**1.428E+00**	**5.175E-01**	**slope bound hit (slope = 1)**
log-probit	3	0.300	163.040	6.321E+00	3.127E+00	slope bound hit (slope = 1)
multistage, 4-degree	3	0.410	162.280	3.401E+00	1.889E+00	final ß = 0
probit	3	0.350	162.656	4.548E+00	2.889E+00	
Weibull	3	0.410	162.280	3.401E+00	1.889E+00	power bound hit (power = 1)
gamma, unrestricted	2	0.798	161.801	3.374E-03	8.884E-242	unrestricted (power = 0.215)
log-logistic, unrestricted [b]	2	0.728	161.983	4.942E-02	error	unrestricted (slope = 0.465)
log-probit, unrestricted	2	0.732	161.972	6.495E-02	error	unrestricted (slope = 0.289)
Weibull, unrestricted	2	0.766	161.884	1.792E-02	error	unrestricted (power = 0.324)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.2.28.2. *Output for Selected Model: Log-Logistic*

Miettinen et al., 2006: Cariogenic Lesions, Pups

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\Blood\36_Miet_2006_Cariogenic_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\36_Miet_2006_Cariogenic_LogLogistic_1.plt
                                              Mon Feb 08 10:56:59 2010
 ================================================================

 Table 2 converting the percentage into the number of animals, and control is Control II from the
study. Dose is in ng per kg and is from Table 1
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
```

```
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                  Default Initial Parameter Values
                     background =     0.595238
                      intercept =       -2.494
                          slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             background    intercept

background            1        -0.66

 intercept        -0.66            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background         0.644165            *                *                   *
      intercept         -2.55354            *                *                   *
          slope                1            *                *                   *

* - Indicates that this value is not calculated.



                         Analysis of Deviance Table

        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -77.6769         5
   Fitted model          -78.646         2       1.93832      3          0.5853
  Reduced model         -83.2067         1       11.0597      4          0.0259

           AIC:         161.292


                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
    0.0000     0.6442        27.055    25.000          42       -0.662
    2.2195     0.6966        20.200    23.000          29        1.131
    6.2259     0.7603        19.007    19.000          25       -0.003
   16.0142     0.8416        20.198    20.000          24       -0.111
   46.6355     0.9231        29.540    29.000          32       -0.358

 Chi^2 = 1.86      d.f. = 3        P-value = 0.6024


   Benchmark Dose Computation
```

```
Specified effect =           0.1

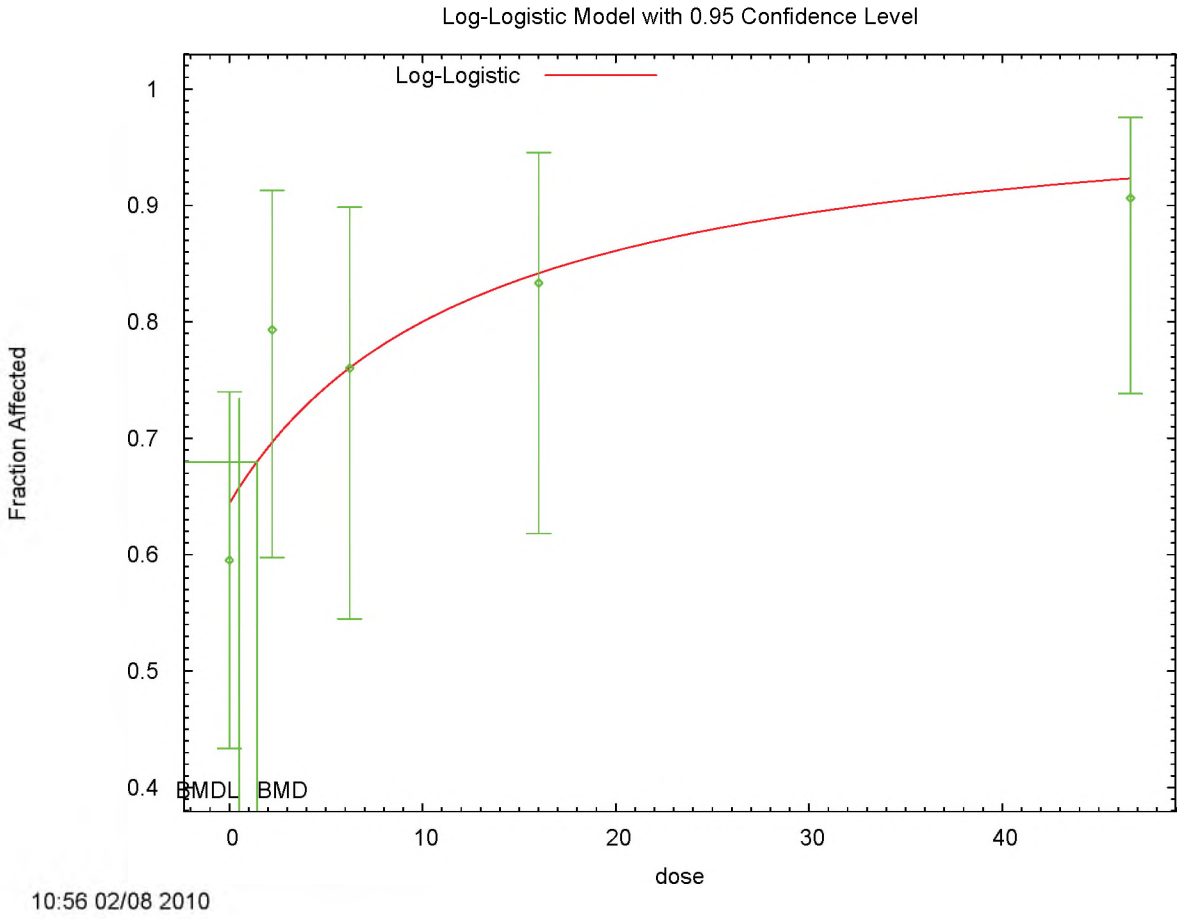
Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       1.42805

            BMDL =      0.517495
```

E.2.28.3. *Figure for Selected Model: Log-Logistic*



E.2.28.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

Miettinen et al., 2006: Cariogenic Lesions, Pups

```
================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\1\Blood\36_Miet_2006_Cariogenic_LogLogistic_U_1.(d)
        Gnuplot Plotting File:  C:\1\Blood\36_Miet_2006_Cariogenic_LogLogistic_U_1.plt
                                          Mon Feb 08 10:56:59 2010
================================================================
```

```
 Table 2 converting the percentage into the number of animals, and control is Control II from the
study. Dose is in ng per kg and is from Table 1
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =     0.595238
                     intercept =    -0.739403
                         slope =     0.442847


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.51         0.24

 intercept        -0.51            1        -0.89

     slope         0.24        -0.89            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background         0.597745            *               *                   *
      intercept        -0.798024            *               *                   *
          slope         0.465259            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -77.6769         5
   Fitted model        -77.9915         3      0.629204      2          0.7301
  Reduced model        -83.2067         1       11.0597      4          0.0259

           AIC:         161.983


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ----------------------------------------------------------------------
    0.0000     0.5977        25.105    25.000          42        -0.033
    2.2195     0.7566        21.940    23.000          29         0.458
```

```
     6.2259      0.8042        20.105     19.000          25        -0.557
    16.0142      0.8474        20.338     20.000          24        -0.192
    46.6355      0.8910        28.512     29.000          32         0.277

 Chi^2 = 0.63      d.f. = 2        P-value = 0.7281


   Benchmark Dose Computation

Specified effect =            0.1

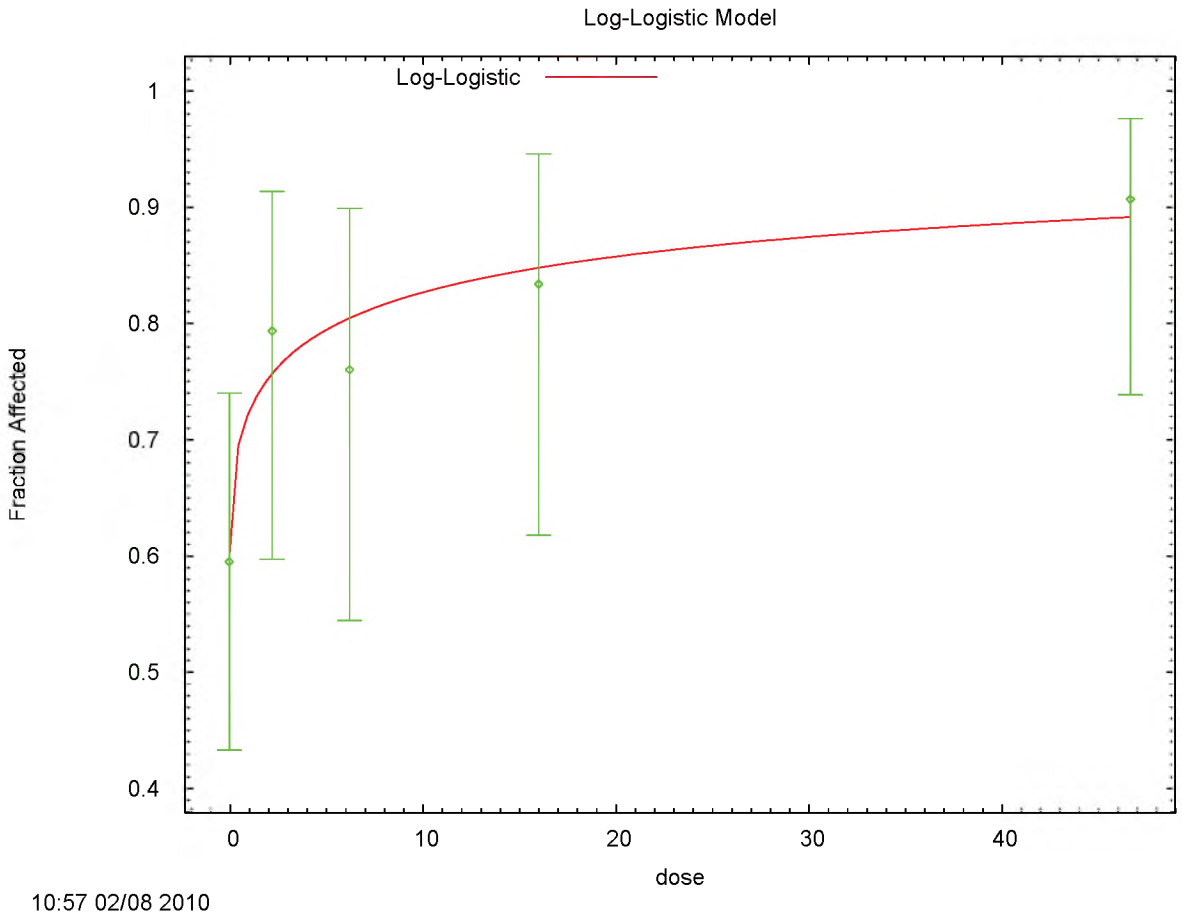
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.049422

           Benchmark dose computation failed.  Lower limit includes zero.
```

E.2.28.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.2.29. Murray et al., 1979: Fertility in F2 Generation

E.2.29.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	0	N/A	61.729	4.481E+00	1.590E+00	
logistic	1	0.051	61.318	2.420E+00	1.722E+00	negative intercept (intercept = -2.567)
log-logistic	0	N/A	61.729	4.971E+00	1.565E+00	
multistage, 1-degree	1	0.031	63.154	1.598E+00	8.747E-01	
multistage, 2-degree [a]	**1**	**0.079**	**60.464**	**2.733E+00**	**1.366E+00**	
probit	1	0.048	61.544	2.250E+00	1.590E+00	negative intercept (intercept = -1.459)
Weibull	0	N/A	61.729	5.042E+00	1.604E+00	
log-probit, unrestricted	0	N/A	61.729	4.244E+00	1.506E+00	unrestricted (slope = 3.182)

[a] Best-fitting model, BMDS output presented in this appendix

E.2.29.2. *Output for Selected Model: Multistage, 2-Degree*

Murray et al., 1979: Fertility in F2 Generation

```
 ======================================================================
         Multistage Model. (Version: 3.0;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\Murray_1979_fert_index_f2_Multi2_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\Murray_1979_fert_index_f2_Multi2_1.plt
                                                 Wed Feb 10 16:06:28 2010
 ======================================================================

 Table 1 but expressed as number of dams who do not produce offspring
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2
```

```
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =    0.0567204
                        Beta(1) =            0
                        Beta(2) =    0.0155037


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(1)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(2)

Background            1        -0.45

   Beta(2)        -0.45            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0780188           *               *                   *
        Beta(1)                0           *               *                   *
        Beta(2)        0.0141051           *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
      Full model        -25.8194         3
    Fitted model        -28.2318         2       4.82474      1         0.02805
   Reduced model        -34.0009         1        16.363      2       0.0002798

           AIC:         60.4636


                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ---------------------------------------------------------------------
    0.0000     0.0780         2.497     4.000          32         0.991
    1.1242     0.0943         1.886     0.000          20        -1.443
    5.8831     0.4341         8.683     9.000          20         0.143

 Chi^2 = 3.08      d.f. = 1        P-value = 0.0790


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =           0.95

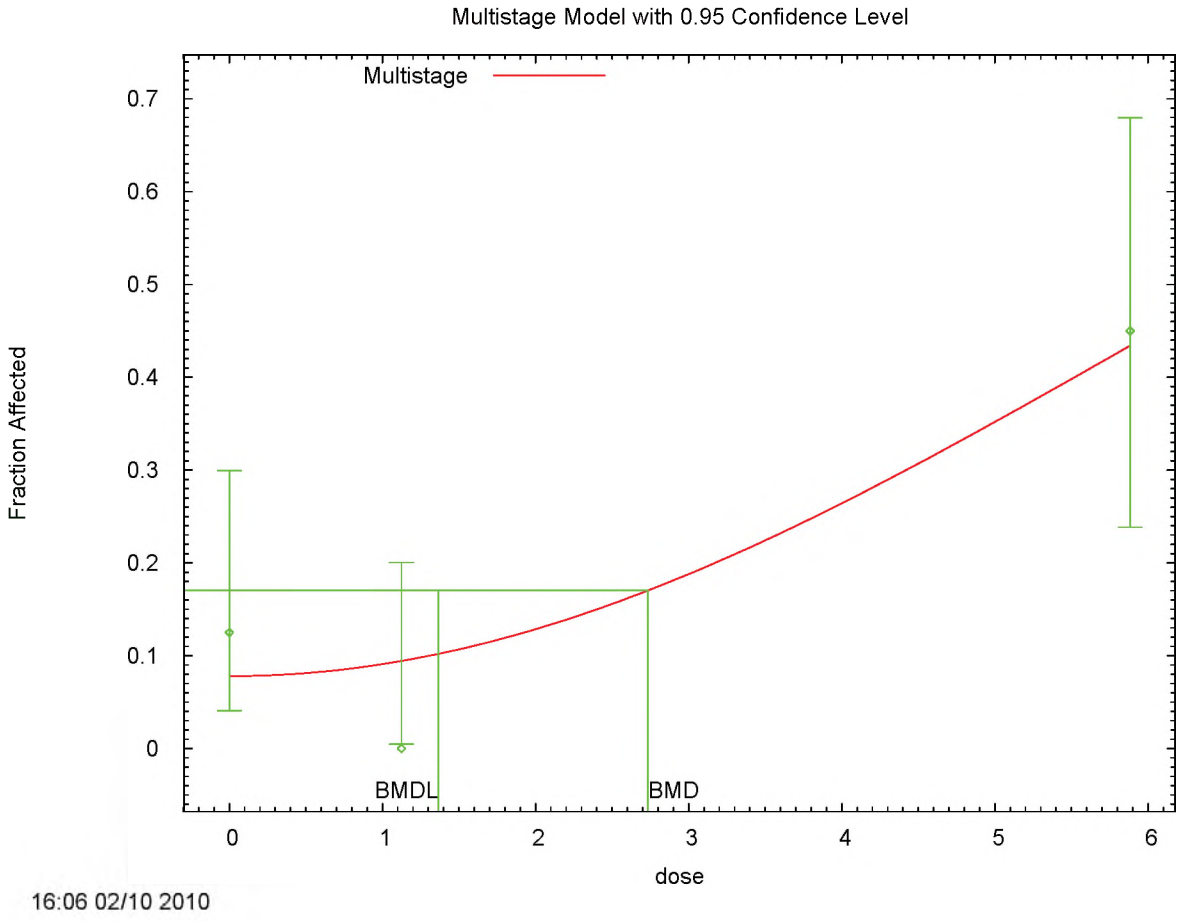
             BMD =        2.73307
```

```
                BMDL =        1.36619

                BMDU =        4.10938

Taken together, (1.36619, 4.10938) is a 90      % two-sided confidence
interval for the BMD
```

E.2.29.3. *Figure for Selected Model: Multistage, 2-Degree*



E.2.30. National Toxicology Program, 1982: Toxic Hepatitis, Male Mice

E.2.30.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	1	0.027	113.103	3.823E+00	2.005E+00	
logistic	2	0.092	110.352	3.108E+00	2.465E+00	negative intercept (intercept = -3.388)
log-logistic	1	0.026	113.089	3.797E+00	2.141E+00	
log-probit	1	0.027	113.111	3.565E+00	2.294E+00	
multistage, 3-degree [a]	**1**	**0.036**	**112.045**	**2.782E+00**	**1.343E+00**	
probit	2	0.082	110.512	2.763E+00	2.241E+00	negative intercept (intercept = -1.894)
Weibull	1	0.025	113.044	3.967E+00	1.704E+00	

[a] Best-fitting model, BMDS output presented in this appendix

E.2.30.2. *Output for Selected Model: Multistage, 3-Degree*

National Toxicology Program, 1982: Toxic Hepatitis, Male Mice

```
 ================================================================
         Multistage Model. (Version: 3.0;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\37_NTP_1982_ToxHep_Multi3_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\37_NTP_1982_ToxHep_Multi3_1.plt
                                          Mon Feb 08 10:57:32 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0471757
Beta(1) = 0.00749116
Beta(2) = 0
Beta(3) = 0.00139828

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(1)	Beta(3)
Background	1	-0.77	0.69
Beta(1)	-0.77	1	-0.95
Beta(3)	0.69	-0.95	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0267933	*	*	*
Beta(1)	0.0283198	*	*	*
Beta(2)	0	*	*	*
Beta(3)	0.0012342	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-51.0633	4			
Fitted model	-53.0224	3	3.91812	1	0.04777
Reduced model	-121.743	1	141.358	3	<.0001

AIC: 112.045

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0268	1.956	1.000	73	-0.693
0.7665	0.0482	2.363	5.000	49	1.759
2.2711	0.1005	4.925	3.000	49	-0.915
11.2437	0.8775	43.877	44.000	50	0.053

Chi^2 = 4.41 d.f. = 1 P-value = 0.0357

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

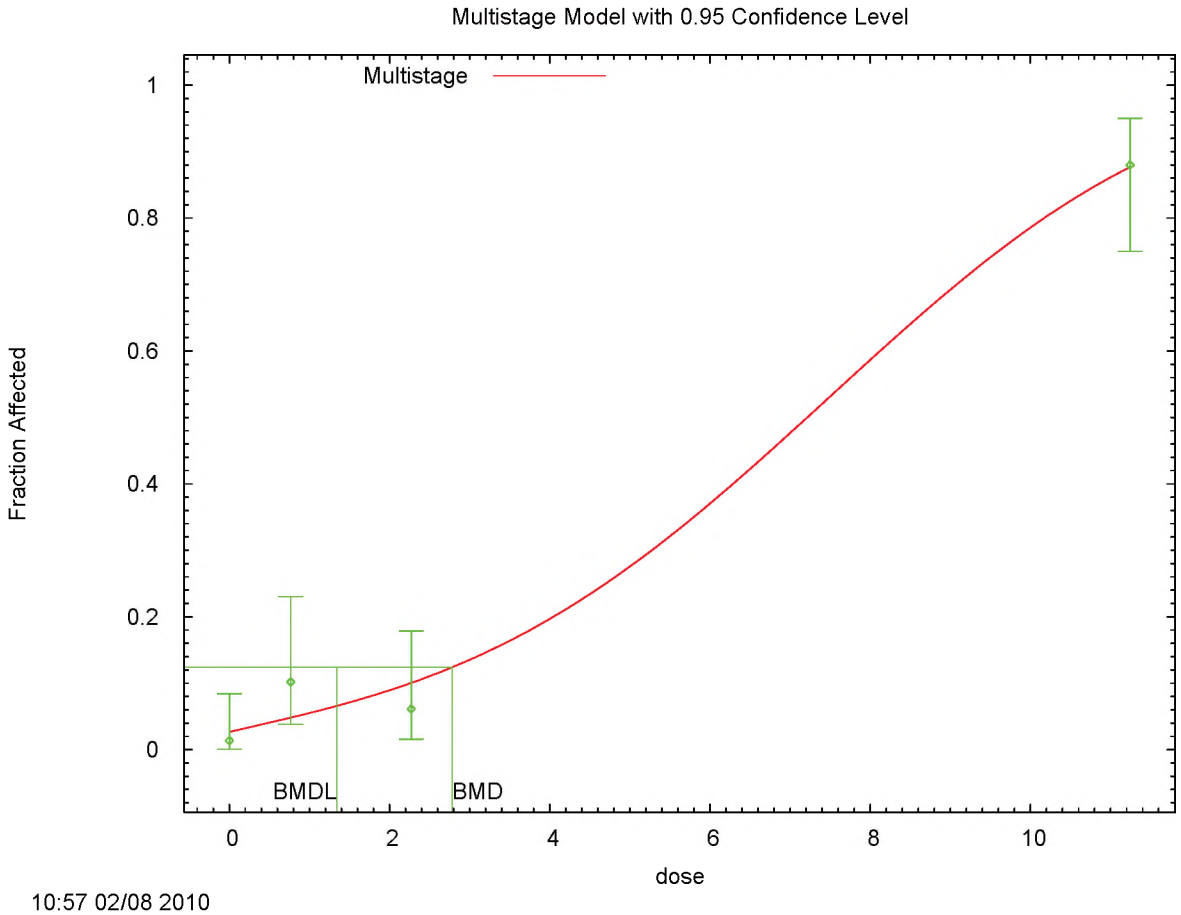
```
                BMD =        2.78201

               BMDL =        1.34308

               BMDU =         4.5214

Taken together, (1.34308, 4.5214 ) is a 90     % two-sided confidence
interval for the BMD
```

E.2.30.3. *Figure for Selected Model: Multistage, 3-Degree*



E.2.31. National Toxicology Program, 2006: Alveolar Metaplasia

E.2.31.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	4	0.010	320.093	9.886E-01	8.393E-01	power bound hit (power = 1)
logistic	4	<0.001	343.283	2.389E+00	2.052E+00	negative intercept (intercept = -1.059)
log-logistic [a]	**3**	**0.723**	**312.558**	**6.497E-01**	**3.751E-01**	
log-probit	4	0.024	318.680	1.566E+00	1.318E+00	slope bound hit (slope = 1)
multistage, 5-degree	4	0.010	320.093	9.886E-01	8.393E-01	final ß = 0
probit	4	<0.001	347.071	2.542E+00	2.219E+00	negative intercept (intercept = -0.599)
Weibull	4	0.010	320.093	9.886E-01	8.393E-01	power bound hit (power = 1)
gamma, unrestricted	3	0.426	314.011	1.642E-01	1.874E-02	unrestricted (power = 0.503)
log-probit, unrestricted	3	0.696	312.677	6.818E-01	2.740E-01	unrestricted (slope = 0.677)
Weibull, unrestricted	3	0.522	313.492	2.644E-01	6.947E-02	unrestricted (power = 0.661)

[a] Best-fitting model, BMDS output presented in this appendix

E.2.31.2. *Output for Selected Model: Log-Logistic*

National Toxicology Program, 2006: Alveolar Metaplasia

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\Blood\40_NTP_2006_AlvMeta_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\40_NTP_2006_AlvMeta_LogLogistic_1.plt
                                              Mon Feb 08 10:58:58 2010
 ================================================================

 0
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   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
```

Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.0377358
intercept = -1.69494
slope = 1.12282

Asymptotic Correlation Matrix of Parameter Estimates

	background	intercept	slope
background	1	-0.21	0.1
intercept	-0.21	1	-0.93
slope	0.1	-0.93	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0373462	*	*	*
intercept	-1.70923	*	*	*
slope	1.13164	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-152.615	6			
Fitted model	-153.279	3	1.32728	3	0.7227
Reduced model	-216.802	1	128.374	5	<.0001

AIC: 312.558

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0373	1.979	2.000	53	0.015
2.5565	0.3682	19.881	19.000	54	-0.249
5.6937	0.5807	30.776	33.000	53	0.619
9.7882	0.7162	37.243	35.000	52	-0.690
16.5688	0.8197	43.446	45.000	53	0.555
29.6953	0.8976	46.674	46.000	52	-0.308

Chi^2 = 1.33 d.f. = 3 P-value = 0.7232

Benchmark Dose Computation

Specified effect = 0.1

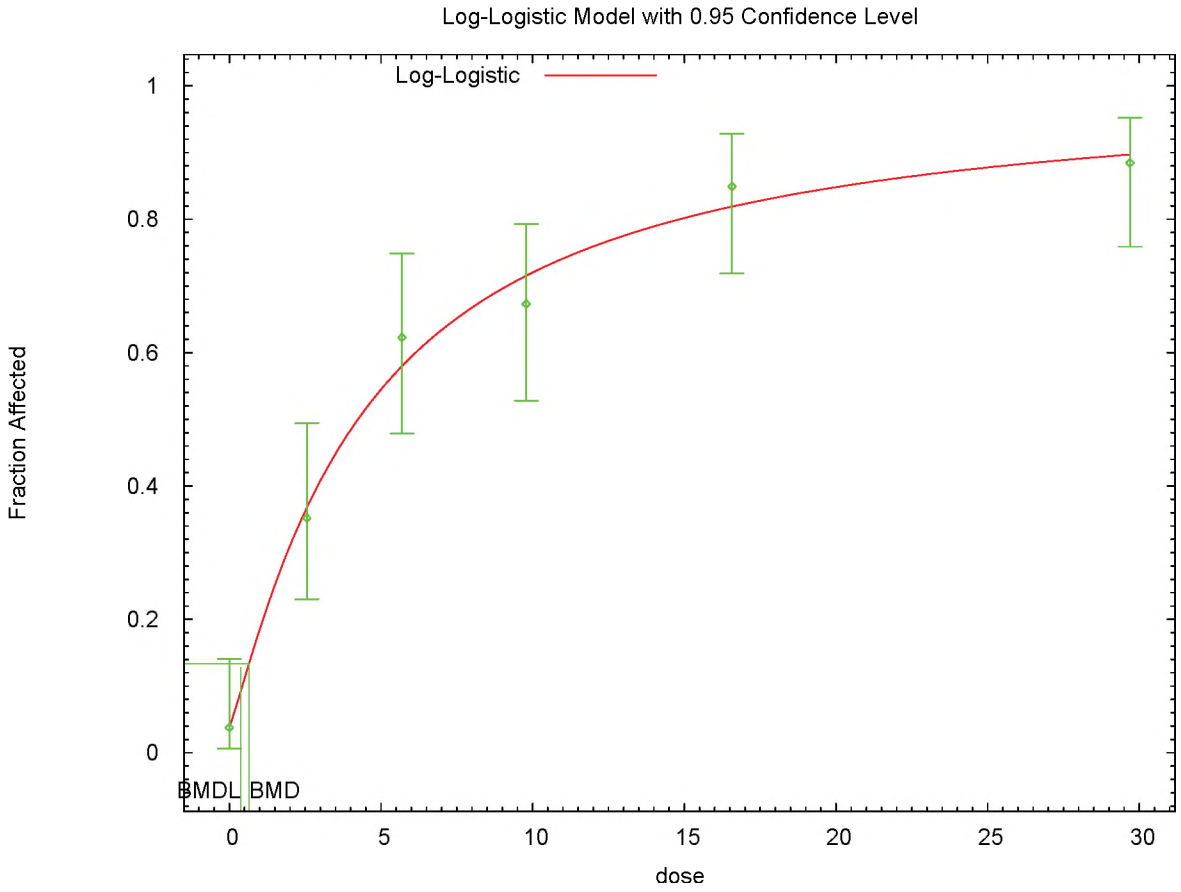
Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.64971

BMDL = 0.375051

E.2.31.3. *Figure for Selected Model: Log-Logistic*



10:58 02/08 2010

E.2.32. National Toxicology Program, 2006: Eosinophilic Focus, Liver

E.2.32.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	3	0.293	331.902	3.573E+00	2.225E+00	
logistic	4	0.405	330.400	5.949E+00	5.137E+00	negative intercept (intercept = -2.043)
log-logistic	3	0.152	333.515	4.139E+00	2.077E+00	
log-probit	4	0.192	332.312	4.889E+00	3.980E+00	slope bound hit (slope = 1)
multistage, 5-degree	3	0.752	329.328	3.393E+00	2.466E+00	
probit [a]	**4**	**0.459**	**329.945**	**5.583E+00**	**4.864E+00**	**negative intercept (intercept = -1.235)**
Weibull	3	0.324	331.628	3.770E+00	2.249E+00	
log-probit, unrestricted	3	0.116	334.150	4.146E+00	2.152E+00	unrestricted (slope = 0.895)

[a] Best-fitting model, BMDS output presented in this appendix

E.2.32.2. *Output for Selected Model: Probit*

National Toxicology Program, 2006: Eosinophilic Focus, Liver

```
 ================================================================
         Probit Model. (Version: 3.1;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\45_NTP_2006_LivEosFoc_Probit_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\45_NTP_2006_LivEosFoc_Probit_1.plt
                                                Mon Feb 08 11:00:54 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial (and Specified) Parameter Values

background =	0	Specified
intercept =	-1.28017	
slope =	0.0712441	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.77
slope	-0.77	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-1.23453	0.125132	-1.47979	-0.989279
slope	0.0688678	0.00823346	0.0527305	0.085005

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-161.07	6			
Fitted model	-162.972	2	3.80461	4	0.4331
Reduced model	-202.816	1	83.4925	5	<.0001

AIC: 329.945

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1085	5.751	3.000	53	-1.215
2.5565	0.1449	7.826	8.000	54	0.067
5.6937	0.1998	10.588	14.000	53	1.172
9.7882	0.2876	15.242	17.000	53	0.533
16.5688	0.4628	24.526	22.000	53	-0.696
29.6953	0.7912	41.932	42.000	53	0.023

Chi^2 = 3.62 d.f. = 4 P-value = 0.4593

Benchmark Dose Computation

Specified effect = 0.1

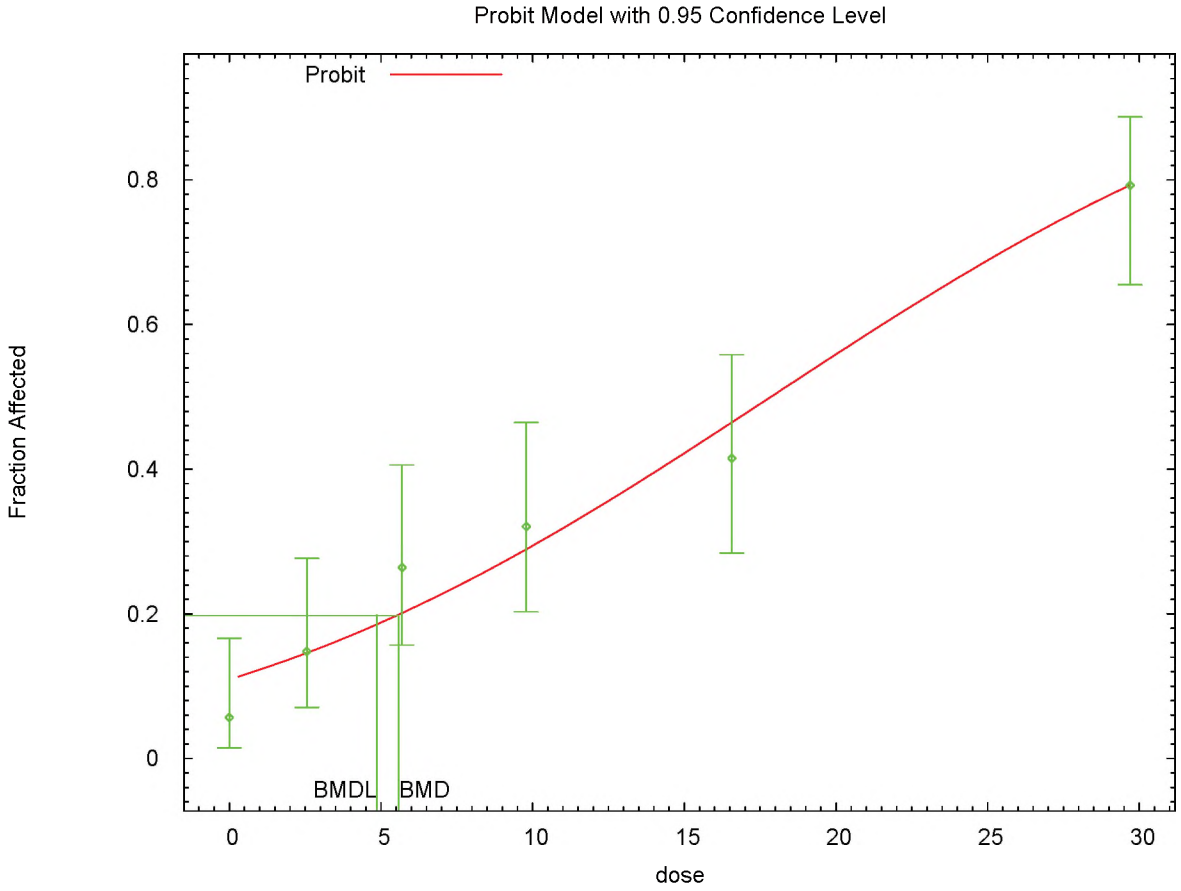
Risk Type = Extra risk

Confidence level = 0.95

BMD = 5.58309

BMDL = 4.86394

E.2.32.3. *Figure for Selected Model: Probit*



E.2.33. National Toxicology Program, 2006: Fatty Change Diffuse, Liver

E.2.33.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	4	0.659	252.348	4.028E+00	2.923E+00	
logistic	4	0.056	262.132	5.890E+00	5.042E+00	negative intercept (intercept = -2.825)
log-logistic	4	0.359	254.413	4.254E+00	3.228E+00	
log-probit	4	0.367	254.428	4.204E+00	3.277E+00	
multistage, 5-degree	3	0.581	254.045	3.524E+00	2.234E+00	
probit	4	0.075	260.915	5.567E+00	4.784E+00	negative intercept (intercept = -1.665)
Weibull [a]	**4**	**0.724**	**251.989**	**3.917E+00**	**2.856E+00**	

[a] Best-fitting model, BMDS output presented in this appendix

E.2.33.2. *Output for Selected Model: Weibull*

National Toxicology Program, 2006: Fatty Change Diffuse, Liver

```
 ================================================================
         Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\47_NTP_2006_LivFatDiff_Weibull_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\47_NTP_2006_LivFatDiff_Weibull_1.plt
                                          Mon Feb 08 11:01:56 2010
 ================================================================

 NTP_liver_fatty_change_diffuse
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = DichEff
   Independent variable = Dose
   Power parameter is restricted as power >=1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial (and Specified) Parameter Values
                    Background =   0.00925926
                         Slope =   0.00721355
```

Power = 1.69678

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Slope	Power
Slope	1	-0.98
Power	-0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	0.0135075	0.00640459	0.00095478	0.0260603
Power	1.50444	0.168981	1.17324	1.83564

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-122.992	6			
Fitted model	-123.995	2	2.00444	4	0.7349
Reduced model	-204.846	1	163.708	5	<.0001

AIC: 251.989

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	53	0.000
2.5565	0.0539	2.912	2.000	54	-0.550
5.6937	0.1688	8.949	12.000	53	1.119
9.7882	0.3415	18.102	17.000	53	-0.319
16.5688	0.6024	31.929	30.000	53	-0.542
29.6953	0.8913	47.238	48.000	53	0.336

Chi^2 = 2.06 d.f. = 4 P-value = 0.7243

Benchmark Dose Computation

Specified effect = 0.1

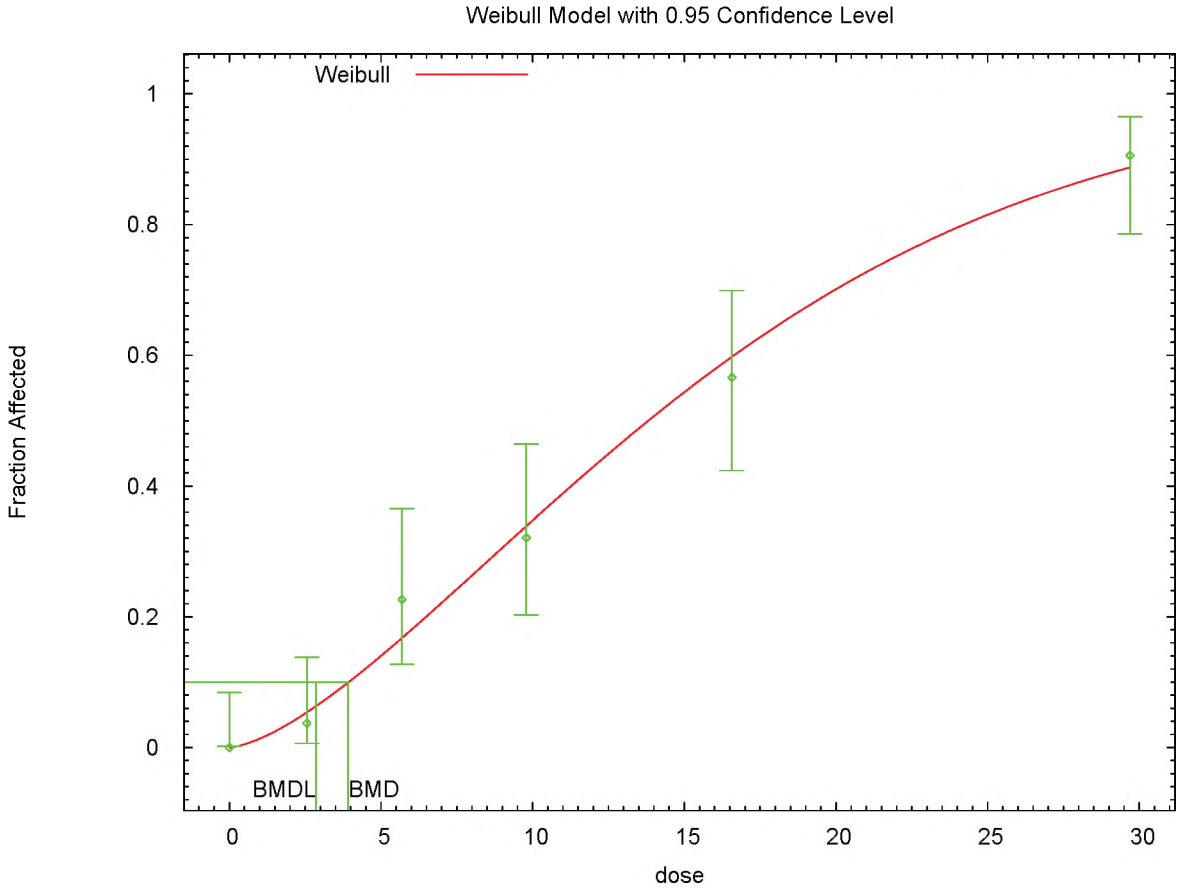
Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.91723

BMDL = 2.85566

E.2.33.3. *Figure for Selected Model: Weibull*



E.2.34. National Toxicology Program, 2006: Gingival Hyperplasia, Squamous, 2 Years

E.2.34.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	4	0.036	314.985	7.743E+00	5.166E+00	power bound hit (power = 1)
logistic	4	0.016	318.602	1.392E+01	1.056E+01	negative intercept (intercept = -1.859)
log-logistic [a]	**4**	**0.055**	**313.351**	**5.850E+00**	**3.730E+00**	**slope bound hit (slope = 1)**
log-probit	4	0.005	321.426	1.535E+01	1.038E+01	slope bound hit (slope = 1)
multistage, 5-degree	4	0.036	314.985	7.743E+00	5.166E+00	final ß = 0
probit	4	0.018	318.240	1.318E+01	9.924E+00	negative intercept (intercept = -1.123)
Weibull	4	0.036	314.985	7.743E+00	5.166E+00	power bound hit (power = 1)
gamma, unrestricted	3	0.633	307.618	5.309E-01	9.859E-07	unrestricted (power = 0.282)
log-logistic, unrestricted [b]	3	0.655	307.507	7.049E-01	1.260E-05	unrestricted (slope = 0.374)
log-probit, unrestricted	3	0.668	307.444	8.357E-01	4.796E-05	unrestricted (slope = 0.22)
Weibull, unrestricted	3	0.644	307.562	6.143E-01	3.872E-06	unrestricted (power = 0.325)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.2.34.2. *Output for Selected Model: Log-Logistic*

National Toxicology Program, 2006: Gingival Hyperplasia, Squamous, 2 Years

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\Blood\42_NTP_2006_GingHypSq_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\42_NTP_2006_GingHypSq_LogLogistic_1.plt
                                              Mon Feb 08 10:59:57 2010
 ================================================================

 [insert study notes]
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   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
```

```
   Slope parameter is restricted as slope >= 1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0188679
                     intercept =     -3.75308
                         slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.79

 intercept        -0.79            1



                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0671812               *                   *                   *
      intercept         -3.96371               *                   *                   *
          slope                1               *                   *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -149.95         6
   Fitted model        -154.675         2       9.45085      4         0.05077
  Reduced model        -162.631         1       25.3627      5       0.0001186

           AIC:         313.351


                                 Goodness  of  Fit
                                                                    Scaled
      Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
     0.0000     0.0672         3.561     1.000          53       -1.405
     2.5565     0.1104         5.960     7.000          54        0.452
     5.6937     0.1582         8.385    14.000          53        2.113
     9.7882     0.2134        11.311    13.000          53        0.566
    16.5688     0.2905        15.394    15.000          53       -0.119
    29.6953     0.4036        21.389    16.000          53       -1.509

 Chi^2 = 9.26      d.f. = 4        P-value = 0.0550


   Benchmark Dose Computation
```

```
Specified effect =          0.1

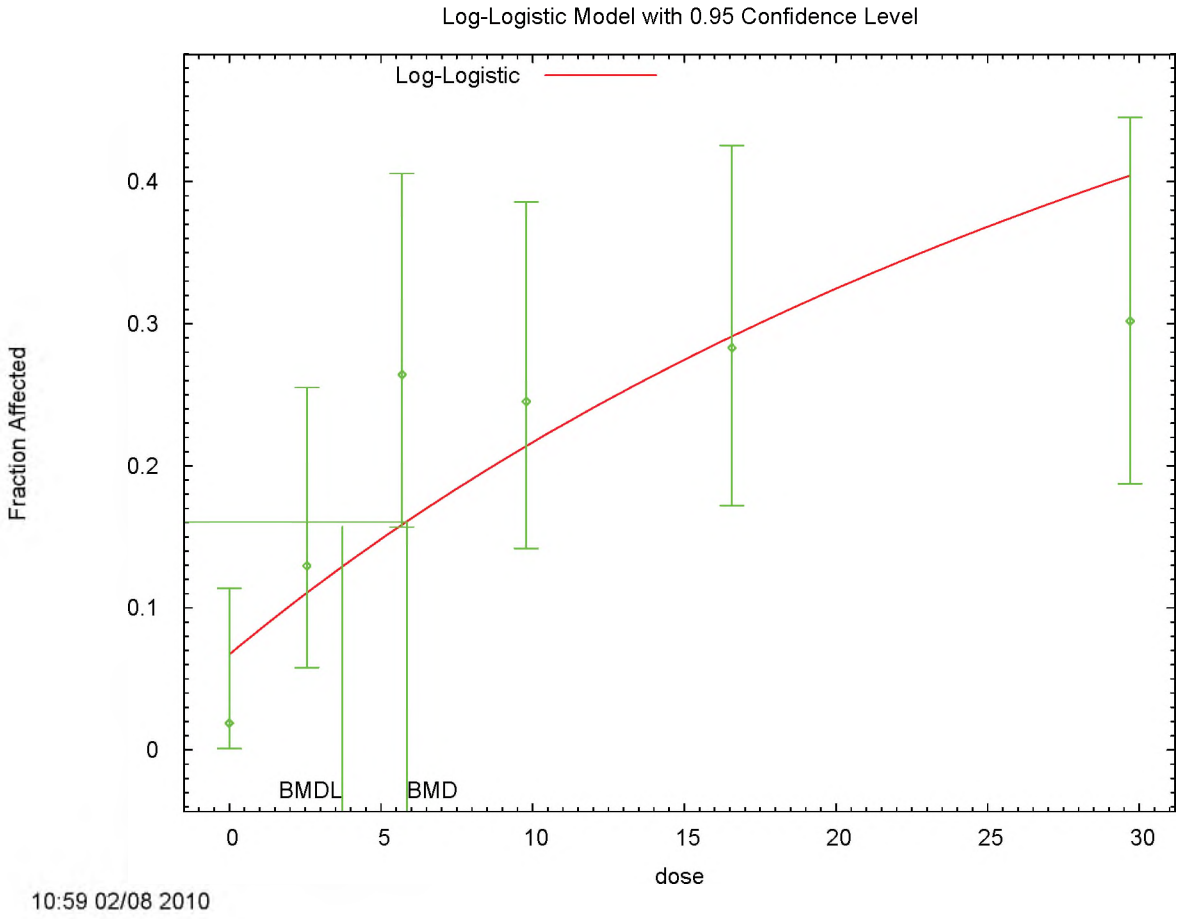
Risk Type        =      Extra risk

Confidence level =         0.95

             BMD =      5.85026

            BMDL =       3.7296
```

E.2.34.3. *Figure for Selected Model: Log-Logistic*



E.2.34.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

National Toxicology Program, 2006: Gingival Hyperplasia, Squamous, 2 Years

```
================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\1\Blood\42_NTP_2006_GingHypSq_LogLogistic_U_1.(d)
        Gnuplot Plotting File:  C:\1\Blood\42_NTP_2006_GingHypSq_LogLogistic_U_1.plt
                                          Mon Feb 08 10:59:57 2010
================================================================

[insert study notes]
```

```
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   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0188679
                     intercept =         -2.2
                         slope =     0.424326


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.27         0.11

 intercept        -0.27            1        -0.93

     slope         0.11        -0.93            1


                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0185138            *               *                   *
      intercept         -2.06653            *               *                   *
          slope         0.373721            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -149.95         6
   Fitted model        -150.753         3       1.60697      3          0.6578
  Reduced model        -162.631         1       25.3627      5       0.0001186

           AIC:         307.507


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
    0.0000     0.0185         0.981     1.000          53        0.019
    2.5565     0.1681         9.078     7.000          54       -0.756
    5.6937     0.2101        11.136    14.000          53        0.966
    9.7882     0.2433        12.893    13.000          53        0.034
```

```
    16.5688      0.2792         14.795     15.000           53         0.063
    29.6953      0.3230         17.117     16.000           53        -0.328

 Chi^2 = 1.62       d.f. = 3         P-value = 0.6554


   Benchmark Dose Computation

Specified effect =            0.1

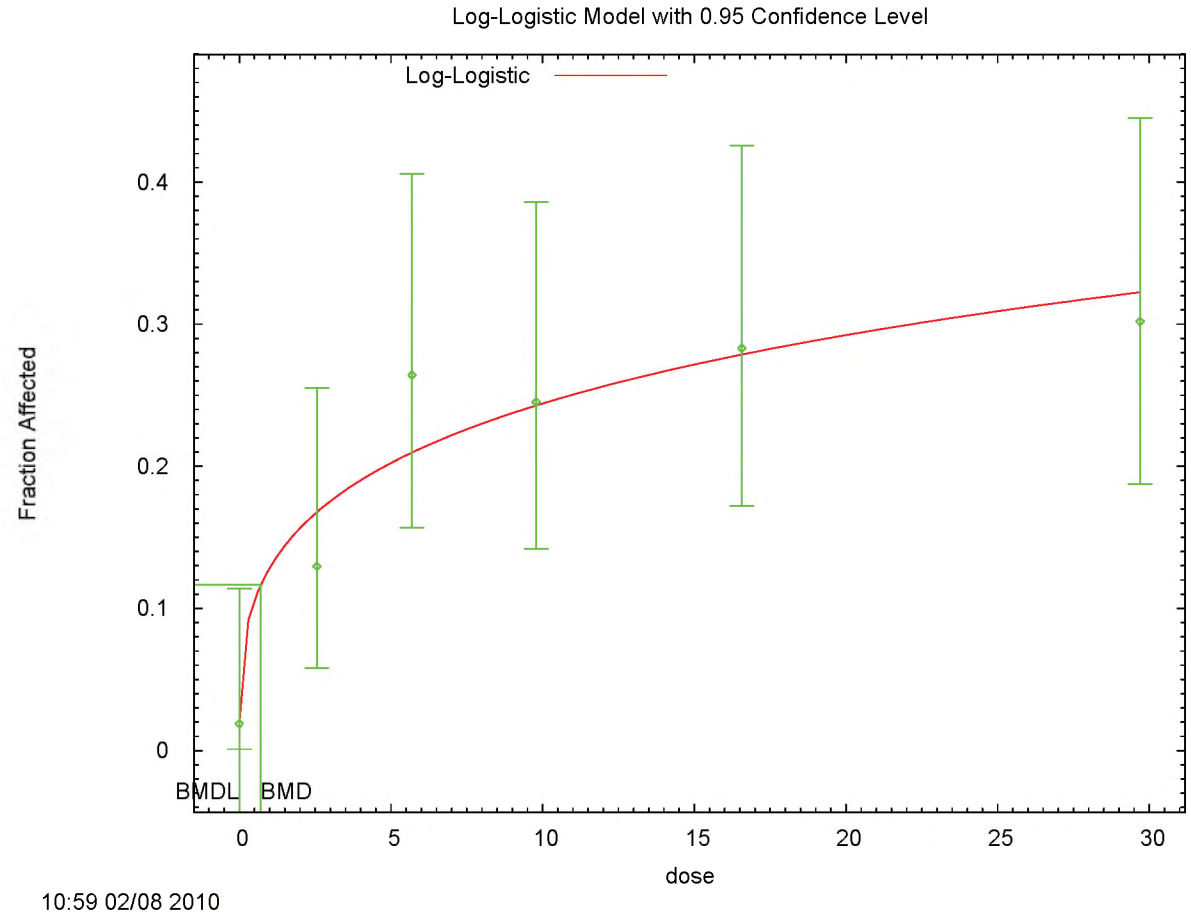
Risk Type        =       Extra risk

Confidence level =           0.95

             BMD =       0.704898

            BMDL =   1.26034e-005
```

E.2.34.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.2.35. National Toxicology Program, 2006: Hepatocyte Hypertrophy, 2 Years

E.2.35.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	5	0.034	273.875	9.091E-01	7.868E-01	power bound hit (power = 1)
logistic	4	<0.001	297.895	2.475E+00	2.122E+00	negative intercept (intercept = -1.685)
log-logistic	4	0.006	279.210	1.137E+00	6.491E-01	
log-probit	5	0.006	277.800	1.530E+00	1.321E+00	
multistage, 5-degree [a]	**4**	**0.018**	**275.693**	**9.272E-01**	**7.906E-01**	
probit	4	<0.001	299.731	2.453E+00	2.137E+00	negative intercept (intercept = -0.985)
Weibull	5	0.034	273.875	9.091E-01	7.868E-01	power bound hit (power = 1)
gamma, unrestricted	4	0.027	275.270	error	error	unrestricted (power = 0.844)
log-probit, unrestricted	4	0.008	278.360	1.191E+00	7.038E-01	unrestricted (slope = 0.864)
Weibull, unrestricted	4	0.024	275.439	7.345E-01	3.588E-01	unrestricted (power = 0.92)

[a] Best-fitting model, BMDS output presented in this appendix

E.2.35.2. *Output for Selected Model: Multistage, 5-Degree*

National Toxicology Program, 2006: Hepatocyte Hypertrophy, 2 Years

```
 ========================================================================
         Multistage Model. (Version: 3.0;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\43_NTP_2006_HepHyper_Multi5_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\43_NTP_2006_HepHyper_Multi5_1.plt
                                                Mon Feb 08 11:00:25 2010
 ========================================================================

 [insert study notes]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4-beta5*dose^5)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 6
```

Total number of records with missing values = 0
Total number of parameters in model = 6
Total number of specified parameters = 0
Degree of polynomial = 5

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.112745
Beta(1) = 0.0950808
Beta(2) = 0
Beta(3) = 0
Beta(4) = 0
Beta(5) = 4.39515e-008

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(2) -Beta(3) -Beta(4)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)	Beta(5)
Beta(1)	1	-0.5
Beta(5)	-0.5	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.113632	*	*	*
Beta(2)	0	*	*	*
Beta(3)	0	*	*	*
Beta(4)	0	*	*	*
Beta(5)	1.71322e-008	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-129.986	6			
Fitted model	-135.847	2	11.7216	4	0.01955
Reduced model	-219.97	1	179.968	5	<.0001

AIC: 275.693

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	53	0.000
2.5565	0.2521	13.614	19.000	54	1.688
5.6937	0.4764	25.251	19.000	53	-1.719
9.7882	0.6717	35.599	42.000	53	1.872
16.5688	0.8510	45.106	41.000	53	-1.584
29.6953	0.9769	51.778	52.000	53	0.203

```
 Chi^2 = 11.86      d.f. = 4        P-value = 0.0184


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

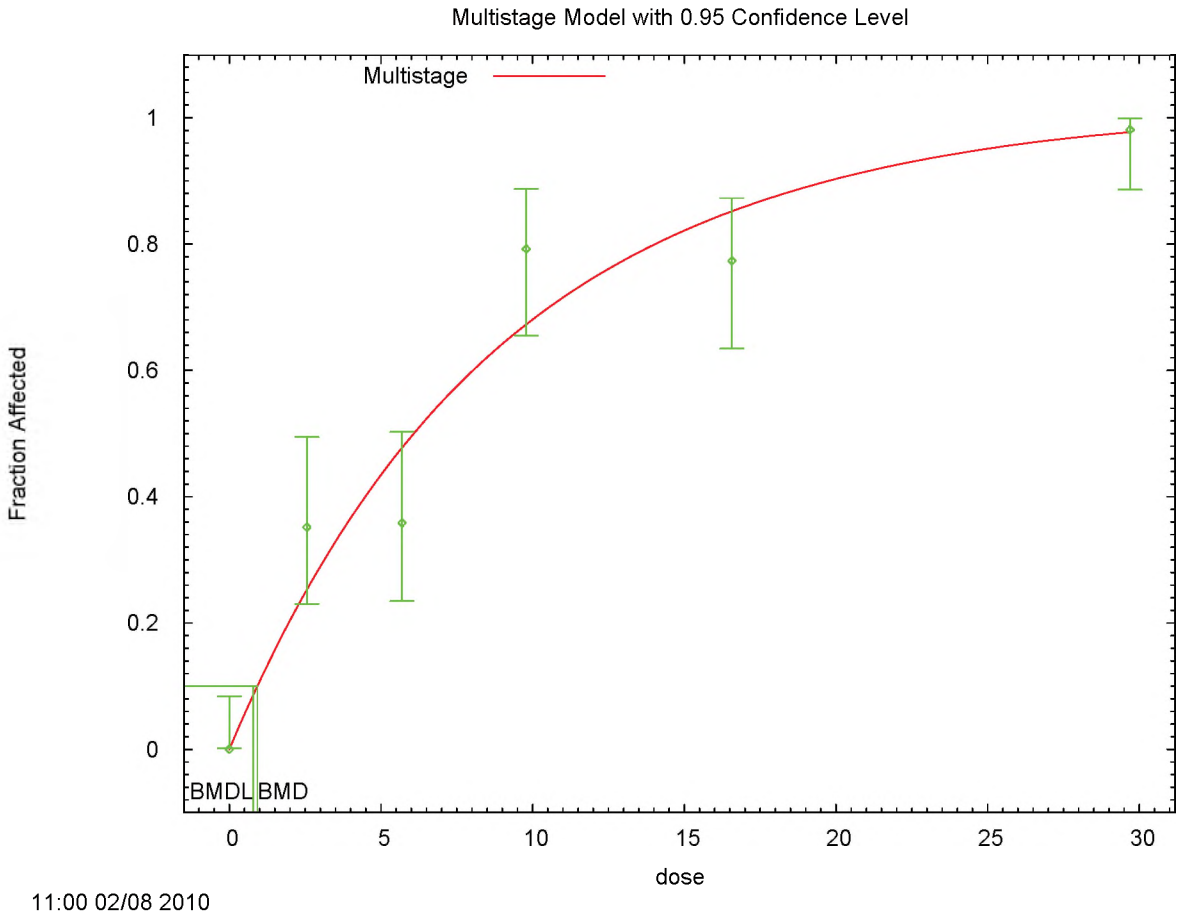
             BMD =       0.92721

            BMDL =      0.790637

            BMDU =       1.14523

Taken together, (0.790637, 1.14523) is a 90      % two-sided confidence
interval for the BMD
```

E.2.35.3. *Figure for Selected Model: Multistage, 5-Degree*



E.2.36. National Toxicology Program, 2006: Necrosis, Liver

E.2.36.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	4	0.939	234.400	8.655E+00	6.340E+00	power bound hit (power = 1)
logistic	4	0.601	236.742	1.484E+01	1.240E+01	negative intercept (intercept = -2.818)
log-logistic	4	0.943	234.382	7.928E+00	5.605E+00	slope bound hit (slope = 1)
log-probit	4	0.572	236.863	1.333E+01	1.024E+01	slope bound hit (slope = 1)
multistage, 5-degree	4	0.939	234.400	8.655E+00	6.340E+00	final ß = 0
probit	4	0.666	236.293	1.393E+01	1.154E+01	negative intercept (intercept = -1.626)
Weibull	4	0.939	234.400	8.655E+00	6.340E+00	power bound hit (power = 1)
gamma, unrestricted	3	0.883	236.290	7.726E+00	3.453E+00	unrestricted (power = 0.87)
log-logistic, unrestricted	3	0.860	236.377	7.733E+00	3.536E+00	unrestricted (slope = 0.974)
log-probit, unrestricted [a]	**3**	**0.805**	**236.598**	**7.501E+00**	**3.504E+00**	**unrestricted (slope = 0.517)**
Weibull, unrestricted	3	0.879	236.302	7.763E+00	3.508E+00	unrestricted (power = 0.895)

[a] Best-fitting model, BMDS output presented in this appendix

E.2.36.2. *Output for Selected Model: Log-Probit, Unrestricted*

National Toxicology Program, 2006: Necrosis, Liver

```
 ================================================================
         Probit Model. (Version: 3.1;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\50_NTP_2006_LivNec_LogProbit_U_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\50_NTP_2006_LivNec_LogProbit_U_1.plt
                                              Mon Feb 08 11:29:30 2010
 ================================================================

 NTP_liver_necrosis
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = DichEff
```

```
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial (and Specified) Parameter Values
                    background =    0.0188679
                     intercept =     -2.16223
                         slope =     0.457376


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.65         0.55

 intercept        -0.65            1        -0.97

     slope         0.55        -0.97            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0221151        0.0221351         -0.0212689            0.065499
      intercept         -2.32352         0.556343           -3.41393           -1.23311
          slope         0.517104         0.185064           0.154385           0.879823


                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -114.813         6
   Fitted model         -115.299         3      0.972184      3           0.808
  Reduced model          -127.98         1       26.3331      5         <.0001

           AIC:         236.598


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ----------------------------------------------------------------------
    0.0000     0.0221         1.172     1.000          53        -0.161
    2.5565     0.0544         2.938     4.000          54         0.637
    5.6937     0.0976         5.174     4.000          53        -0.543
    9.7882     0.1457         7.720     8.000          53         0.109
   16.5688     0.2096        11.106    10.000          53        -0.373
   29.6953     0.3002        15.908    17.000          53         0.327

 Chi^2 = 0.99      d.f. = 3        P-value = 0.8048


   Benchmark Dose Computation

Specified effect =            0.1
```

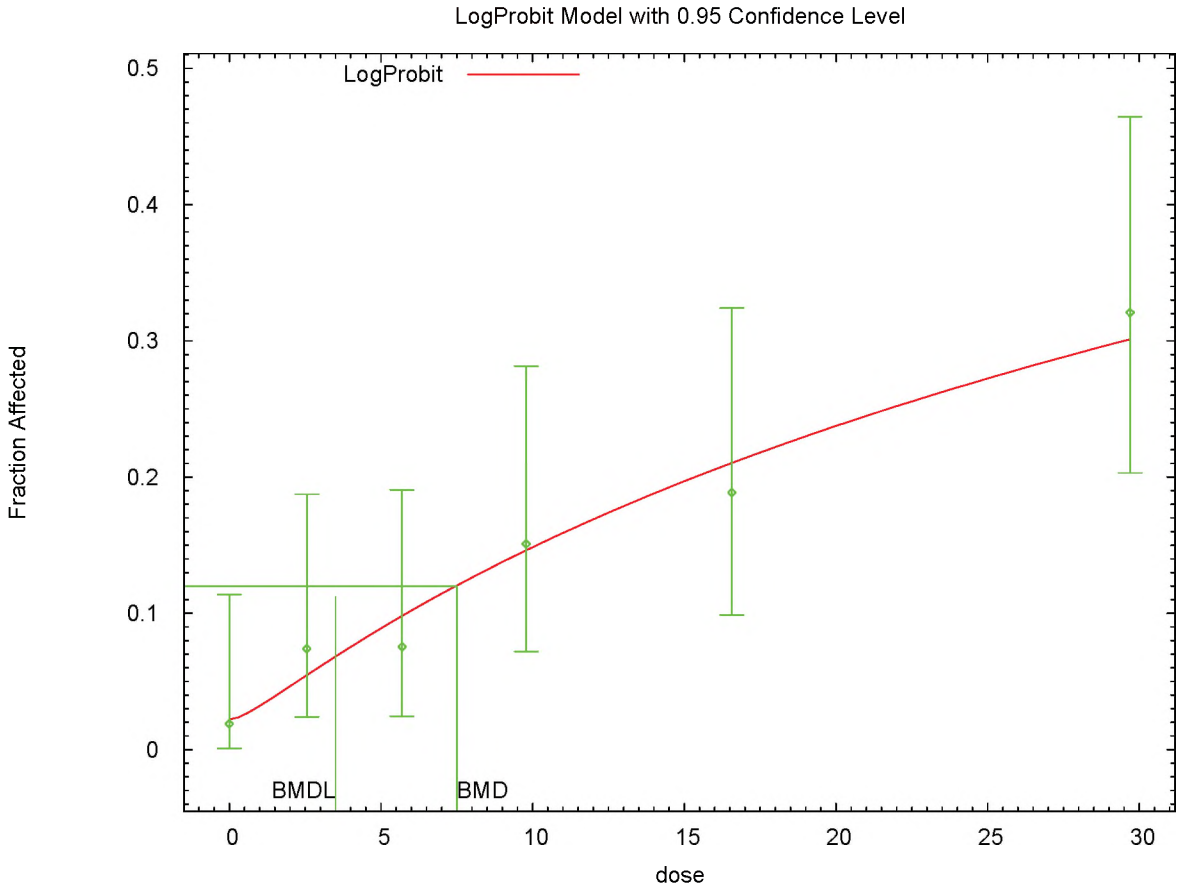
```
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        7.50077

            BMDL =         3.5039
```

E.2.36.3. *Figure for Selected Model: Log-Probit, Unrestricted*



E.2.37. National Toxicology Program, 2006: Oval Cell Hyperplasia

E.2.37.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	3	0.074	199.468	6.739E+00	5.074E+00	
logistic	4	0.171	196.803	6.064E+00	5.145E+00	negative intercept (intercept = -3.834)
log-logistic	3	0.042	201.659	6.936E+00	5.604E+00	
log-probit	3	0.072	200.121	7.090E+00	5.931E+00	
multistage, 5-degree	3	0.207	195.962	4.785E+00	3.105E+00	
probit [a]	**4**	**0.227**	**195.448**	**5.673E+00**	**4.793E+00**	**negative intercept (intercept = -2.19)**
Weibull [b]	3	0.077	198.375	5.718E+00	4.088E+00	

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.2.37.2. *Output for Selected Model: Probit*

National Toxicology Program, 2006: Oval Cell Hyperplasia

```
 ================================================================
         Probit Model. (Version: 3.1;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\53_NTP_2006_OvalHyper_Probit_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\53_NTP_2006_OvalHyper_Probit_1.plt
                                              Mon Feb 08 13:25:23 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial (and Specified) Parameter Values
background = 0 Specified
intercept = -2.29925
slope = 0.169545

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.87
slope	-0.87	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-2.18988	0.208021	-2.5976	-1.78217
slope	0.172453	0.0182446	0.136694	0.208211

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-92.4898	6			
Fitted model	-95.7242	2	6.46873	4	0.1668
Reduced model	-210.191	1	235.402	5	<.0001

AIC: 195.448

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0143	0.756	0.000	53	-0.876
2.5565	0.0401	2.168	4.000	54	1.270
5.6937	0.1135	6.017	3.000	53	-1.306
9.7882	0.3079	16.317	20.000	53	1.096
16.5688	0.7478	39.631	38.000	53	-0.516
29.6953	0.9983	52.911	53.000	53	0.299

Chi^2 = 5.64 d.f. = 4 P-value = 0.2274

Benchmark Dose Computation

Specified effect = 0.1

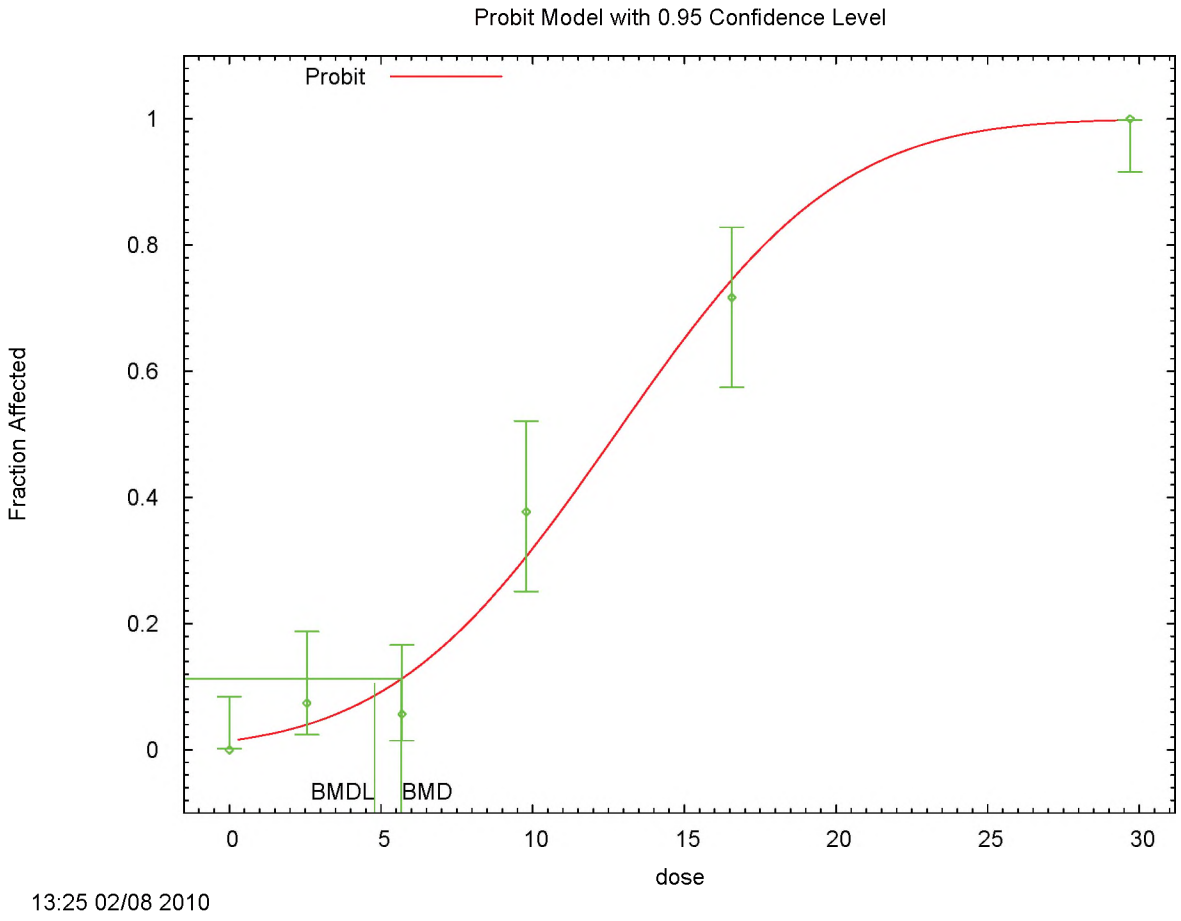
Risk Type = Extra risk

Confidence level = 0.95

BMD = 5.67298

BMDL = 4.79341

E.2.37.3. *Figure for Selected Model: Probit*



E.2.37.4. *Output for Additional Model Presented: Weibull*

National Toxicology Program, 2006: Oval Cell Hyperplasia

```
 ================================================================
         Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\53_NTP_2006_OvalHyper_Weibull_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\53_NTP_2006_OvalHyper_Weibull_1.plt
                                              Mon Feb 08 13:25:23 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = DichEff
   Independent variable = Dose
   Power parameter is restricted as power >=1

   Total number of observations = 6
```

Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.00925926
Slope = 0.00296825
Power = 2.17092

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope	Power
Background	1	-0.72	0.7
Slope	-0.72	1	-0.99
Power	0.7	-0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0164137	0.0221488	-0.0269971	0.0598245
Slope	0.00162074	0.00202897	-0.00235596	0.00559745
Power	2.39427	0.455116	1.50226	3.28628

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-92.4898	6			
Fitted model	-96.1875	3	7.3953	3	0.06031
Reduced model	-210.191	1	235.402	5	<.0001

AIC: 198.375

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0164	0.870	0.000	53	-0.940
2.5565	0.0314	1.695	4.000	54	1.799
5.6937	0.1138	6.034	3.000	53	-1.312
9.7882	0.3285	17.411	20.000	53	0.757
16.5688	0.7440	39.431	38.000	53	-0.450
29.6953	0.9957	52.774	53.000	53	0.476

Chi^2 = 6.85 d.f. = 3 P-value = 0.0770

Benchmark Dose Computation

Specified effect = 0.1

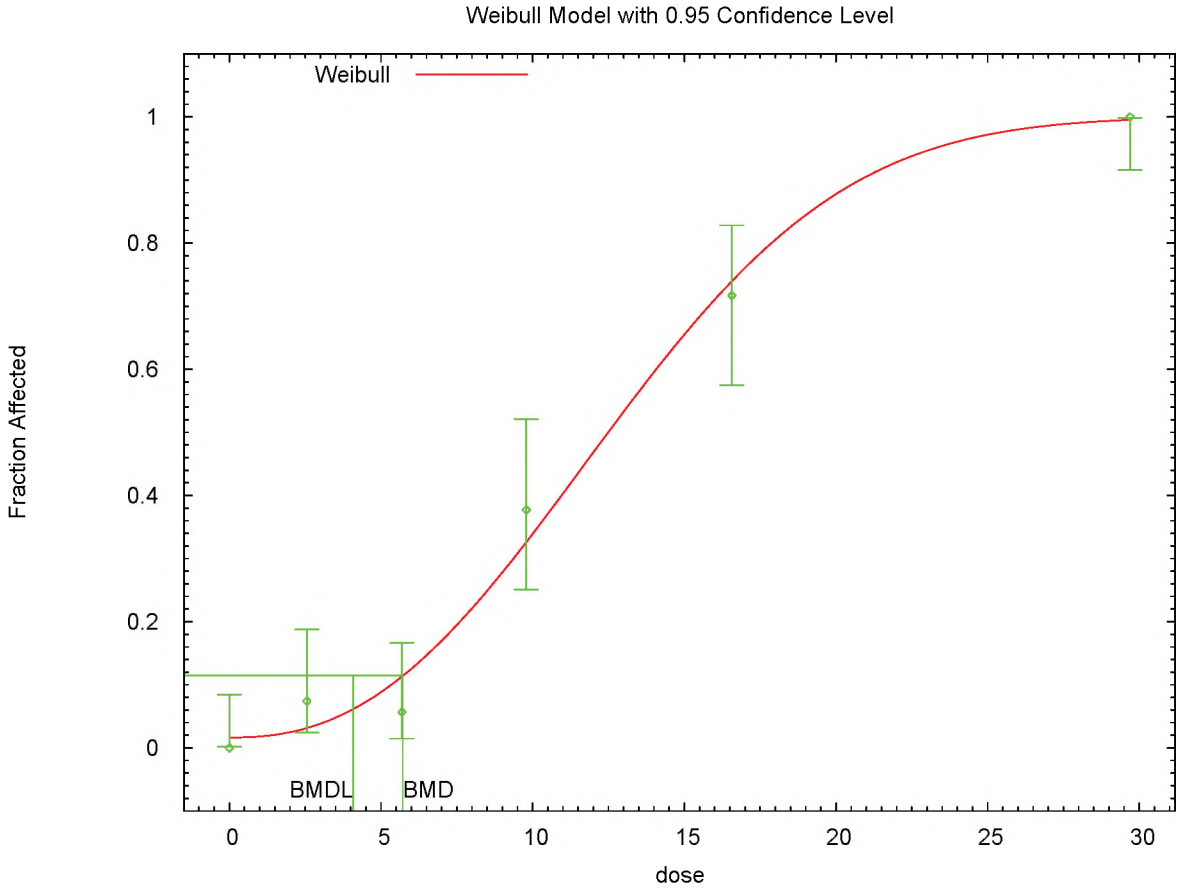
Risk Type = Extra risk

Confidence level = 0.95

BMD = 5.71754

BMDL = 4.08823

E.2.37.5. *Figure for Additional Model Presented: Weibull*



E.2.38. National Toxicology Program, 2006: Pigmentation, Liver

E.2.38.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	3	0.552	196.971	2.172E+00	1.493E+00	
logistic	4	0.247	197.066	1.853E+00	1.521E+00	negative intercept (intercept = -2.51)
log-logistic	3	0.984	195.530	2.566E+00	1.937E+00	
log-probit [a]	**3**	**0.962**	**195.526**	**2.463E+00**	**1.890E+00**	
multistage, 5-degree	3	0.058	199.955	1.822E+00	9.916E-01	final ß = 0
probit	4	0.004	200.504	1.710E+00	1.430E+00	negative intercept (intercept = -1.392)
Weibull	3	0.219	199.007	1.756E+00	1.190E+00	

[a] Best-fitting model, BMDS output presented in this appendix

E.2.38.2. *Output for Selected Model: Log-Probit*

National Toxicology Program, 2006: Pigmentation, Liver

```
 ================================================================
         Probit Model. (Version: 3.1;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\54_NTP_2006_Pigment_LogProbit_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\54_NTP_2006_Pigment_LogProbit_1.plt
                                          Mon Feb 08 13:25:55 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values
background = 0.0754717
intercept = -2.48683
slope = 1.53221

Asymptotic Correlation Matrix of Parameter Estimates

	background	intercept	slope
background	1	-0.42	0.33
intercept	-0.42	1	-0.96
slope	0.33	-0.96	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0725473	0.0338856	0.00613263	0.138962
intercept	-2.93268	0.487158	-3.8875	-1.97787
slope	1.83184	0.246868	1.34798	2.31569

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-94.6177	6			
Fitted model	-94.7632	3	0.291072	3	0.9617
Reduced model	-210.717	1	232.198	5	<.0001

AIC: 195.526

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0725	3.845	4.000	53	0.082
2.5565	0.1769	9.553	9.000	54	-0.197
5.6937	0.6291	33.342	34.000	53	0.187
9.7882	0.9013	47.771	48.000	53	0.105
16.5688	0.9874	52.334	52.000	53	-0.412
29.6953	0.9995	52.974	53.000	53	0.160

Chi^2 = 0.29 d.f. = 3 P-value = 0.9624

Benchmark Dose Computation

Specified effect = 0.1

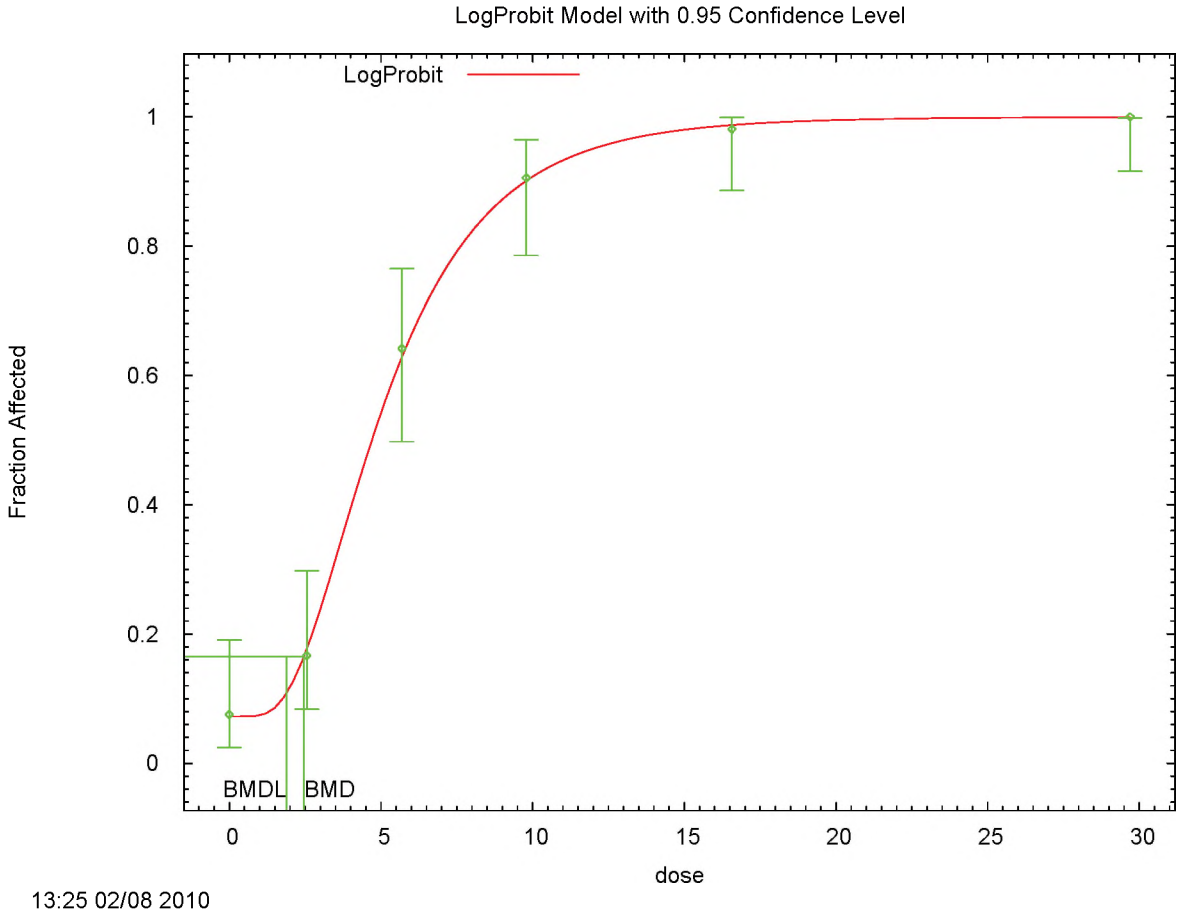
Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.46293

BMDL = 1.88981

E.2.38.3. *Figure for Selected Model: Log-Probit*



E.2.39. National Toxicology Program, 2006: Toxic Hepatopathy

E.2.39.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	4	0.754	185.763	4.302E+00	3.463E+00	
logistic	4	0.159	191.136	4.833E+00	4.068E+00	negative intercept (intercept = -3.756)
log-logistic	3	0.391	189.577	4.697E+00	3.818E+00	
log-probit	3	0.394	189.580	4.972E+00	3.780E+00	
multistage, 5-degree [a]	**4**	**0.693**	**185.924**	**3.980E+00**	**3.059E+00**	**final ß = 0**
probit	4	0.231	189.820	4.621E+00	3.860E+00	negative intercept (intercept = -2.172)
Weibull	4	0.716	185.785	4.089E+00	3.215E+00	

[a] Best-fitting model, BMDS output presented in this appendix

E.2.39.2. *Output for Selected Model: Multistage, 5-Degree*

National Toxicology Program, 2006: Toxic Hepatopathy

```
 ======================================================================
          Multistage Model. (Version: 3.0;  Date: 05/16/2008)
          Input Data File: C:\1\Blood\55_NTP_2006_ToxHepa_Multi5_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\55_NTP_2006_ToxHepa_Multi5_1.plt
                                                Mon Feb 08 13:26:28 2010
 ======================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4-beta5*dose^5)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 0
 Degree of polynomial = 5


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

Background =	0
Beta(1) =	0
Beta(2) =	0
Beta(3) =	0
Beta(4) =	0
Beta(5) =	4.36963e+012

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1) -Beta(4) -Beta(5)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(2)	Beta(3)
Beta(2)	1	-0.95
Beta(3)	-0.95	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.00639021	*	*	*
Beta(3)	6.5404e-005	*	*	*
Beta(4)	0	*	*	*
Beta(5)	0	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-89.8076	6			
Fitted model	-90.9619	2	2.30853	4	0.6792
Reduced model	-218.207	1	256.799	5	<.0001

AIC: 185.924

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	53	0.000
2.5565	0.0420	2.265	2.000	54	-0.180
5.6937	0.1969	10.434	8.000	53	-0.841
9.7882	0.4901	25.976	30.000	53	1.106
16.5688	0.8715	46.189	45.000	53	-0.488
29.6953	0.9994	52.966	53.000	53	0.185

Chi^2 = 2.23 d.f. = 4 P-value = 0.6928

Benchmark Dose Computation

Specified effect = 0.1

```
Risk Type        =      Extra risk

Confidence level =           0.95

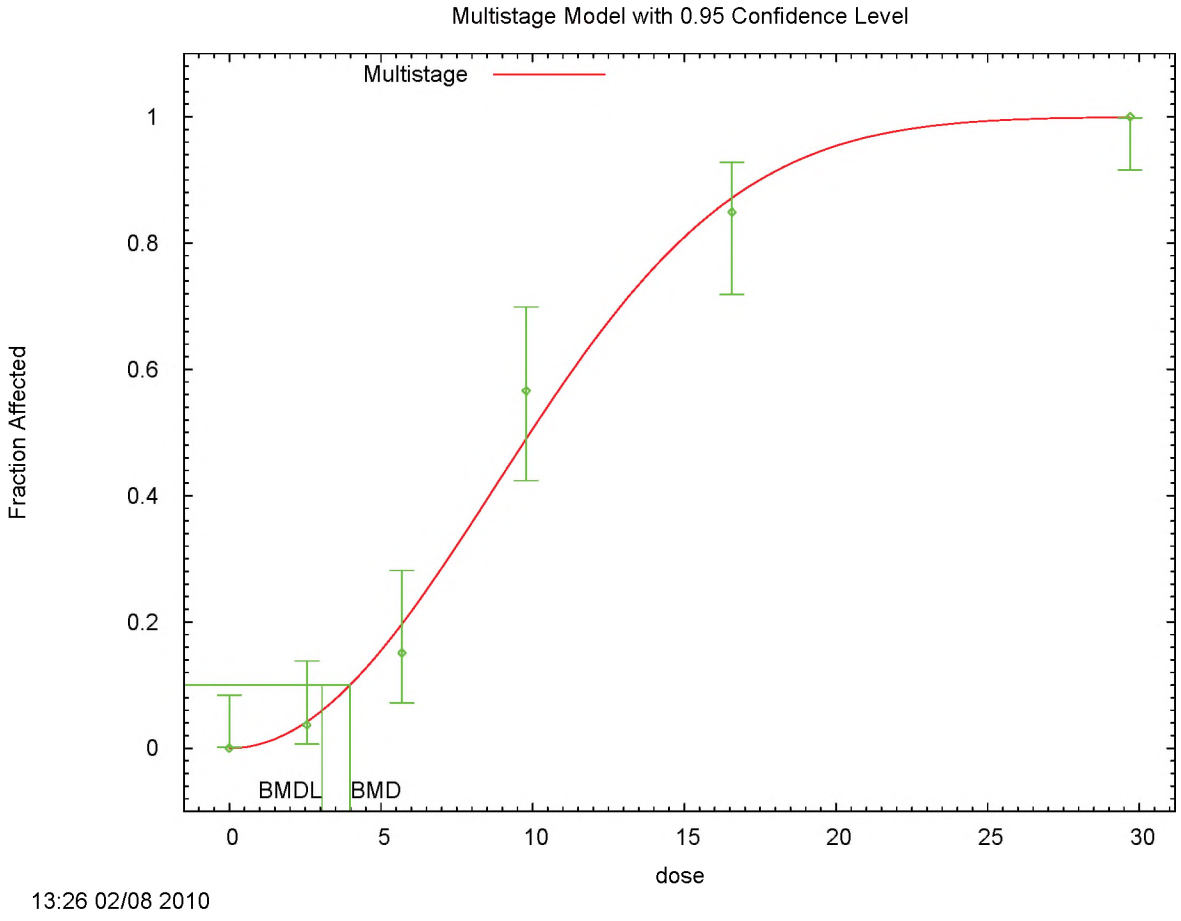
             BMD =        3.98025

            BMDL =        3.05855

            BMDU =        4.89735

Taken together, (3.05855, 4.89735) is a 90      % two-sided confidence
interval for the BMD
```

E.2.39.3. *Figure for Selected Model: Multistage, 5-Degree*



E.2.40. Ohsako et al., 2001: Ano-Genital Length, PND 120

E.2.40.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	3	0.027	171.073	2.592E+01	1.750E+01	
exponential (M3)	3	0.027	171.073	2.592E+01	1.750E+01	power hit bound (d = 1)
exponential (M4)	2	0.106	168.392	2.248E+00	8.445E-01	
exponential (M5)	1	0.049	169.789	2.193E+00	9.382E-01	
Hill [b]	**2**	**0.154**	**167.647**	**2.879E+00**	**8.028E-01**	**n lower bound hit (n = 1)**
linear	3	0.025	171.258	2.700E+01	1.881E+01	
polynomial, 4-degree	3	0.025	171.258	2.700E+01	1.881E+01	
power	3	0.025	171.258	2.700E+01	1.881E+01	power bound hit (power = 1)
Hill, unrestricted [c]	1	0.056	169.555	3.494E+00	3.046E-01	unrestricted (n = 0.591)
power, unrestricted	2	0.153	167.654	4.151E+00	2.395E-01	unrestricted (power = 0.291)

[a] Constant variance model selected ($p = 0.165$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.40.2. *Output for Selected Model: Hill*

Ohsako et al., 2001: Ano-Genital Length, PND 120

```
 ======================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\Blood\56_Ohsako_2001_Anogen_HillCV_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\56_Ohsako_2001_Anogen_HillCV_1.plt
                                                Mon Feb 08 13:27:02 2010
 ======================================================================

 Figure 7
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
```

```
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                        alpha =      7.27386
                          rho =            0   Specified
                    intercept =       28.905
                            v =      -5.1065
                            n =      1.57046
                            k =       2.4317


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho    -n
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha    intercept            v            k

      alpha           1     4.4e-008    -9.8e-008     7.2e-008

  intercept     4.4e-008            1        -0.57        -0.52

          v    -9.8e-008        -0.57            1        -0.23

          k     7.2e-008        -0.52        -0.23            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha          7.07394          1.36138            4.40568              9.7422
       intercept          28.9732          0.74996            27.5034             30.4431
               v         -5.02686          1.05086           -7.08651             -2.9672
               n                1               NA
               k          2.56203          2.11462           -1.58255             6.70661

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.



     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    12       28.9           29         3.13         2.66        -0.0889
 1.04    10       27.9         27.5          2.5         2.66          0.495
3.471    10       25.2         26.1         3.21         2.66          -1.09
11.36    10         26         24.9         2.85         2.66           1.35
38.42    12       23.8         24.3         1.56         2.66         -0.602


 Model Descriptions for likelihoods calculated
```

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          -77.952340           6     167.904680
              A2          -74.703868          10     169.407736
              A3          -77.952340           6     167.904680
          fitted          -79.823277           4     167.646555
               R          -89.824703           2     183.649405


                    Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              30.2417          8       0.0001916
  Test 2              6.49694          4           0.165
  Test 3              6.49694          4           0.165
  Test 4              3.74187          2           0.154

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =             1

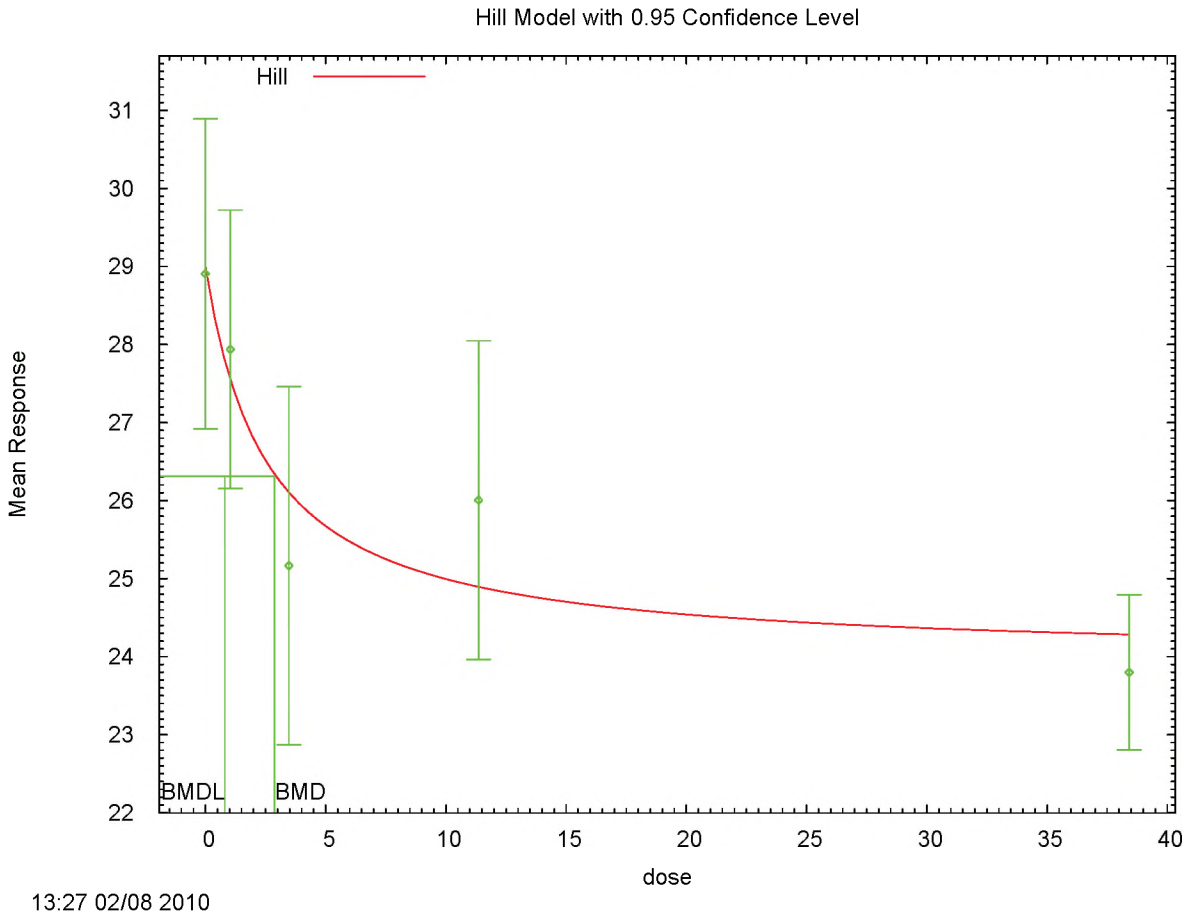
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        2.87863

            BMDL =      0.802782
```

E.2.40.3. *Figure for Selected Model: Hill*



E.2.40.4. *Output for Additional Model Presented: Hill, Unrestricted*

Ohsako et al., 2001: Ano-Genital Length, PND 120

```
 ================================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\Blood\56_Ohsako_2001_Anogen_HillCV_U_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\56_Ohsako_2001_Anogen_HillCV_U_1.plt
                                                 Mon Feb 08 13:27:04 2010
 ================================================================================

 Figure 7
 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter is not restricted
   A constant variance model is fit
```

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	7.27386	
rho =	0	Specified
intercept =	28.905	
v =	-5.1065	
n =	1.57046	
k =	2.4317	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	n	k
alpha	1	-3.1e-008	7.5e-009	1.7e-008	-8.8e-009
intercept	-3.1e-008	1	0.001	0.0016	-0.13
v	7.5e-009	0.001	1	0.98	-0.99
n	1.7e-008	0.0016	0.98	1	-0.97
k	-8.8e-009	-0.13	-0.99	-0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	7.06192	1.35907	4.3982	9.72564
intercept	28.9618	0.754441	27.4831	30.4404
v	-6.82284	11.1104	-28.5989	14.9532
n	0.591421	1.04	-1.44695	2.62979
k	7.47064	48.002	-86.6115	101.553

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	12	28.9	29	3.13	2.66	-0.074
1.04	10	27.9	27.3	2.5	2.66	0.71
3.471	10	25.2	26.3	3.21	2.66	-1.36
11.36	10	26	25.1	2.85	2.66	1.04
38.42	12	23.8	24	1.56	2.66	-0.284

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)

```
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -77.952340           6     167.904680
             A2          -74.703868          10     169.407736
             A3          -77.952340           6     167.904680
          fitted         -79.777354           5     169.554709
              R          -89.824703           2     183.649405


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              30.2417          8       0.0001916
   Test 2              6.49694          4           0.165
   Test 3              6.49694          4           0.165
   Test 4              3.65003          1         0.05607

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =            1

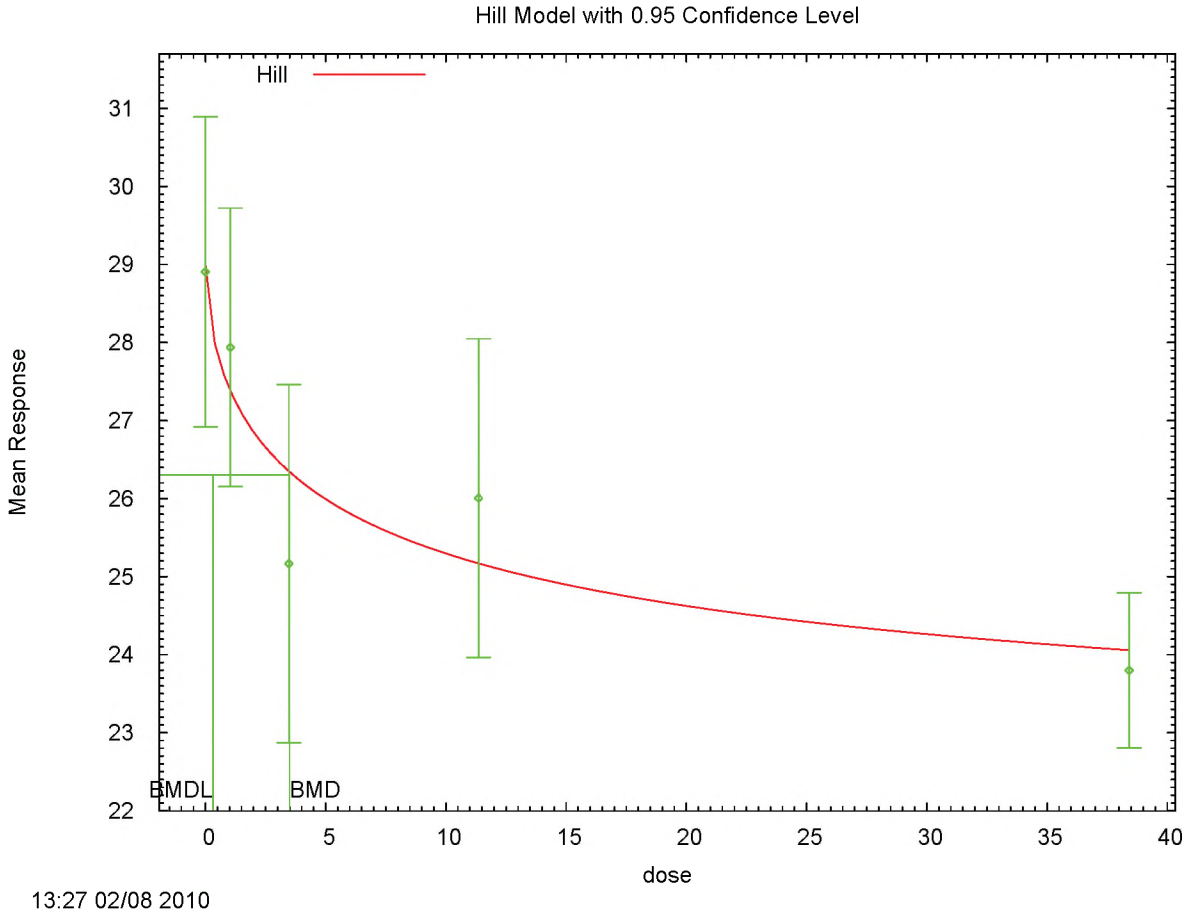
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       3.49389

            BMDL =      0.304602
```

E.2.40.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.2.41. Sewall et al., 1995: T4 In Serum

E.2.41.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	3	0.722	204.495	1.869E+01	1.243E+01	
exponential (M3)	3	0.722	204.495	1.869E+01	1.243E+01	power hit bound (d = 1)
exponential (M4)	2	0.854	205.483	1.106E+01	4.650E+00	
exponential (M5)	2	0.854	205.483	1.106E+01	4.650E+00	power hit bound (d = 1)
Hill [b]	**2**	**0.898**	**205.382**	**1.031E+01**	**3.603E+00**	**n lower bound hit (n = 1)**
linear	3	0.576	205.150	2.238E+01	1.619E+01	
polynomial, 4-degree	3	0.576	205.150	2.238E+01	1.619E+01	
power	3	0.576	205.150	2.238E+01	1.619E+01	power bound hit (power = 1)
Hill, unrestricted [c]	1	0.864	207.196	9.706E+00	1.973E+00	unrestricted (n = 0.569)
power, unrestricted	2	0.985	205.197	9.726E+00	1.914E+00	unrestricted (power = 0.538)

[a] Constant variance model selected ($p = 0.4078$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.41.2. *Output for Selected Model: Hill*

Sewall et al., 1995: T4 In Serum

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\Blood\58_Sewall_1995_T4_HillCV_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\58_Sewall_1995_T4_HillCV_1.plt
                                          Mon Feb 08 13:28:15 2010
 ================================================================

 Figure 1, Saline noninitiated
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
```

```
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                        alpha =      33.0913
                          rho =            0   Specified
                    intercept =      30.6979
                            v =     -12.2937
                            n =     0.950815
                            k =      12.5808


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho    -n
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                   alpha    intercept            v            k

      alpha            1    -1.2e-009    -1.8e-008     1.5e-008

  intercept    -1.2e-009            1          0.3        -0.65

          v    -1.8e-008          0.3            1        -0.89

          k     1.5e-008        -0.65        -0.89            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha          29.5556          6.23087             17.3433             41.7679
       intercept          30.3957          1.68747             27.0883             33.7031
               v         -18.2488          7.72836            -33.3961            -3.10154
               n                1               NA
               k          24.2883           26.743             -28.127             76.7035

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0       9       30.7         30.4         4.66         5.44          0.167
3.291       9       27.9         28.2         7.17         5.44         -0.188
7.107       9       25.9         26.3         6.81         5.44         -0.204
16.63       9       23.6           23         5.38         5.44          0.319
44.66       9       18.4         18.6         4.12         5.44        -0.0942


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
```

```
            Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2
        Model A3 uses any fixed variance parameters that
        were specified by the user

 Model  R:         Yi = Mu + e(i)
             Var{e(i)} = Sigma^2


                       Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1          -98.583448           6     209.166896
              A2          -96.590204          10     213.180407
              A3          -98.583448           6     209.166896
           fitted         -98.691143           4     205.382286
               R         -109.013252           2     222.026503


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              24.8461          8        0.001651
   Test 2              3.98649          4          0.4078
   Test 3              3.98649          4          0.4078
   Test 4              0.21539          2          0.8979

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =             1

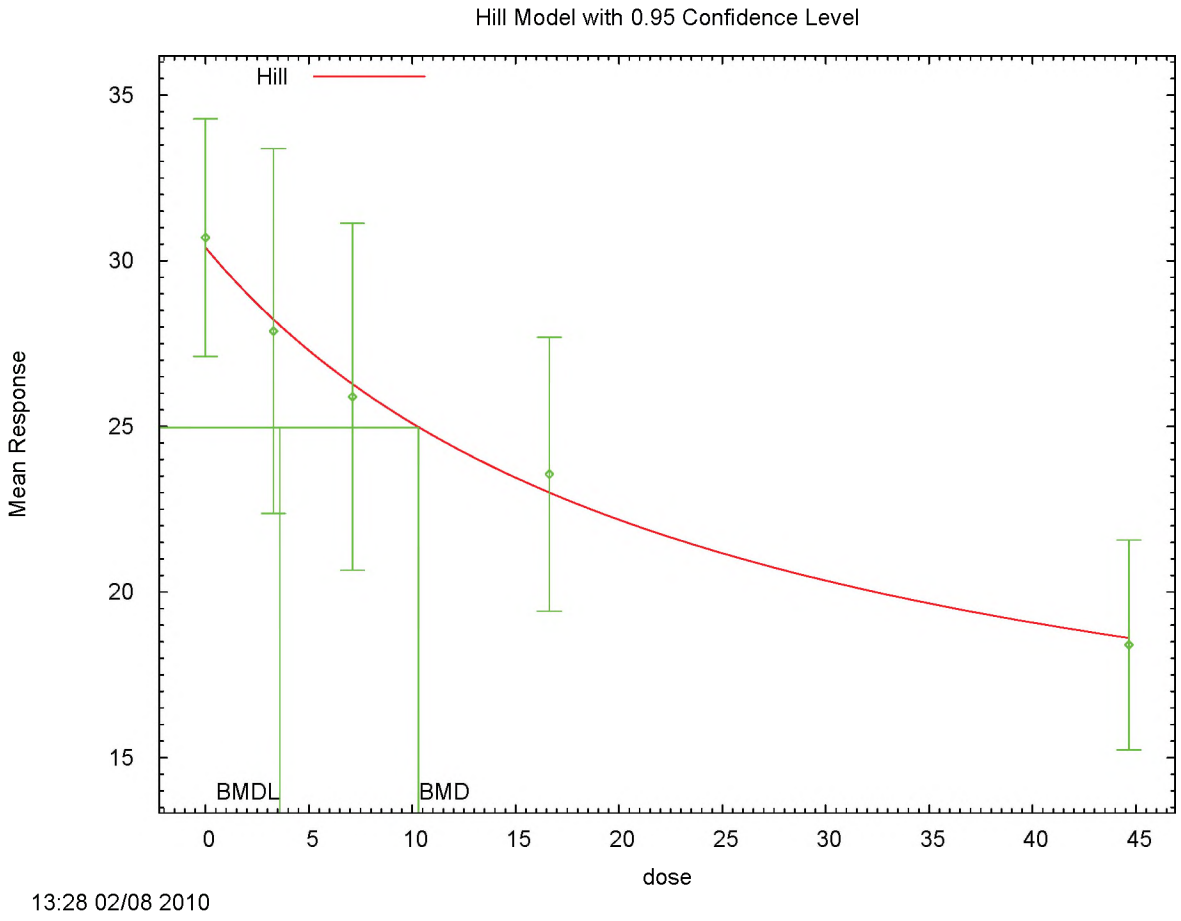
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =         10.306

            BMDL =       3.60269
```

E.2.41.3. *Figure for Selected Model: Hill*



E.2.41.4. *Output for Additional Model Presented: Hill, Unrestricted*

Sewall et al., 1995: T4 In Serum

```
 ================================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\Blood\58_Sewall_1995_T4_HillCV_U_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\58_Sewall_1995_T4_HillCV_U_1.plt
                                                 Mon Feb 08 13:28:15 2010
 ================================================================================

 Figure 1, Saline noninitiated
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter is not restricted
   A constant variance model is fit
```

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	33.0913	
rho =	0	Specified
intercept =	30.6979	
v =	-12.2937	
n =	0.950815	
k =	12.5808	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	n	k
alpha	1	-3.9e-005	0.00022	0.00021	-0.00022
intercept	-3.9e-005	1	-0.17	-0.31	0.18
v	0.00022	-0.17	1	0.97	-1
n	0.00021	-0.31	0.97	1	-0.98
k	-0.00022	0.18	-1	-0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	29.4337	6.20518	17.2718	41.5957
intercept	30.7096	1.79801	27.1855	34.2336
v	-143.244	3972.28	-7928.78	7642.29
n	0.569063	0.947248	-1.28751	2.42564
k	2856.29	171186	-332662	338374

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	9	30.7	30.7	4.66	5.43	-0.00646
3.291	9	27.9	27.7	7.17	5.43	0.0842
7.107	9	25.9	26.1	6.81	5.43	-0.134
16.63	9	23.6	23.4	5.38	5.43	0.0657
44.66	9	18.4	18.4	4.12	5.43	-0.00948

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)

```
            Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
             Var{e(i)} = Sigma^2


                        Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
               A1          -98.583448           6     209.166896
               A2          -96.590204          10     213.180407
               A3          -98.583448           6     209.166896
           fitted          -98.598183           5     207.196367
                R         -109.013252           2     222.026503


                     Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                       Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              24.8461          8        0.001651
   Test 2              3.98649          4          0.4078
   Test 3              3.98649          4          0.4078
   Test 4            0.0294713          1          0.8637

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =            1

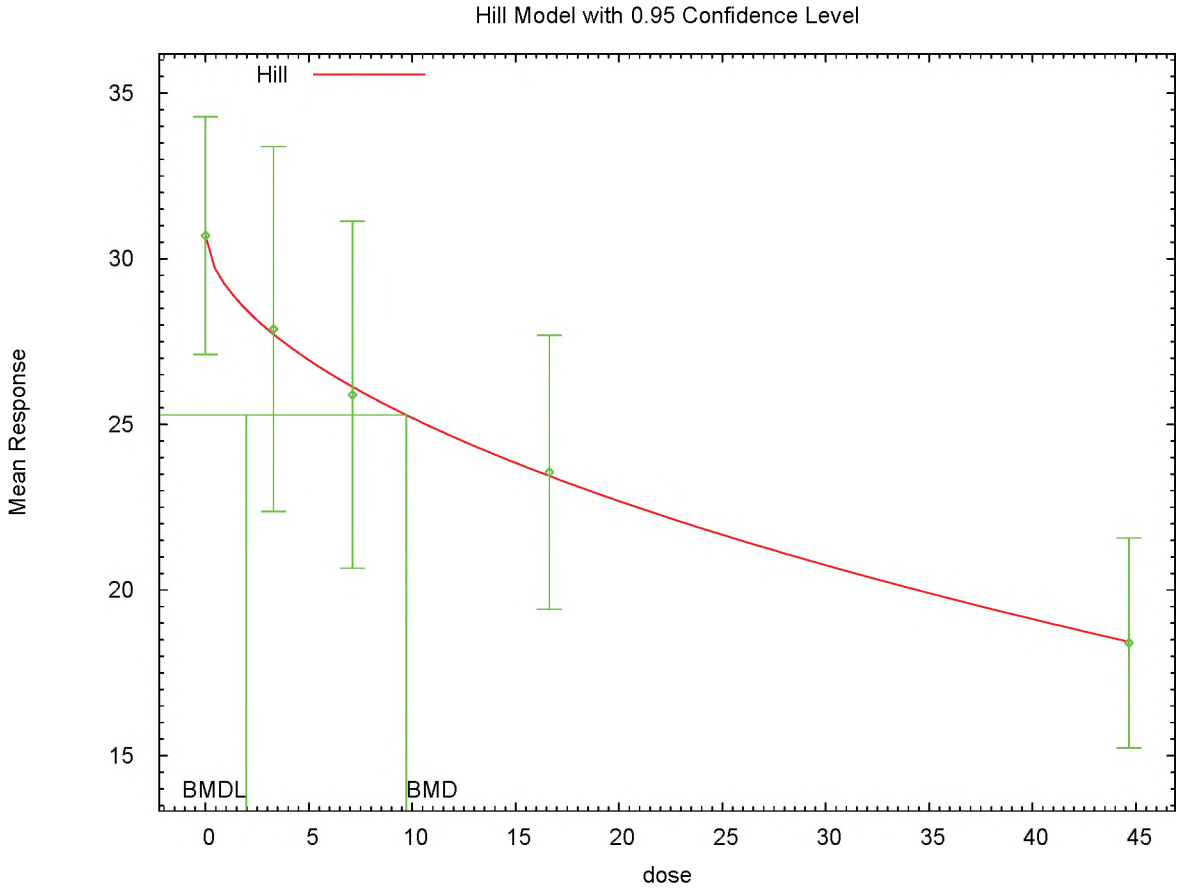
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       9.70574

            BMDL =       1.97319
```

E.2.41.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.2.42. Shi et al., 2007: Estradiol 17B, PE9

E.2.42.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	3	0.010	391.638	6.976E+00	3.761E+00	
exponential (M3)	3	0.010	391.638	6.976E+00	3.761E+00	power hit bound (d = 1)
exponential (M4) [b]	**2**	**0.690**	**382.969**	**8.068E-01**	**3.544E-01**	
exponential (M5)	2	0.690	382.969	8.068E-01	3.544E-01	power hit bound (d = 1)
Hill	2	0.975	382.278	7.239E-01	error	n lower bound hit (n = 1)
linear	3	0.003	394.308	9.841E+00	6.687E+00	
polynomial, 4-degree	3	0.003	394.308	9.841E+00	6.687E+00	
power	3	0.003	394.308	9.841E+00	6.687E+00	power bound hit (power = 1)
Hill, unrestricted	1	0.897	384.243	7.086E-01	error	unrestricted (n = 0.875)
power, unrestricted	2	0.506	383.590	6.280E-01	3.304E-02	unrestricted (power = 0.222)

[a] Non-constant variance model selected ($p = 0.0521$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.42.2. *Output for Selected Model: Exponential (M4)*

Shi et al., 2007: Estradiol 17B, PE9

```
=============================================================================
        Exponential Model. (Version: 1.61;  Date: 7/24/2009)
        Input Data File: C:\1\Blood\59_Shi_2007_Estradiol_Exp_1.(d)
        Gnuplot Plotting File:
                                                  Mon Feb 08 13:28:52 2010
=============================================================================

 Figure 4 PE9 only
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.
```

```
   Model 2 is nested within Models 3 and 4.
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


                Initial Parameter Values

                Variable          Model 4
                --------          --------
                  lnalpha            2.65881
                      rho            0.913414
                        a                 108
                        b            0.277637
                        c            0.340136
                        d                   1



                  Parameter Estimates

                 Variable          Model 4
                 --------          -------
                  lnalpha            1.66773
                      rho            1.15314
                        a            103.146
                        b            1.00685
                        c           0.418742
                        d                  1


         Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0     10        102.9        41.41
 0.3418      10        86.19        19.58
 1.075     10        63.33        29.36
  5.23     10         48.1        18.82
 13.91     10        38.57        22.59


              Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0         103.1        33.35         -0.02738
 0.3418         85.69        29.96          0.05296
  1.075         63.51        25.21         -0.02238
   5.23          43.5        20.27           0.7167
  13.91         43.19        20.19          -0.7237


Other models for which likelihoods are calculated:
```

```
  Model A1:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                    Model      Log(likelihood)      DF         AIC
                    -------    -----------------    ----   ------------
                       A1       -188.3615           6      388.7231
                       A2        -183.667          10      387.3339
                       A3       -186.1132           7      386.2263
                        R       -203.3606           2      410.7211
                        4       -186.4844           5      382.9687


Additive constant for all log-likelihoods =     -45.95.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                         39.39           8            < 0.0001
  Test 2                         9.389           4             0.05208
  Test 3                         4.892           3              0.1798
 Test 6a                        0.7424           2              0.6899


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

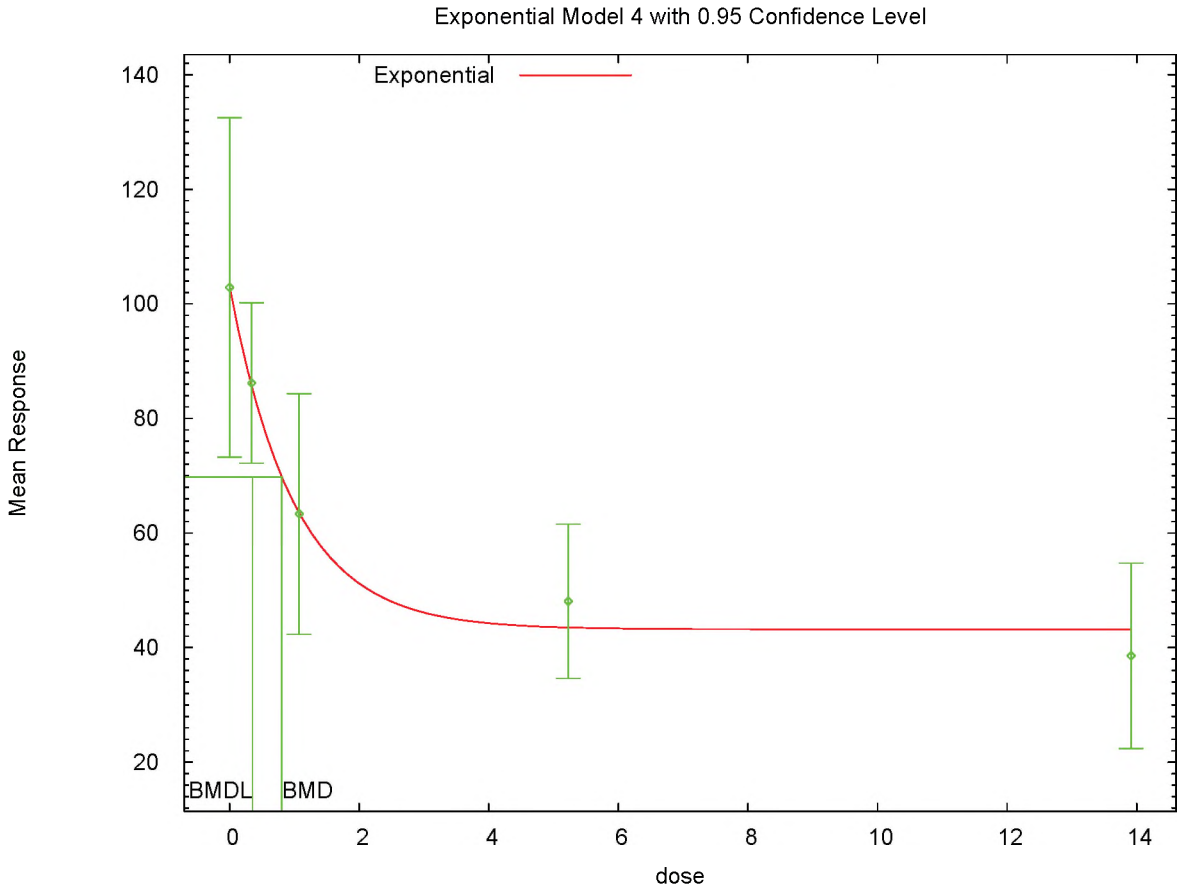
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000
```

BMD = 0.806817

BMDL = 0.354366

E.2.42.3. *Figure for Selected Model: Exponential (M4)*



13:28 02/08 2010

E.2.43. Smialowicz et al., 2008: PFC per 10^6 Cells

E.2.43.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	3	0.101	901.897	8.343E+00	5.064E+00	
exponential (M3)	3	0.101	901.897	8.343E+00	5.064E+00	power hit bound (d = 1)
exponential (M4)	2	0.044	903.897	8.325E+00	1.465E+00	
exponential (M5)	2	0.044	903.897	8.325E+00	1.465E+00	power hit bound (d = 1)
Hill	2	0.063	903.192	3.669E+00	6.970E-01	n lower bound hit (n = 1)
linear	3	0.048	903.585	1.373E+01	1.053E+01	
polynomial, 4-degree	3	0.048	903.585	1.374E+01	1.053E+01	
power	3	0.048	903.585	1.373E+01	1.053E+01	power bound hit (power = 1)
Hill, unrestricted	1	0.213	901.219	1.928E+00	2.208E-01	unrestricted (n = 0.35)
power, unrestricted [b]	**2**	**0.481**	**899.130**	**1.902E+00**	**2.158E-01**	**unrestricted (power = 0.333)**

[a] Constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.43.2. *Output for Selected Model: Power, Unrestricted*

Smialowicz et al., 2008: PFC per 10^6 Cells

```
 ================================================================
          Power Model. (Version: 2.15;  Date: 04/07/2008)
          Input Data File: C:\1\Blood\60_Smial_2008_PFCcells_PwrCV_U_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\60_Smial_2008_PFCcells_PwrCV_U_1.plt
                                          Mon Feb 08 13:29:38 2010
 ================================================================

 Anti Response to SRBCs, PFC per 10to6 cells, Table 4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The power is not restricted
   A constant variance model is fit
```

```
Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                         alpha =       232385
                           rho =            0   Specified
                       control =         1491
                         slope =     -491.716
                         power =     0.288021


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha      control        slope        power

    alpha             1    -3.4e-009     1.8e-009    -1.2e-010

  control    -3.4e-009            1        -0.82        -0.65

    slope     1.8e-009        -0.82            1         0.94

    power    -1.2e-010        -0.65         0.94            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         alpha           219793          37974.5             145365              294222
       control          1470.48           123.73            1227.98             1712.99
         slope         -378.406          157.002           -686.125            -70.6872
         power         0.333124         0.113501           0.110666            0.555581


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    15  1.49e+003    1.47e+003          716          469          0.169
0.438    14  1.13e+003    1.18e+003          171          469         -0.431
2.464    15        945          959          516          469          -0.12
 13.4    15        677          572          465          469          0.867
31.65     8        161          274          117          469         -0.684


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
```

```
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -444.832859           6     901.665718
             A2         -425.402825          10     870.805651
             A3         -444.832859           6     901.665718
          fitted        -445.564823           4     899.129647
              R         -463.753685           2     931.507371


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              76.7017          8          <.0001
   Test 2              38.8601          4          <.0001
   Test 3              38.8601          4          <.0001
   Test 4              1.46393          2           0.481

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  Consider running a
non-homogeneous variance model

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


               Benchmark Dose Computation

Specified effect =             1

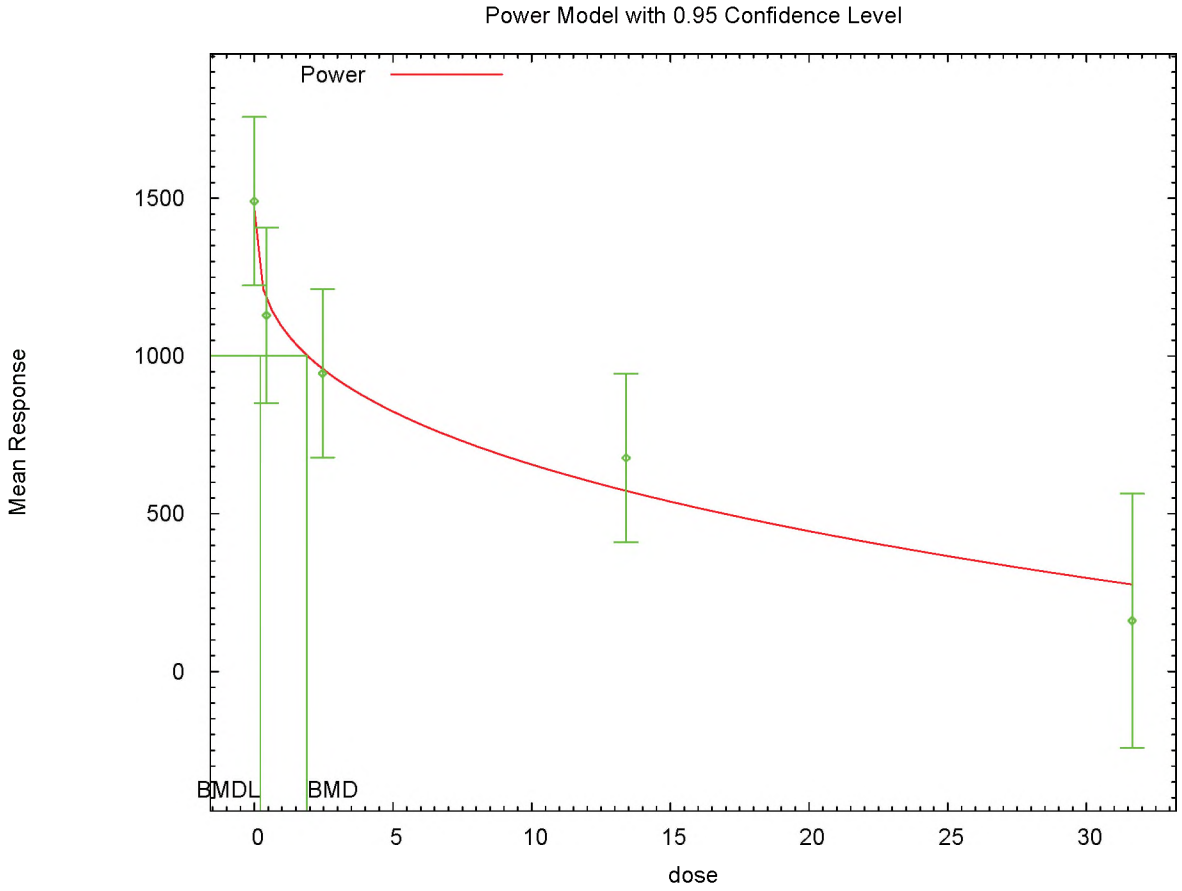
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 1.90249


            BMDL = 0.215843
```

E.2.43.3. *Figure for Selected Model: Power, Unrestricted*



E.2.44. Smialowicz et al., 2008: PFC per Spleen

E.2.44.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	3	0.124	377.565	1.334E+01	8.593E+00	
exponential (M3)	2	0.069	379.138	1.536E+01	8.895E+00	
exponential (M4)	3	0.124	377.565	1.334E+01	8.593E+00	
exponential (M5)	1	0.021	381.138	1.536E+01	8.895E+00	
Hill	2	0.116	378.108	1.568E+01	error	n lower bound hit (n = 1)
linear	3	0.126	377.522	2.055E+01	1.624E+01	
polynomial, 4-degree	3	0.126	377.522	2.055E+01	1.624E+01	
power	3	0.126	377.522	2.055E+01	1.624E+01	power bound hit (power = 1)
Hill, unrestricted	1	0.103	378.463	1.202E+01	error	unrestricted (n = 0.544)
power, unrestricted [b]	**2**	**0.270**	**376.420**	**1.187E+01**	**3.762E+00**	**unrestricted (power = 0.531)**

[a] Non-constant variance model selected ($p = 0.0011$)

[b] Best-fitting model, BMDS output presented in this appendix

E.2.44.2. *Output for Selected Model: Power, Unrestricted*

Smialowicz et al., 2008: PFC per Spleen

```
 ======================================================================
          Power Model. (Version: 2.15;  Date: 04/07/2008)
          Input Data File: C:\1\Blood\61_Smial_2008_PFCspleen_Pwr_U_1.(d)
          Gnuplot Plotting File:  C:\1\Blood\61_Smial_2008_PFCspleen_Pwr_U_1.plt
                                                Mon Feb 08 13:30:16 2010
 ======================================================================

 Anti Response to SRBCs - PFC x 10 to the 4 per spleen, Table 4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   The power is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
```

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	4.76607
rho =	0
control =	27.8
slope =	-9.21898
power =	0.286443

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.98	0.25	-0.28	-0.22
rho	-0.98	1	-0.3	0.28	0.22
control	0.25	-0.3	1	-0.83	-0.74
slope	-0.28	0.28	-0.83	1	0.99
power	-0.22	0.22	-0.74	0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	0.746922	1.02058	-1.25337	2.74721
rho	1.36826	0.355827	0.67085	2.06567
control	25.3816	2.96691	19.5666	31.1967
slope	-3.5662	2.52558	-8.51626	1.38385
power	0.531216	0.175728	0.186796	0.875637

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	15	27.8	25.4	13.4	13.3	0.706
0.438	14	21	23.1	13.6	12.4	-0.626
2.464	15	17.6	19.6	9.4	11.1	-0.704
13.4	15	12.6	11.2	8.7	7.6	0.702
31.65	8	3	3.03	3.1	3.1	-0.0313

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

```
Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1         -190.565019           6     393.130038
            A2         -181.476284          10     382.952569
            A3         -181.900030           7     377.800059
         fitted        -183.210137           5     376.420274
             R         -204.636496           2     413.272993


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              46.3204          8          <.0001
   Test 2              18.1775          4        0.001139
   Test 3              0.84749          3          0.8381
   Test 4              2.62021          2          0.2698

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


               Benchmark Dose Computation

Specified effect =            1

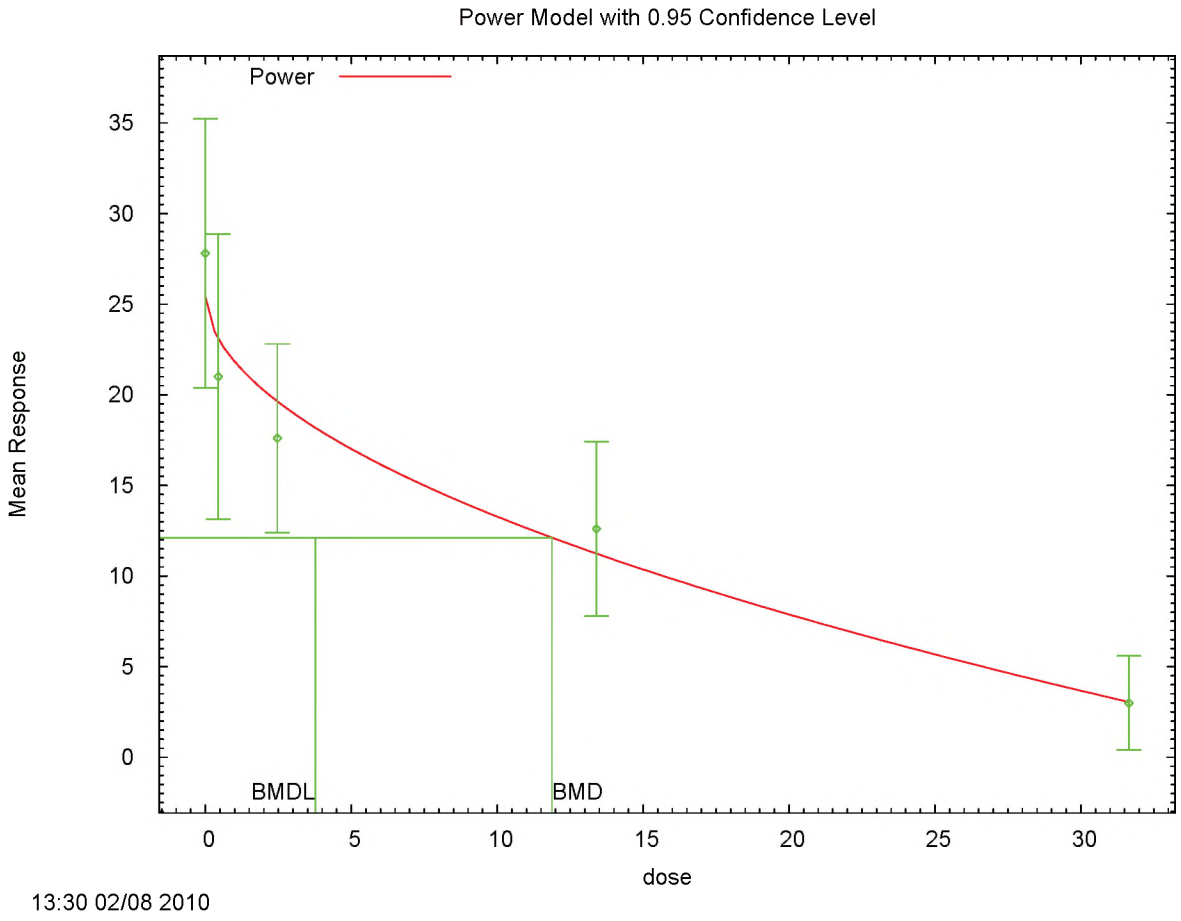
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 11.8748


            BMDL = 3.76161
```

E.2.44.3. *Figure for Selected Model: Power, Unrestricted*



E.2.45. Toth et al., 1979: Amyloidosis

E.2.45.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	2	0.040	149.120	1.965E+01	1.283E+01	power bound hit (power = 1)
logistic	2	0.019	151.340	3.701E+01	2.858E+01	negative intercept (intercept = -2.16)
log-logistic [a]	**2**	**0.053**	**148.269**	**1.503E+01**	**8.747E+00**	**slope bound hit (slope = 1)**
log-probit	2	0.009	152.855	3.782E+01	2.502E+01	slope bound hit (slope = 1)
multistage, 3-degree	2	0.040	149.120	1.965E+01	1.283E+01	final ß = 0
probit	2	0.021	151.115	3.467E+01	2.657E+01	negative intercept (intercept = -1.276)
Weibull	2	0.040	149.120	1.965E+01	1.283E+01	power bound hit (power = 1)
gamma, unrestricted	2	0.959	140.119	4.349E-01	2.891E-03	unrestricted (power = 0.254)
log-logistic, unrestricted [b]	2	0.903	140.240	4.843E-01	5.312E-03	unrestricted (slope = 0.326)
log-probit, unrestricted	2	0.870	140.315	4.960E-01	7.292E-03	unrestricted (slope = 0.186)
Weibull, unrestricted	2	0.933	140.174	4.641E-01	4.069E-03	unrestricted (power = 0.289)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.2.45.2. *Output for Selected Model: Log-Logistic*

Toth et al., 1979: Amyloidosis

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\Blood\62_Toth_1979_Amylyr_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\62_Toth_1979_Amylyr_LogLogistic_1.plt
                                                Mon Feb 08 13:30:54 2010
 ================================================================

 Table 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
```

```
    Slope parameter is restricted as slope >= 1

    Total number of observations = 4
    Total number of records with missing values = 0
    Maximum number of iterations = 250
    Relative Function Convergence has been set to: 1e-008
    Parameter Convergence has been set to: 1e-008



    User has chosen the log transformed model


                  Default Initial Parameter Values
                     background =            0
                      intercept =     -4.54593
                          slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             background    intercept

background            1        -0.49

 intercept        -0.49            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0699918            *               *                   *
      intercept         -4.90704            *               *                   *
          slope                1            *               *                   *

* - Indicates that this value is not calculated.



                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -68.017         4
   Fitted model        -72.1346         2       8.23525      2         0.01628
  Reduced model        -82.0119         1         27.99      3         <.0001

           AIC:         148.269


                                 Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
    0.0000     0.0700         2.660     0.000          38       -1.691
    0.5732     0.0739         3.252     5.000          44        1.007
   14.2123     0.1584         6.971    10.000          44        1.251
   91.2070     0.4446        19.117    17.000          43       -0.650

 Chi^2 = 5.86      d.f. = 2        P-value = 0.0534


   Benchmark Dose Computation

Specified effect =           0.1
```

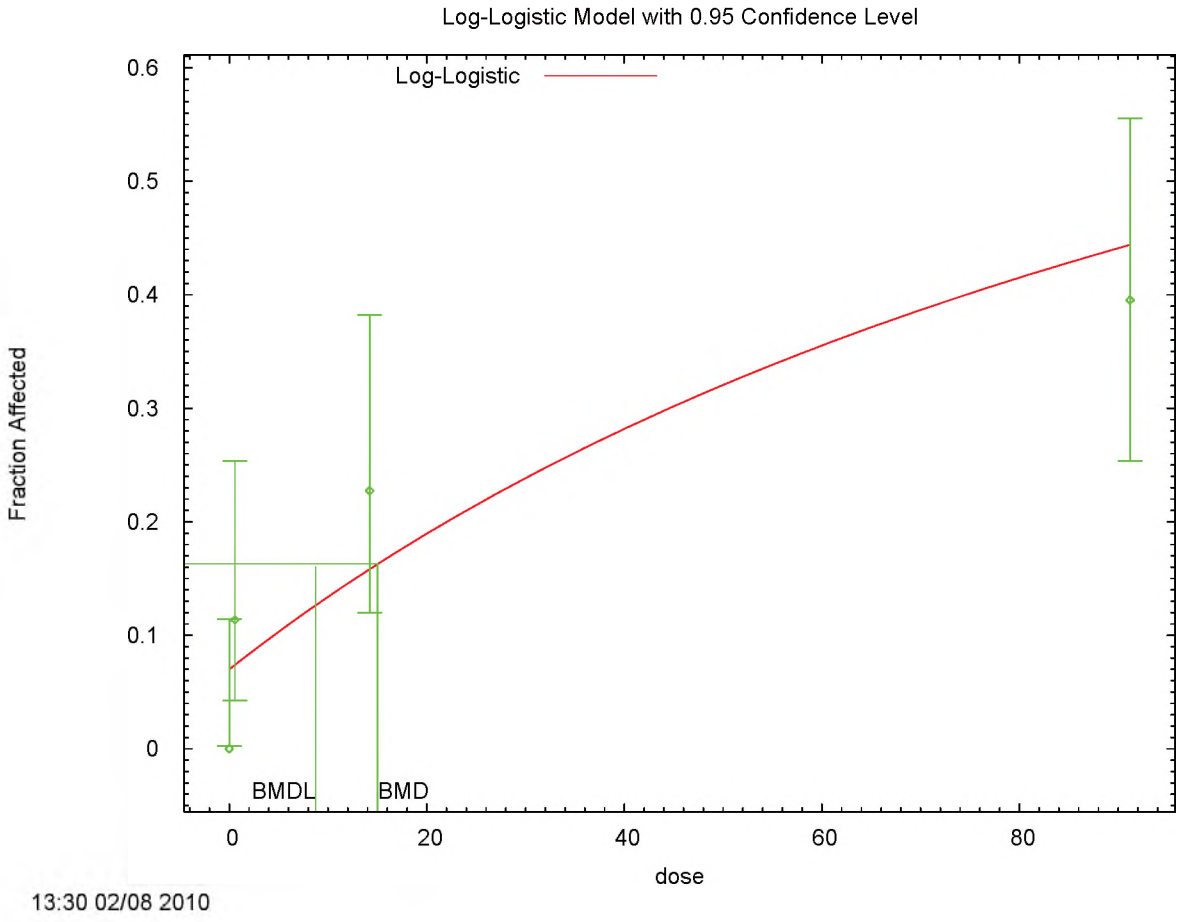
```
Risk Type          =      Extra risk

Confidence level =           0.95

              BMD =        15.0264

             BMDL =        8.74665
```

E.2.45.3. *Figure for Selected Model: Log-Logistic*



E.2.45.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

Toth et al., 1979: Amyloidosis

```
 ========================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\Blood\62_Toth_1979_Amy1yr_LogLogistic_U_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\62_Toth_1979_Amy1yr_LogLogistic_U_1.plt
                                                Mon Feb 08 13:30:54 2010
 ========================================================================

 Table 2
 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

```
The form of the probability function is:

P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


Dependent variable = DichEff
Independent variable = Dose
Slope parameter is not restricted

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



User has chosen the log transformed model


                Default Initial Parameter Values
                   background =            0
                    intercept =     -1.92722
                        slope =     0.314472


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -background
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

             intercept        slope

 intercept            1        -0.84

     slope        -0.84            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background                0            *               *                   *
      intercept         -1.96073            *               *                   *
          slope         0.326156            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -68.017         4
   Fitted model        -68.1201         2      0.206341      2           0.902
  Reduced model        -82.0119         1         27.99      3         <.0001

          AIC:          140.24


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
 ----------------------------------------------------------------------
   0.0000     0.0000         0.000     0.000          38        0.000
   0.5732     0.1051         4.623     5.000          44        0.186
  14.2123     0.2507        11.029    10.000          44       -0.358
  91.2070     0.3802        16.348    17.000          43        0.205
```

```
 Chi^2 = 0.20      d.f. = 2        P-value = 0.9028


   Benchmark Dose Computation

Specified effect =            0.1

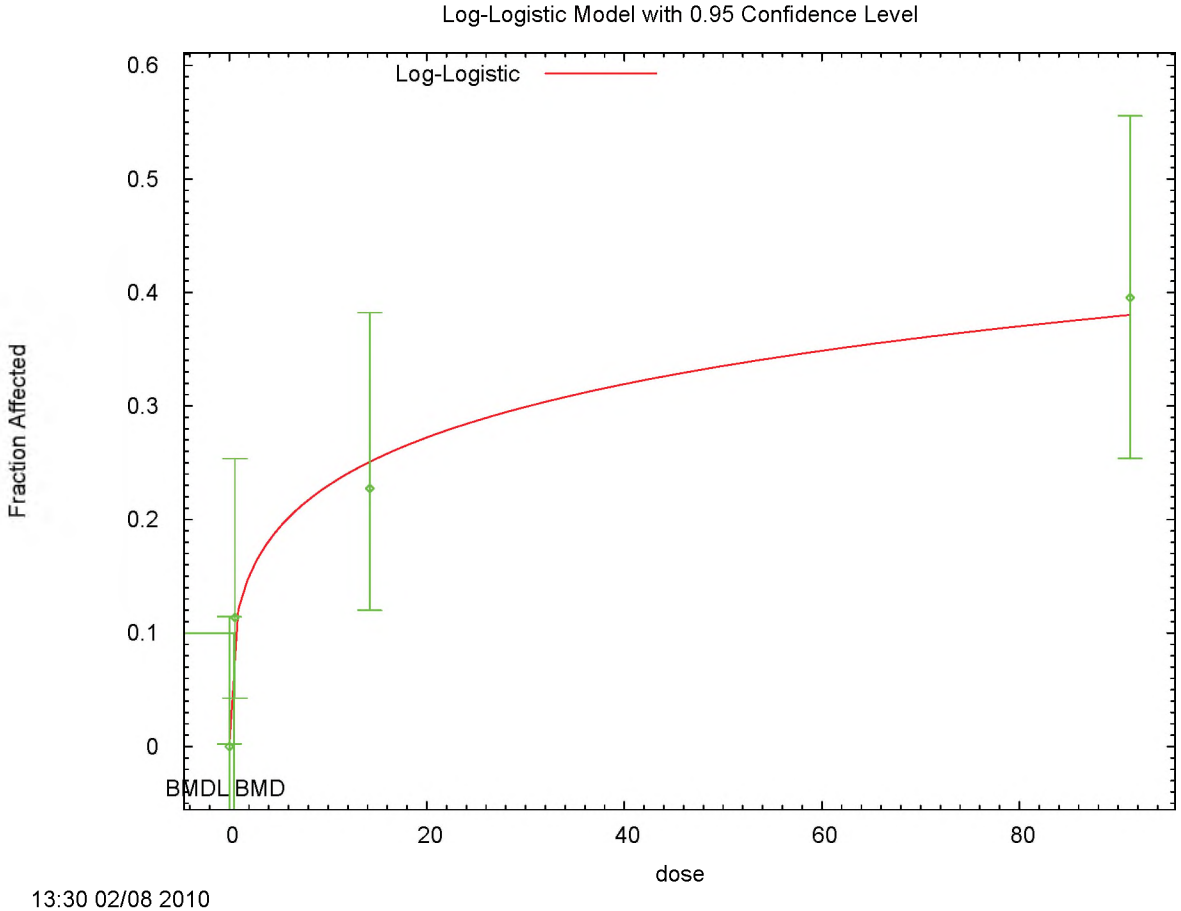
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.484272

            BMDL =     0.00531211
```

E.2.45.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.2.46. Toth et al., 1979: Skin Lesions

E.2.46.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	2	0.032	156.346	1.037E+01	7.470E+00	power bound hit (power = 1)
logistic	2	0.005	161.421	2.487E+01	1.982E+01	negative intercept (intercept = -1.999)
log-logistic [a]	**2**	**0.078**	**153.963**	**6.413E+00**	**4.025E+00**	**slope bound hit (slope = 1)**
log-probit	2	0.003	161.788	1.887E+01	1.280E+01	slope bound hit (slope = 1)
multistage, 3-degree	2	0.032	156.346	1.037E+01	7.470E+00	final ß = 0
probit	2	0.006	160.991	2.309E+01	1.858E+01	negative intercept (intercept = -1.198)
Weibull	2	0.032	156.346	1.037E+01	7.470E+00	power bound hit (power = 1)
gamma, unrestricted	2	0.945	147.148	error	error	unrestricted (power = 0.341)
log-logistic, unrestricted [b]	2	0.744	147.631	5.969E-01	6.773E-02	unrestricted (slope = 0.48)
log-probit, unrestricted	2	0.670	147.844	5.939E-01	8.147E-02	unrestricted (slope = 0.279)
Weibull, unrestricted	2	0.866	147.324	5.539E-01	5.181E-02	unrestricted (power = 0.405)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.2.46.2. *Output for Selected Model: Log-Logistic*

Toth et al., 1979: Skin Lesions

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\Blood\63_Toth_1979_SkinLes_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\63_Toth_1979_SkinLes_LogLogistic_1.plt
                                              Wed Feb 10 14:47:53 2010
 ================================================================

 Table 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
```

```
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =            0
                     intercept =     -3.94312
                         slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             background    intercept

background            1        -0.43

 intercept        -0.43            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0564562            *               *                   *
      intercept         -4.05558            *               *                   *
          slope                1            *               *                   *

* - Indicates that this value is not calculated.



                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -71.5177         4
   Fitted model         -74.9813         2       6.92722      2         0.03132
  Reduced model         -95.8498         1       48.6642      3         <.0001

           AIC:          153.963


                                  Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ---------------------------------------------------------------------
    0.0000     0.0565         2.145     0.000          38        -1.508
    0.5732     0.0657         2.892     5.000          44         1.282
   14.2123     0.2429        10.687    13.000          44         0.813
   91.2070     0.6343        27.275    25.000          43        -0.720

 Chi^2 = 5.10      d.f. = 2        P-value = 0.0782


   Benchmark Dose Computation

Specified effect =           0.1
```

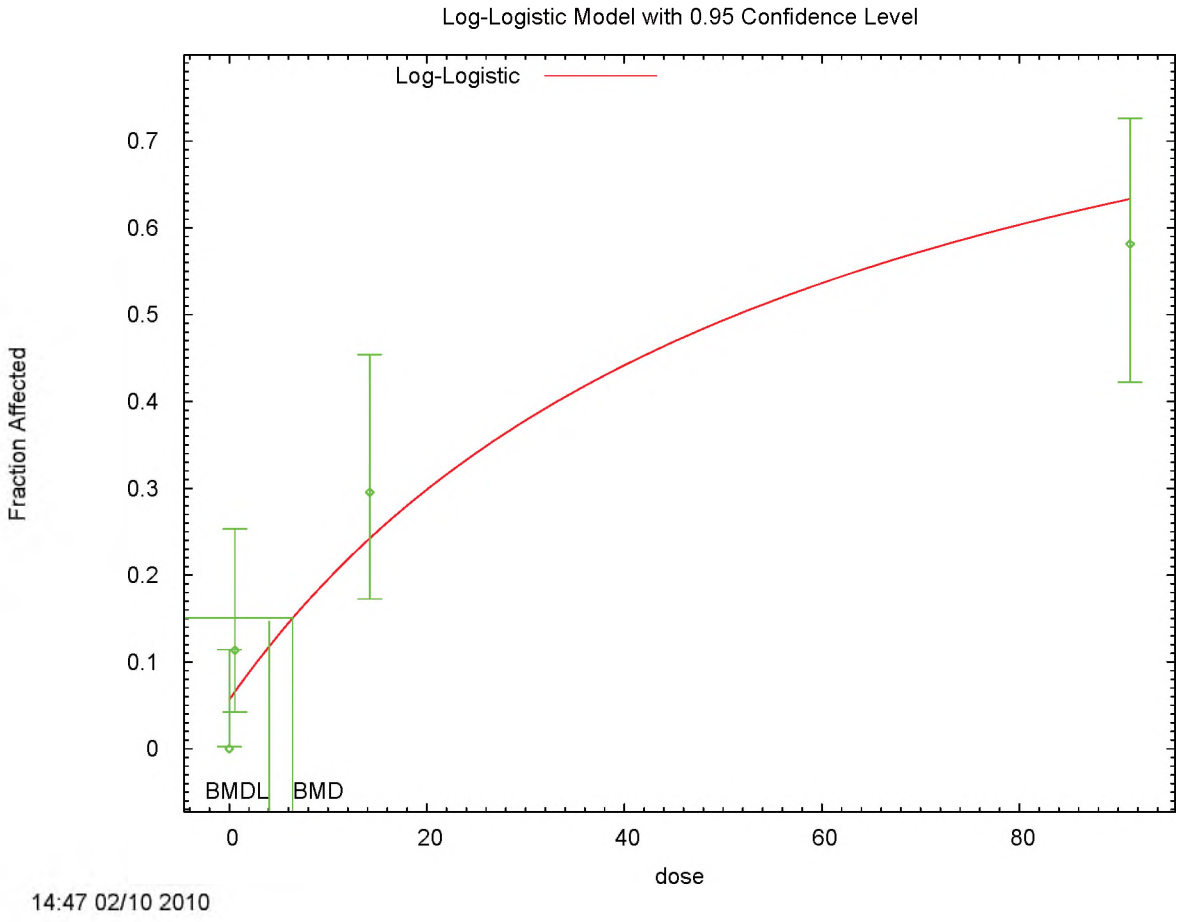
```
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         6.4132

            BMDL =         4.0249
```

E.2.46.3. *Figure for Selected Model: Log-Logistic*



E.2.46.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

Toth et al., 1979: Skin Lesions

```
================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\1\Blood\63_Toth_1979_SkinLes_LogLogistic_U_1.(d)
        Gnuplot Plotting File:  C:\1\Blood\63_Toth_1979_SkinLes_LogLogistic_U_1.plt
                                          Wed Feb 10 14:47:54 2010
================================================================

Table 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

The form of the probability function is:

P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

Dependent variable = DichEff
Independent variable = Dose
Slope parameter is not restricted

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = -1.87608
slope = 0.458888

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.86
slope	-0.86	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-1.94946	*	*	*
slope	0.4802	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-71.5177	4			
Fitted model	-71.8153	2	0.59526	2	0.7426
Reduced model	-95.8498	1	48.6642	3	<.0001

AIC: 147.631

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	38	0.000
0.5732	0.0983	4.323	5.000	44	0.343
14.2123	0.3374	14.845	13.000	44	-0.588
91.2070	0.5542	23.832	25.000	43	0.358

```
 Chi^2 = 0.59      d.f. = 2        P-value = 0.7438


   Benchmark Dose Computation

Specified effect =           0.1

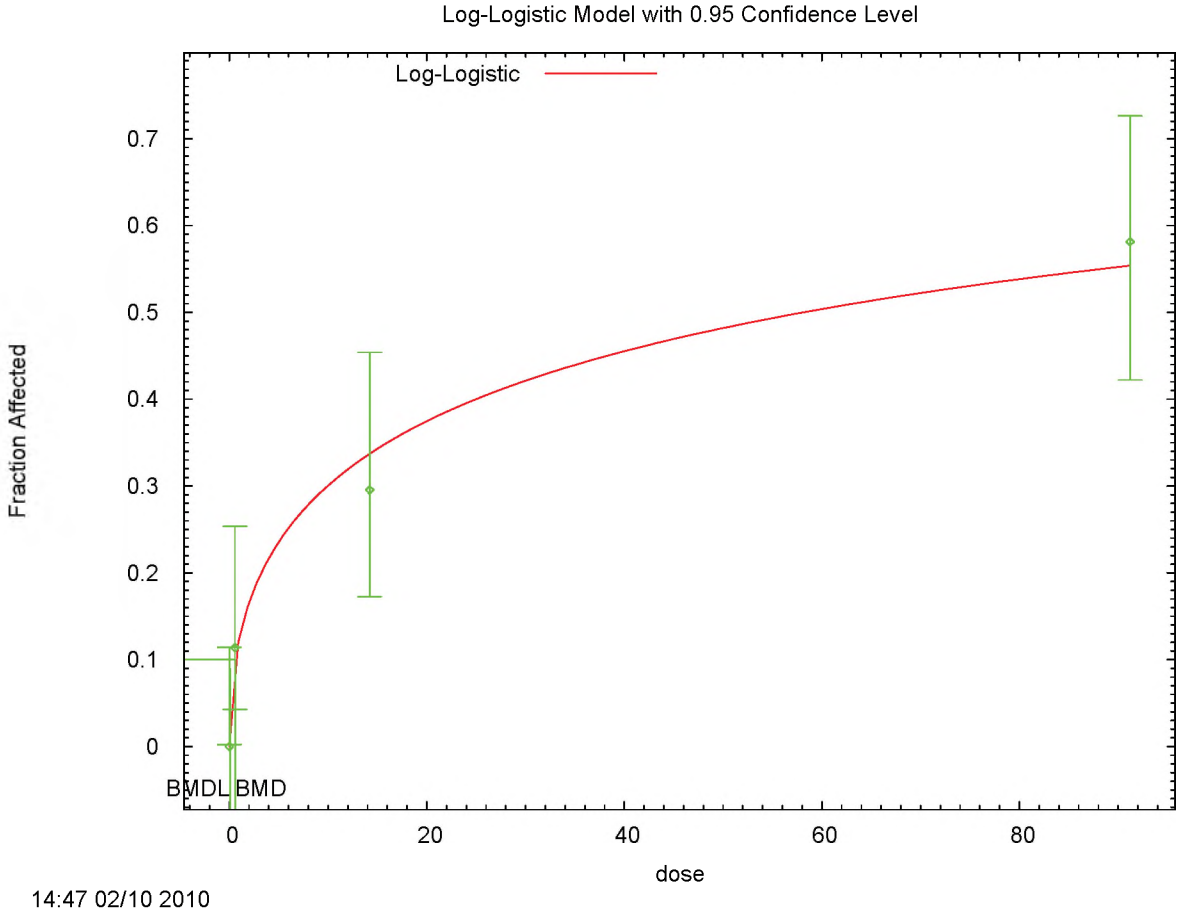
Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =      0.596932

            BMDL =       0.06773
```

E.2.46.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.2.47. Van Birgelen et al., 1995a: Hepatic Retinol

E.2.47.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	4	<0.0001	159.735	7.790E+00	4.150E+00	
exponential (M3)	4	<0.0001	3222.700	5.542E+01	error	power hit bound (d = 1)
exponential (M4) [b]	**3**	**<0.001**	**141.454**	**2.488E+01**	**3.363E+00**	
exponential (M5)	3	<0.001	141.454	2.488E+01	3.363E+00	power hit bound (d = 1)
Hill	3	0.239	124.865	5.316E+00	error	n lower bound hit (n = 1)
linear	4	<0.0001	176.828	1.877E+02	1.437E+02	
polynomial, 5-degree	4	<0.0001	176.828	1.877E+02	1.437E+02	
power	4	<0.0001	176.828	1.877E+02	1.437E+02	power bound hit (power = 1)
Hill, unrestricted	2	0.241	125.495	3.595E+00	error	unrestricted (n = 0.763)
power, unrestricted [c]	3	0.011	131.771	3.802E-01	1.393E-02	unrestricted (power = 0.14)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.47.2. *Output for Selected Model: Exponential (M4)*

Van Birgelen et al., 1995a: Hepatic Retinol

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\65_VanB_1995a_HepRet_Exp_1.(d)
         Gnuplot Plotting File:
                                                Mon Feb 08 13:32:00 2010
 ================================================================

 Tbl3, hepatic retinol
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
```

```
      sign = -1 for decreasing trend.

   Model 2 is nested within Models 3 and 4.
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


              Initial Parameter Values

              Variable          Model 4
              --------          --------
                lnalpha            -1.16065
                    rho             1.53688
                      a              15.645
                      b           0.0254351
                      c           0.0365247
                      d                   1



                Parameter Estimates

               Variable          Model 4
               --------          -------
                lnalpha           -0.92683
                    rho            1.77262
                      a            11.5049
                      b          0.0286598
                      c          0.0653043
                      d                  1


        Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0      8         14.9        8.768
  7.204      8          8.4        3.394
  11.76      8          8.2        2.263
  18.09      8          5.1       0.8485
  86.41      8          2.2       0.8485
  250.2      8          0.6       0.5657


              Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0          11.5        5.483            1.751
  7.204         9.499        4.627          -0.6719
  11.76         8.428        4.161          -0.1552
  18.09         7.154        3.599           -1.615
  86.41         1.655       0.9832            1.568
  250.2        0.7596       0.4931          -0.9155
```

Other models for which likelihoods are calculated:

```
  Model A1:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
                Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-87.1567	7	188.3134
A2	-47.28742	12	118.5748
A3	-55.32422	8	126.6484
R	-109.967	2	223.934
4	-65.72714	5	141.4543

Additive constant for all log-likelihoods = -44.11. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	125.4	10	< 0.0001
Test 2	79.74	5	< 0.0001
Test 3	16.07	4	0.002922
Test 6a	20.81	3	0.0001155

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 6a is less than .1. Model 4 may not adequately describe the data; you may want to consider another model.

Benchmark Dose Computations:

Specified Effect = 1.000000

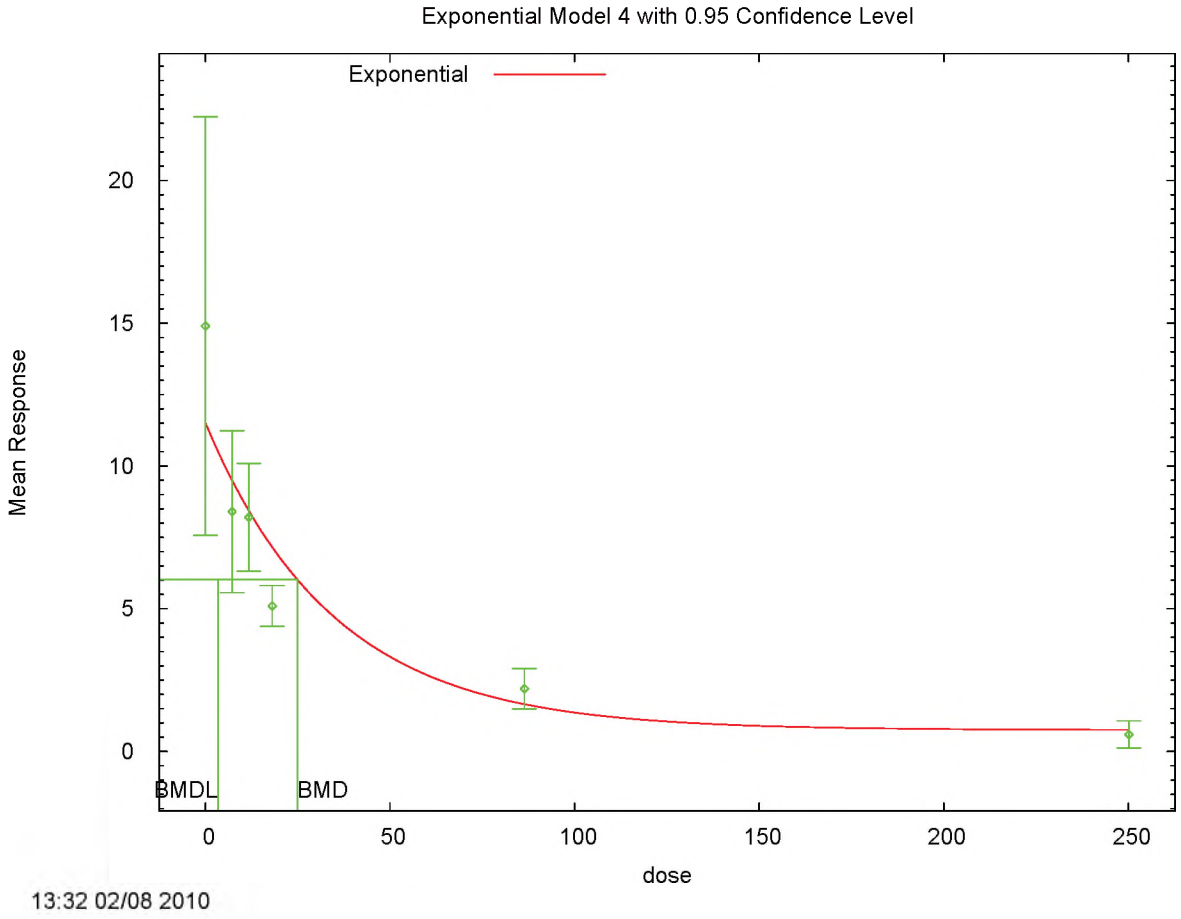
```
            Risk Type = Estimated standard deviations from control

      Confidence Level = 0.950000

                   BMD =      24.8811

                  BMDL =      3.36281
```

E.2.47.3. *Figure for Selected Model: Exponential (M4)*



E.2.47.4. *Output for Additional Model Presented: Power, Unrestricted*

Van Birgelen et al., 1995a: Hepatic Retinol

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\1\Blood\65_VanB_1995a_HepRet_Pwr_U_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\65_VanB_1995a_HepRet_Pwr_U_1.plt
                                              Mon Feb 08 13:32:03 2010
 ================================================================

 Tbl3, hepatic retinol
 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = Mean
Independent variable = Dose
The power is not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	2.76506
rho =	0
control =	14.9
slope =	-3.98831
power =	0.231232

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.8	-0.042	0.038	0.063
rho	-0.8	1	-0.089	0.0044	-0.1
control	-0.042	-0.089	1	-0.95	-0.81
slope	0.038	0.0044	-0.95	1	0.95
power	0.063	-0.1	-0.81	0.95	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-0.986251	0.394722	-1.75989	-0.212609
rho	1.67858	0.202896	1.28091	2.07625
control	16.9266	2.23237	12.5513	21.302
slope	-7.51118	2.04379	-11.5169	-3.50543
power	0.139871	0.0269576	0.0870351	0.192707

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	14.9	16.9	8.77	6.56	-0.874
7.204	8	8.4	7.03	3.39	3.14	1.24
11.76	8	8.2	6.32	2.26	2.87	1.85
18.09	8	5.1	5.67	0.849	2.62	-0.611
86.41	8	2.2	2.91	0.849	1.5	-1.34
250.2	8	0.6	0.666	0.566	0.434	-0.427

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          -87.156698           7     188.313395
              A2          -47.287416          12     118.574833
              A3          -55.324218           8     126.648436
          fitted          -60.885746           5     131.771493
               R         -109.967018           2     223.934036


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              125.359         10          <.0001
  Test 2              79.7386          5          <.0001
  Test 3              16.0736          4        0.002922
  Test 4              11.1231          3         0.01108

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is less than .1.  You may want to try a different
model


               Benchmark Dose Computation

Specified effect =             1

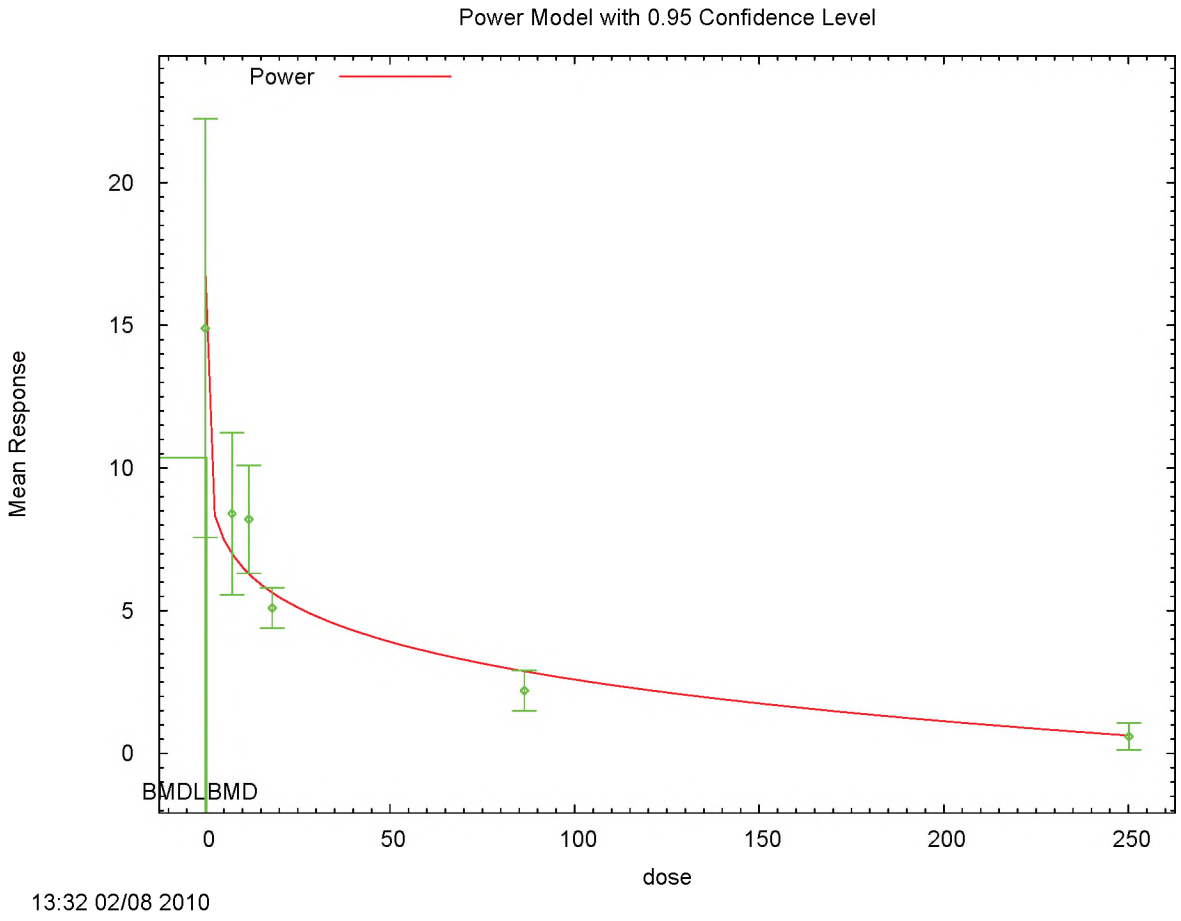
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 0.380208


             BMDL = 0.013927
```

E.2.47.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.2.48. Van Birgelen et al., 1995a: Hepatic Retinol Palmitate

E.2.48.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	4	<0.0001	460.282	error	error	
exponential (M3)	4	<0.0001	460.282	error	error	power hit bound (d = 1)
exponential (M4) [b]	**3**	**<0.0001**	**446.995**	**1.415E+02**	**3.647E+01**	
exponential (M5)	3	<0.0001	446.995	1.415E+02	3.647E+01	power hit bound (d = 1)
Hill	3	0.009	416.233	3.657E+00	error	n lower bound hit (n = 1)
linear	4	<0.0001	486.375	3.487E+02	2.412E+02	
polynomial, 5-degree	0	N/A	584.170	error	5.617E+02	
power	4	<0.0001	486.375	3.487E+02	2.412E+02	power bound hit (power = 1)
Hill, unrestricted	3	<0.0001	527.310	6.875E-14	6.875E-14	unrestricted (n = 0.613)
power, unrestricted [c]	3	0.239	408.982	5.262E-02	5.889E-05	unrestricted (power = 0.064)

[a] Non-constant variance model selected (*p* = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.48.2. *Output for Selected Model: Exponential (M4)*

Van Birgelen et al., 1995a: Hepatic Retinol Palmitate

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\Blood\66_VanB_1995a_HepRetPalm_Exp_1.(d)
         Gnuplot Plotting File:
                                                 Mon Feb 08 13:32:41 2010
 ================================================================

 Tbl3, hepatic retinol palmitate
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
```

```
      sign = -1 for decreasing trend.

   Model 2 is nested within Models 3 and 4.
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


              Initial Parameter Values

              Variable          Model 4
              --------          --------
                lnalpha          0.284674
                    rho           1.77158
                      a             495.6
                      b         0.0337826
                      c        0.00576502
                      d                 1


                Parameter Estimates

               Variable          Model 4
               --------          -------
                lnalpha         -0.241601
                    rho           2.03456
                      a           223.848
                      b         0.0300737
                      c         0.0129253
                      d                 1

  NC = No Convergence


        Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0      8           472        271.5
  7.204      8            94        67.88
  11.76      8           107        76.37
  18.09      8            74         39.6
  86.41      8            22        22.63
  250.2      8             3        2.828


              Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
  ------    ----------    ---------    ----------------
      0         223.8        217.8            3.222
  7.204         180.8        175.3           -1.401
  11.76           158        152.9          -0.9443
  18.09         131.1        126.4           -1.278
  86.41         19.33        18.03           0.4197
```

```
  250.2         3.013        2.721         -0.01317


Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                  Model      Log(likelihood)      DF         AIC
                 -------    -----------------    ----   ------------
                     A1       -250.5548           7      515.1096
                     A2       -196.7557          12      417.5115
                     A3       -197.3832           8      410.7663
                      R       -276.7896           2      557.5793
                      4       -218.4977           5      446.9954


Additive constant for all log-likelihoods =     -44.11.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                              Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                          Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                      160.1            10           < 0.0001
  Test 2                      107.6             5           < 0.0001
  Test 3                      1.255             4              0.869
 Test 6a                      42.23             3           < 0.0001


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is less than .1.  Model 4 may not adequately
  describe the data; you may want to consider another model.


Benchmark Dose Computations:
```

```
Specified Effect = 1.000000

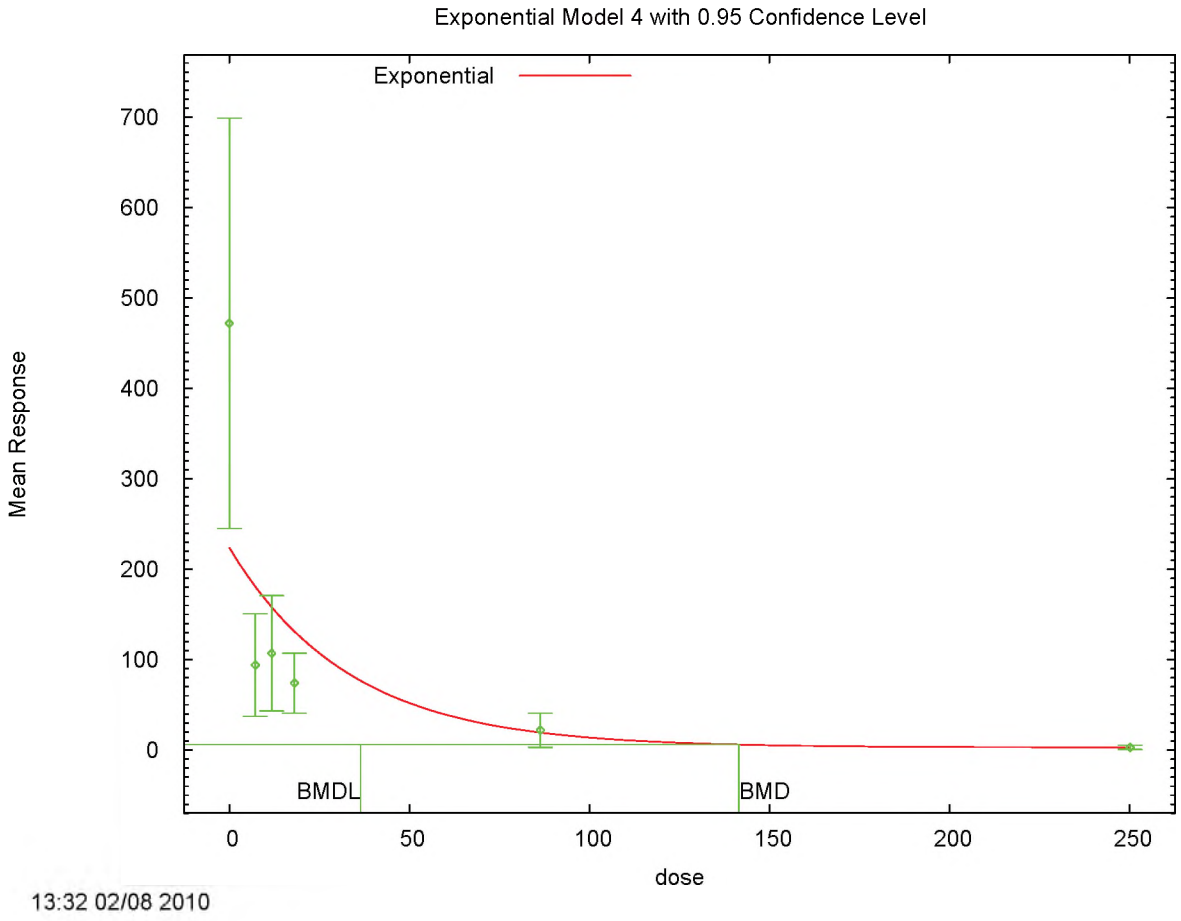
        Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

             BMD =      141.528

            BMDL =      36.4721
```

E.2.48.3. *Figure for Selected Model: Exponential (M4)*



E.2.48.4. *Output for Additional Model Presented: Power, Unrestricted*

Van Birgelen et al., 1995a: Hepatic Retinol Palmitate

```
================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\1\Blood\66_VanB_1995a_HepRetPalm_Pwr_U_1.(d)
        Gnuplot Plotting File:  C:\1\Blood\66_VanB_1995a_HepRetPalm_Pwr_U_1.plt
                                          Mon Feb 08 13:32:47 2010
================================================================

Tbl3, hepatic retinol palmitate
```

~~~~~~

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = Mean
Independent variable = Dose
The power is not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	9.57332
rho =	0
control =	472
slope =	-320.514
power =	0.0711173

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.95	0.3	-0.31	-0.3
rho	-0.95	1	-0.41	0.39	0.29
control	0.3	-0.41	1	-0.98	-0.82
slope	-0.31	0.39	-0.98	1	0.9
power	-0.3	0.29	-0.82	0.9	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
lalpha	0.0640168	0.859472	-1.62052	1.74855
rho	1.81132	0.197468	1.42429	2.19835
control	464.29	87.5705	292.655	635.925
slope	-324.216	83.3327	-487.545	-160.887
power	0.0639088	0.0139778	0.0365129	0.0913048

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	472	464	272	269	0.0812
7.204	8	94	96.5	67.9	64.7	-0.108
11.76	8	107	84.8	76.4	57.6	1.09
18.09	8	74	74.2	39.6	51	-0.00941
86.41	8	22	33.2	22.6	24.6	-1.28
250.2	8	3	2.86	2.83	2.68	0.145
~~~~~~

```
Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -250.554817           7     515.109634
              A2         -196.755746          12     417.511491
              A3         -197.383174           8     410.766347
          fitted         -199.490808           5     408.981615
               R         -276.789644           2     557.579287


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              160.068         10          <.0001
   Test 2              107.598          5          <.0001
   Test 3              1.25486          4           0.869
   Test 4              4.21527          3          0.2391

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


               Benchmark Dose Computation

Specified effect =             1

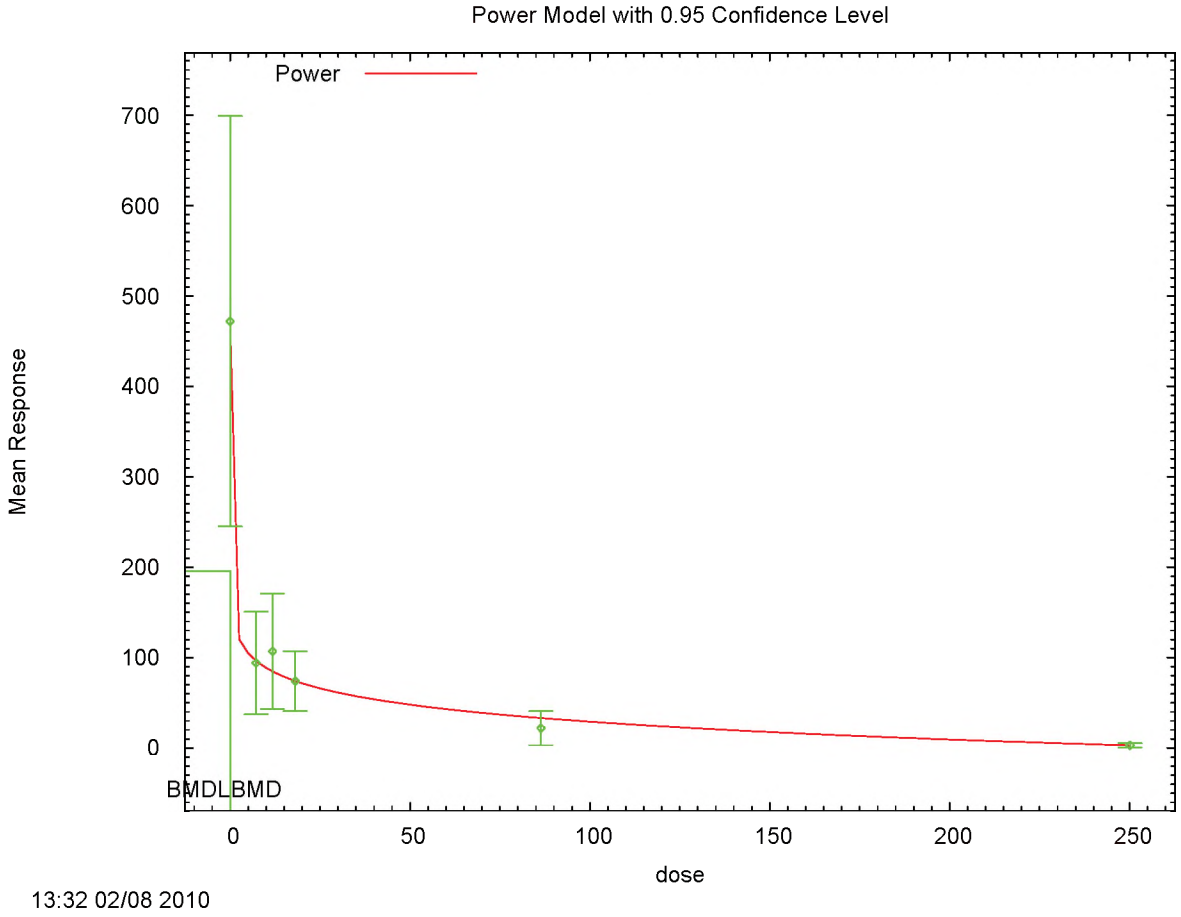
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 0.0526247
```

BMDL = 5.88883e-005

E.2.48.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.2.49. White et al., 1986: CH50

E.2.49.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	5	0.002	389.664	1.957E+01	1.261E+01	
exponential (M3)	5	0.002	389.664	1.957E+01	1.261E+01	power hit bound (d = 1)
exponential (M4)	4	0.001	390.632	1.411E+01	5.177E+00	
exponential (M5)	4	0.001	390.632	1.411E+01	5.177E+00	power hit bound (d = 1)
Hill [b]	**4**	**0.002**	**389.601**	**8.632E+00**	**1.498E+00**	**n lower bound hit (n = 1)**
linear	5	<0.001	394.446	3.497E+01	2.568E+01	
polynomial, 6-degree	5	<0.001	394.446	3.497E+01	2.568E+01	
power	5	<0.001	394.446	3.497E+01	2.568E+01	power bound hit (power = 1)
Hill, unrestricted [c]	3	0.071	381.520	1.481E-01	4.351E-03	unrestricted (n = 0.246)
power, unrestricted	4	0.148	379.265	1.211E-01	1.225E-03	unrestricted (power = 0.227)

[a] Non-constant variance model selected ($p = 0.0871$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.2.49.2. *Output for Selected Model: Hill*

White et al., 1986: CH50

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\Blood\71_White_1986_CH50_Hill_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\71_White_1986_CH50_Hill_1.plt
                                                Mon Feb 08 13:35:56 2010
 ================================================================

 [insert study notes]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
```

```
The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

Total number of dose groups = 7
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                       lalpha =      5.60999
                          rho =            0
                    intercept =           91
                            v =          -74
                            n =     0.118036
                            k =        1.094


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -n
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 lalpha          rho    intercept            v            k

    lalpha            1        -0.99         0.27         0.23        -0.32

       rho        -0.99            1        -0.28        -0.24         0.33

 intercept         0.27        -0.28            1         0.39        -0.78

         v         0.23        -0.24         0.39            1        -0.85

         k        -0.32         0.33        -0.78        -0.85            1


                                 Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha            4.581          1.66273            1.32211             7.83989
            rho          0.31293         0.431616          -0.533022             1.15888
      intercept          74.6365          6.33673            62.2167             87.0562
              v         -66.2096          14.7876           -95.1928            -37.2264
              n                1               NA
              k          20.8286          21.3237             -20.965             62.6223

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     8         91         74.6         14.1         19.4           2.39
1.094     8         54         71.3         8.49         19.3          -2.54
4.085     8         63         63.8         11.3         18.9         -0.117
 7.14     8         56         57.7         25.5         18.6         -0.263
26.81     8         41         37.4           17         17.4          0.589
48.72     8         32         28.3           17         16.7          0.636
90.56     8         17         20.8           17         15.9         -0.678
```

```
Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -181.340979           8     378.681959
              A2         -175.820265          14     379.640529
              A3         -181.238690           9     380.477380
          fitted         -189.800288           5     389.600575
               R         -212.367055           2     428.734109


                    Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              73.0936         12          <.0001
   Test 2              11.0414          6          0.0871
   Test 3              10.8369          5         0.05471
   Test 4              17.1232          4        0.001829

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =             1

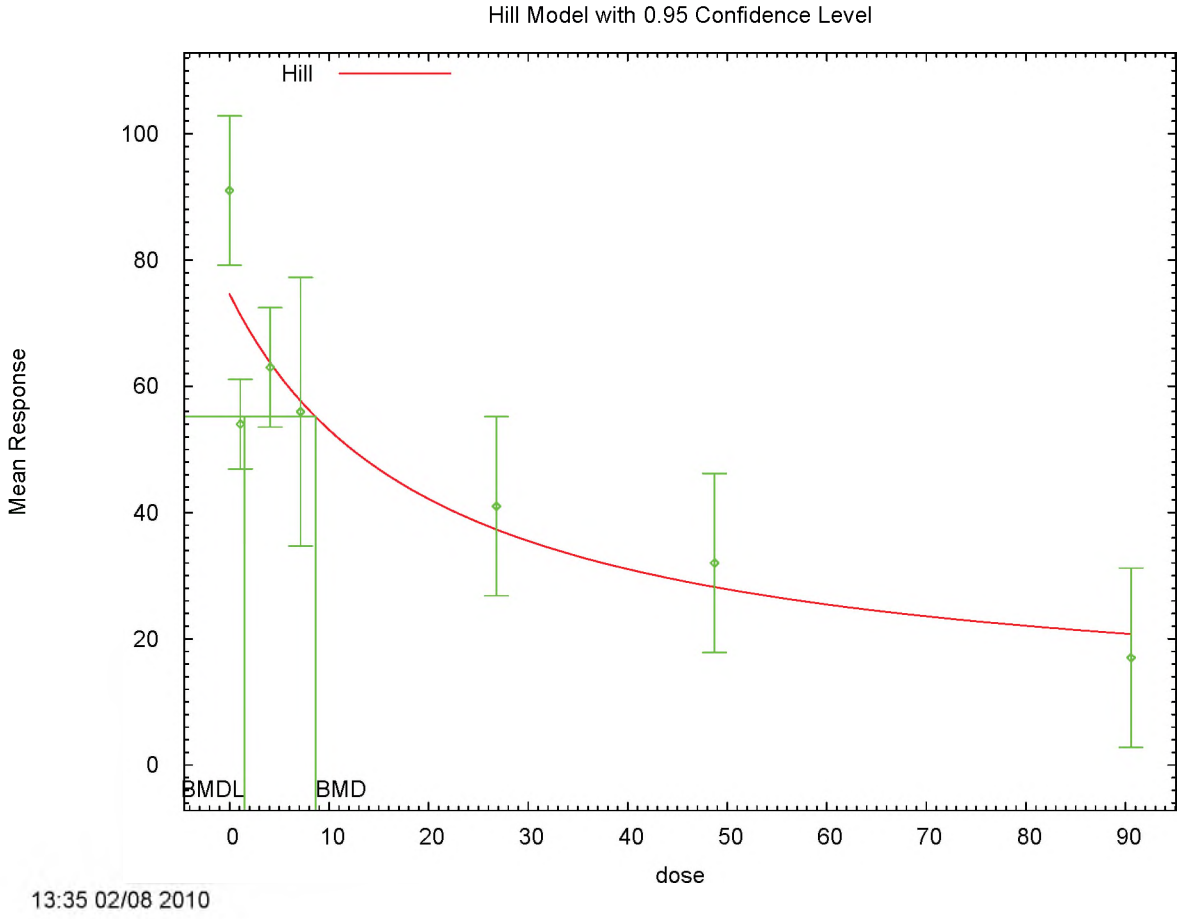
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       8.63239
```

```
BMDL =        1.49823
```

E.2.49.3. *Figure for Selected Model: Hill*



E.2.49.4. *Output for Additional Model Presented: Hill, Unrestricted*

White et al., 1986: CH50

```
=============================================================================
        Hill Model. (Version: 2.14;  Date: 06/26/2008)
        Input Data File: C:\1\Blood\71_White_1986_CH50_Hill_U_1.(d)
        Gnuplot Plotting File:  C:\1\Blood\71_White_1986_CH50_Hill_U_1.plt
                                                Mon Feb 08 13:35:57 2010
=============================================================================

[insert study notes]

  The form of the response function is:

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = Mean
```

Independent variable = Dose
Power parameter is not restricted
The variance is to be modeled as Var(i) = exp(lalpha + rho * ln(mean(i)))

Total number of dose groups = 7
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	5.60999
rho =	0
intercept =	91
v =	-74
n =	0.118036
k =	1.094

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	intercept	v	n	k
lalpha	1	-1	0.16	0.19	-0.4	-0.014
rho	-1	1	-0.16	-0.19	0.4	0.011
intercept	0.16	-0.16	1	0.15	-0.58	0.015
v	0.19	-0.19	0.15	1	-0.02	-0.93
n	-0.4	0.4	-0.58	-0.02	1	-0.35
k	-0.014	0.011	0.015	-0.93	-0.35	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	6.54093	2.08879	2.44698	10.6349
rho	-0.245847	0.541645	-1.30745	0.815757
intercept	89.6302	5.59428	78.6656	100.595
v	-628.486	727.973	-2055.29	798.315
n	0.246409	0.058636	0.131484	0.361333
k	493877	2.74838e+006	-4.89284e+006	5.88059e+006

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	91	89.6	14.1	15.1	0.256
1.094	8	54	65.2	8.49	15.8	-2.01
4.085	8	63	56.3	11.3	16	1.17
7.14	8	56	51.7	25.5	16.2	0.746
26.81	8	41	38.3	17	16.8	0.453
48.72	8	32	30.9	17	17.3	0.175
90.56	8	17	22.3	17	18	-0.831

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -181.340979           8     378.681959
              A2         -175.820265          14     379.640529
              A3         -181.238690           9     380.477380
          fitted         -184.759769           6     381.519538
               R         -212.367055           2     428.734109


                    Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              73.0936         12          <.0001
  Test 2              11.0414          6          0.0871
  Test 3              10.8369          5         0.05471
  Test 4              7.04216          3         0.07057

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =             1

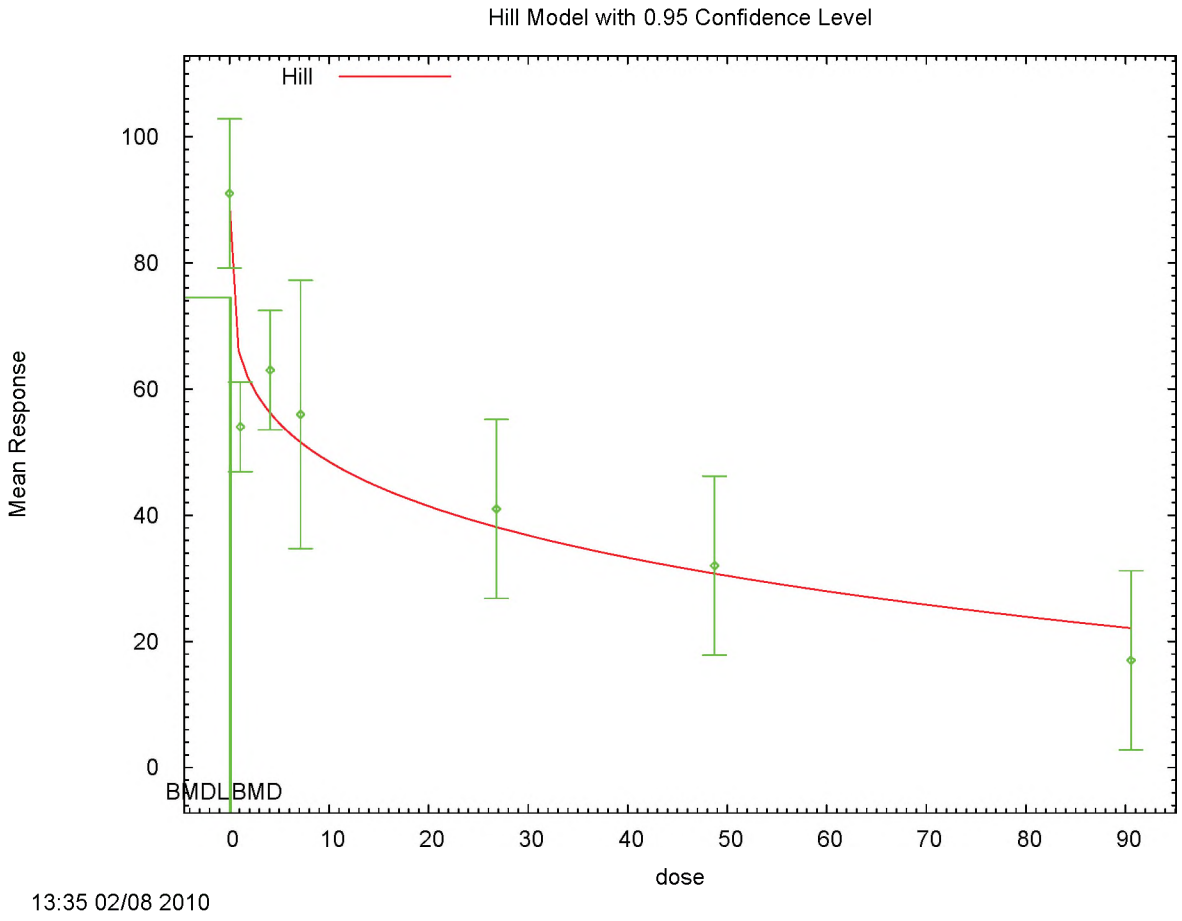
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       0.148074

            BMDL =    0.00435112
```

E.2.49.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.3. ADMINISTERED DOSE BMDS RESULTS

E.3.1. Amin et al., 2000: 0.25% Saccharin Consumed, Female

E.3.1.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
linear [b]	**1**	**0.358**	**179.702**	**8.816E+01**	**5.890E+01**	
polynomial, 2-degree	1	0.358	179.702	8.816E+01	5.890E+01	
power	1	0.358	179.702	8.816E+01	5.890E+01	power bound hit (power = 1)
power, unrestricted [c]	0	N/A	180.858	7.530E+01	2.537E+01	unrestricted (power = 0.605)

[a] Non-constant variance model selected ($p = 0.0005$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.1.2. *Output for Selected Model: Linear*

Amin et al., 2000: 0.25% Saccharin Consumed, Female

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\1_Amin_2000_25_SC_Linear_1.(d)
         Gnuplot Plotting File:  C:\1\1_Amin_2000_25_SC_Linear_1.plt
                                                Tue Feb 16 17:22:16 2010
 ================================================================

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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                        lalpha =      5.29482
                           rho =            0
                        beta_0 =      30.8266
                        beta_1 =    -0.204134
```

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-0.99	-0.016	0.03
rho	-0.99	1	0.013	-0.026
beta_0	-0.016	0.013	1	-0.94
beta_1	0.03	-0.026	-0.94	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-2.55843	1.66185	-5.8156	0.698746
rho	2.42056	0.545617	1.35117	3.48995
beta_0	30.3968	4.03582	22.4868	38.3069
beta_1	-0.196699	0.0443352	-0.283594	-0.109803

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	31.7	30.4	20.6	17.3	0.233
25	10	24.6	25.5	12	14	-0.2
100	10	10.7	10.7	5.33	4.92	-0.0204

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-92.841935	4	193.683870
A2	-85.255316	6	182.510632
A3	-85.429148	5	180.858295
fitted	-85.851107	4	179.702213
R	-98.136607	2	200.273213

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)

```
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              25.7626          4          <.0001
   Test 2              15.1732          2       0.0005072
   Test 3             0.347663          1          0.5554
   Test 4             0.843918          1          0.3583

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


              Benchmark Dose Computation

Specified effect =             1

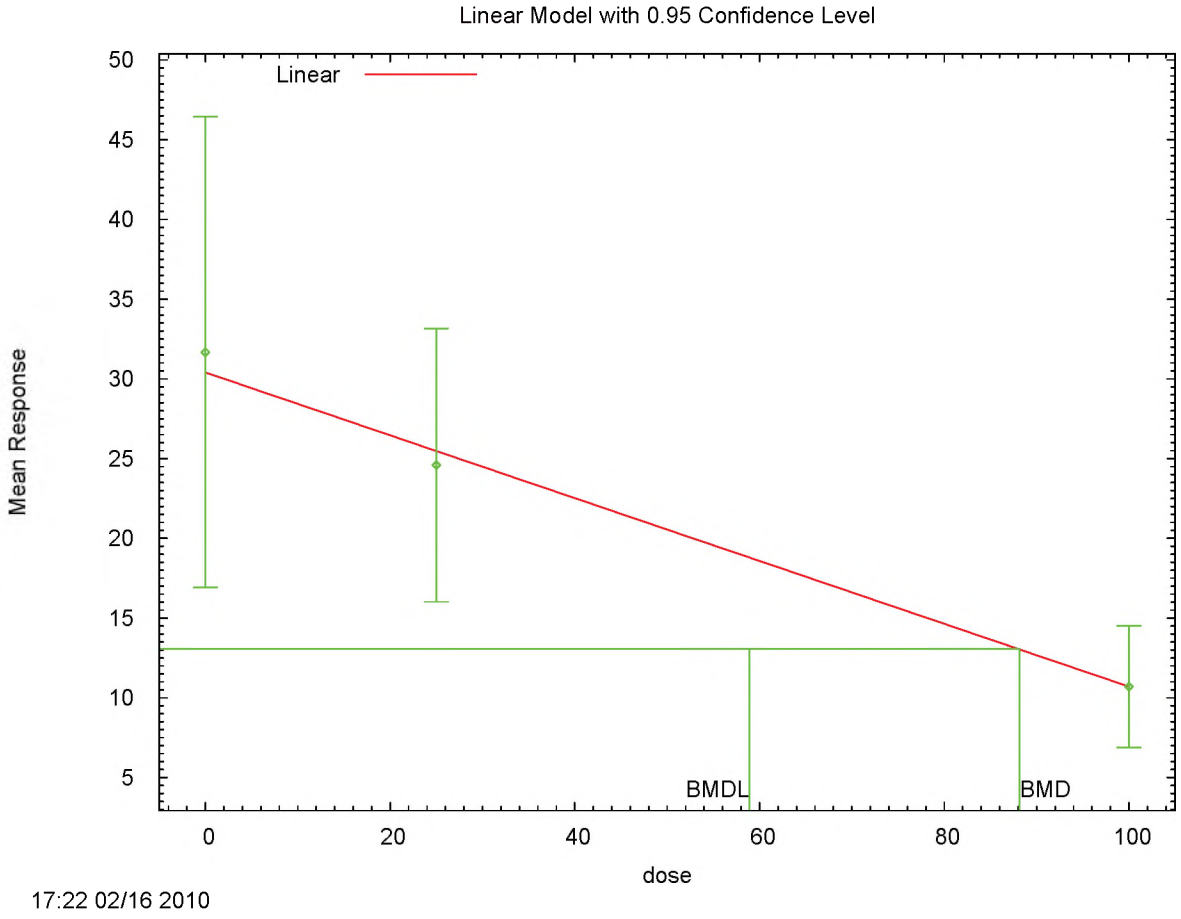
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD =        88.1623


             BMDL =        58.9029
```

E.3.1.3. *Figure for Selected Model: Linear*



E.3.1.4. *Output for Additional Model Presented: Power, Unrestricted*

Amin et al., 2000: 0.25% Saccharin Consumed, Female

```
================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\1\1_Amin_2000_25_SC_Pwr_U_1.(d)
        Gnuplot Plotting File:  C:\1\1_Amin_2000_25_SC_Pwr_U_1.plt
                                          Tue Feb 16 17:22:17 2010
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  The form of the response function is:

  Y[dose] = control + slope * dose^power


  Dependent variable = Mean
  Independent variable = Dose
  The power is not restricted
  The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
```

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	5.29482
rho =	0
control =	31.6727
slope =	-0.567889
power =	0.783745

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.99	0.34	-0.14	-0.061
rho	-0.99	1	-0.42	0.15	0.068
control	0.34	-0.42	1	-0.67	-0.56
slope	-0.14	0.15	-0.67	1	0.99
power	-0.061	0.068	-0.56	0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-2.48291	2.08669	-6.57274	1.60693
rho	2.38455	0.692047	1.02817	3.74094
control	32.99	5.40754	22.3914	43.5886
slope	-1.36469	2.01258	-5.30927	2.5799
power	0.605364	0.288476	0.0399625	1.17077

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	31.7	33	20.6	18.7	-0.223
25	10	24.6	23.4	12	12.4	0.302
100	10	10.7	10.8	5.33	4.94	-0.08

Warning: Likelihood for fitted model larger than the Likelihood for model A3.

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that

```
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -92.841935            4     193.683870
             A2          -85.255316            6     182.510632
             A3          -85.429148            5     180.858295
          fitted         -85.429148            5     180.858295
              R          -98.136607            2     200.273213


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              25.7626          4          <.0001
   Test 2              15.1732          2       0.0005072
   Test 3             0.347663          1          0.5554
   Test 4         -8.2423e-013          0             NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


               Benchmark Dose Computation

Specified effect =            1

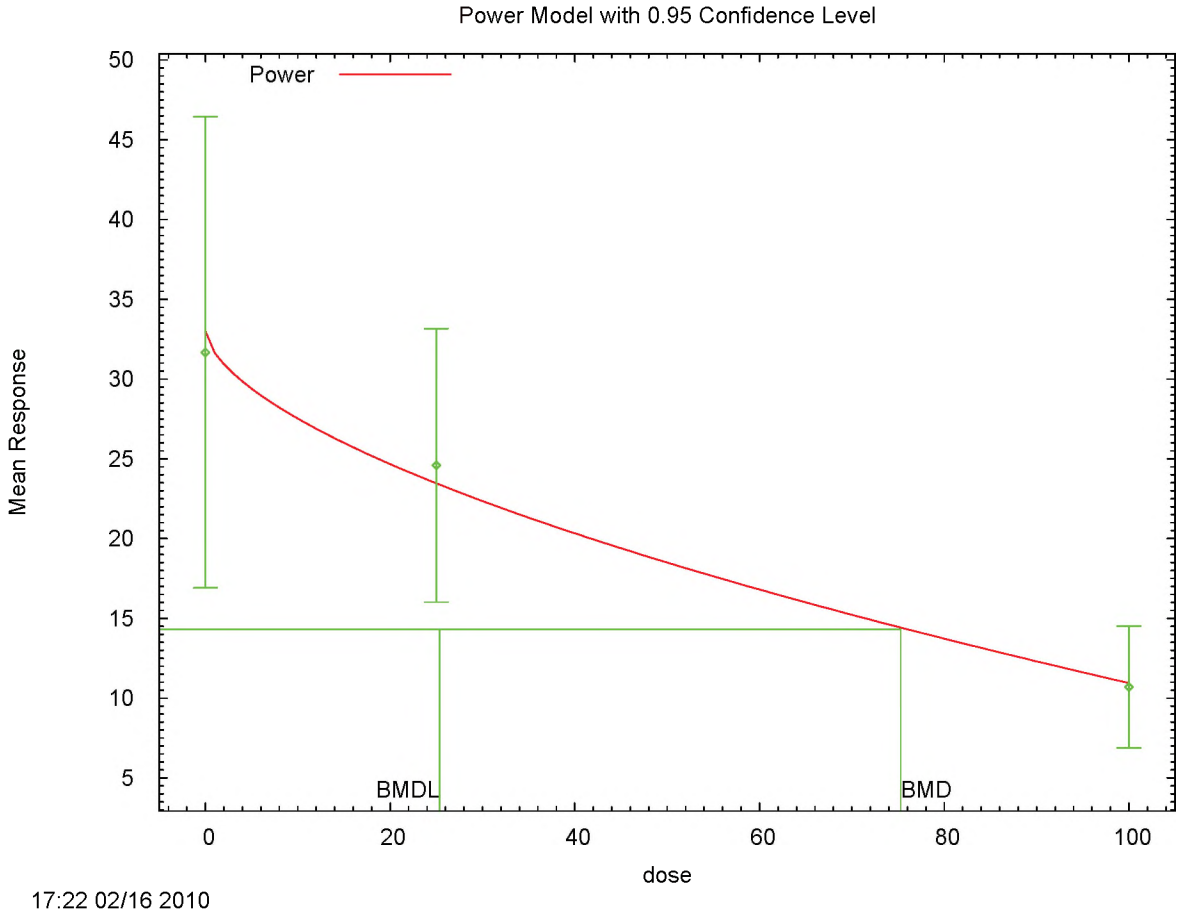
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 75.2994


            BMDL = 25.3717
```

E.3.1.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.3.2. Amin et al., 2000: 0.25% Saccharin Preference Ratio, Female

E.3.2.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
linear [b]	**1**	**0.002**	**228.094**	**1.264E+02**	**6.128E+01**	
polynomial, 2-degree	1	0.002	228.094	1.264E+02	6.128E+01	
power	1	0.002	228.094	1.264E+02	6.128E+01	power bound hit (power = 1)

[a] Non-constant variance model selected ($p = 0.0135$)

[b] Best-fitting model, BMDS output presented in this appendix

E.3.2.2. *Output for Selected Model: Linear*

Amin et al., 2000: 0.25% Saccharin Preference Ratio, Female

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\2_Amin_2000_25_SP_Linear_1.(d)
         Gnuplot Plotting File:  C:\1\2_Amin_2000_25_SP_Linear_1.plt
                                                 Tue Feb 16 17:22:44 2010
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                        lalpha =      6.34368
                           rho =            0
                        beta_0 =      74.2008
                        beta_1 =    -0.219781


          Asymptotic Correlation Matrix of Parameter Estimates

                   lalpha          rho       beta_0       beta_1

    lalpha            1           -1          0.2        -0.28
```

rho	-1	1	-0.19	0.28
beta_0	0.2	-0.19	1	-0.76
beta_1	-0.28	0.28	-0.76	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	0.338774	9.23768	-17.7667	18.4443
rho	1.43998	2.21674	-2.90476	5.78472
beta_0	73.6633	6.6623	60.6054	86.7211
beta_1	-0.207175	0.101074	-0.405276	-0.00907442

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	82.1	73.7	13.3	26.2	1.02
25	10	58.1	68.5	33.9	24.8	-1.32
100	10	54.9	52.9	19.5	20.6	0.295

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-108.574798	4	225.149597
A2	-104.269377	6	220.538754
A3	-105.147952	5	220.295903
fitted	-110.046917	4	228.093834
R	-112.382522	2	228.765045

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

```
   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              16.2263          4         0.00273
   Test 2              8.61084          2          0.0135
   Test 3              1.75715          1           0.185
   Test 4              9.79793          1        0.001747

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


             Benchmark Dose Computation

Specified effect =             1

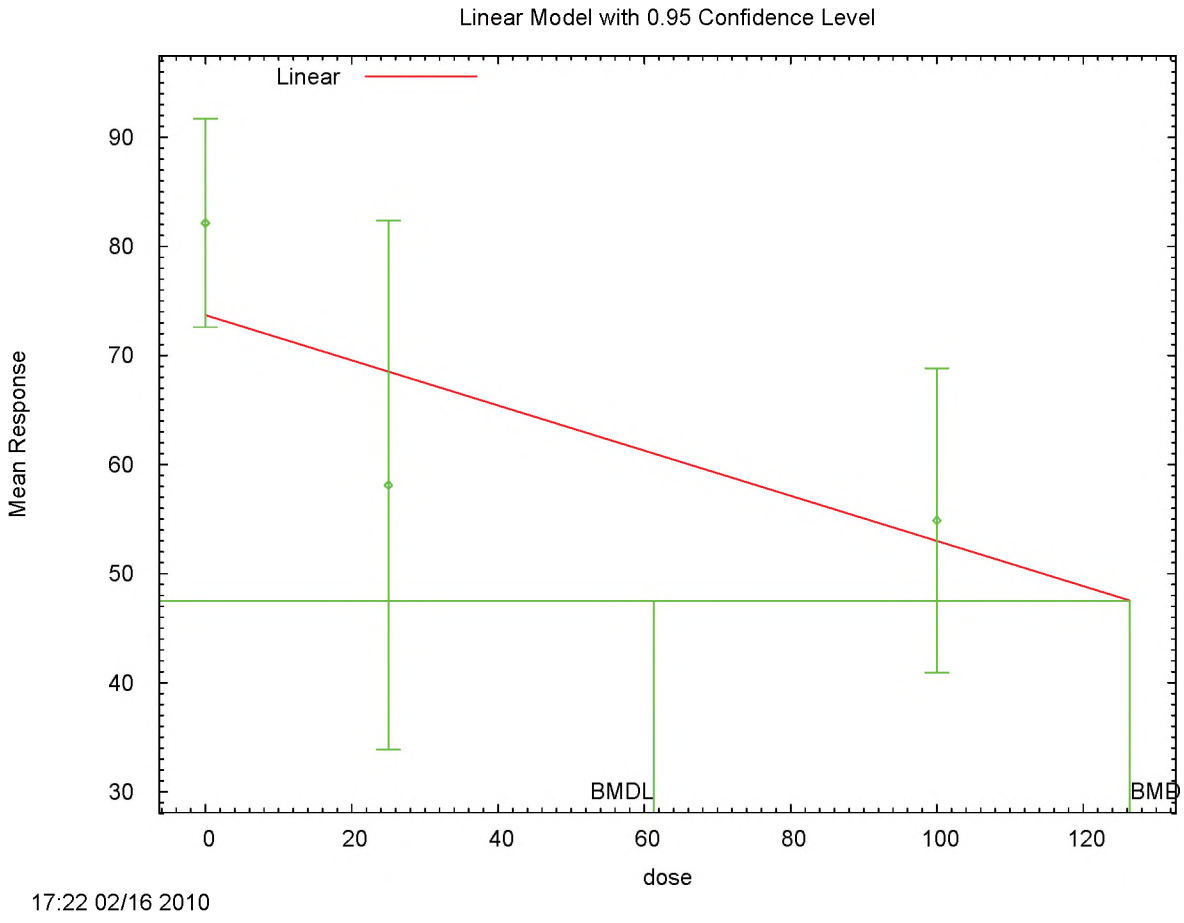
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        126.365


            BMDL =        61.2812
```

E.3.2.3. *Figure for Selected Model: Linear*



E.3.3. Amin et al., 2000: 0.50% Saccharin Consumed, Female

E.3.3.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
linear [b]	**1**	**0.031**	**159.737**	**9.874E+01**	**6.417E+01**	
polynomial, 2-degree	1	0.031	159.737	9.874E+01	6.417E+01	
power	1	0.031	159.737	9.874E+01	6.417E+01	power bound hit (power = 1)
power, unrestricted [c]	0	N/A	157.060	5.610E+01	6.781E+00	unrestricted (power = 0.325)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.3.2. *Output for Selected Model: Linear*

Amin et al., 2000: 0.50% Saccharin Consumed, Female

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\3_Amin_2000_50_SC_Linear_1.(d)
         Gnuplot Plotting File:  C:\1\3_Amin_2000_50_SC_Linear_1.plt
                                                Tue Feb 16 17:23:14 2010
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                        lalpha =      4.68512
                           rho =            0
                        beta_0 =      19.3484
                        beta_1 =    -0.158141


           Asymptotic Correlation Matrix of Parameter Estimates
```

	lalpha	rho	beta_0	beta_1
lalpha	1	-0.97	0.018	-0.0021
rho	-0.97	1	-0.027	0.014
beta_0	0.018	-0.027	1	-0.95
beta_1	-0.0021	0.014	-0.95	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-0.997428	0.992786	-2.94325	0.948397
rho	2.13634	0.404989	1.34257	2.9301
beta_0	18.1144	3.10302	12.0326	24.1962
beta_1	-0.135736	0.0331501	-0.200709	-0.0707631

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	22.4	18.1	16	13.4	1
25	10	11.4	14.7	7.66	10.7	-0.983
100	10	4.54	4.54	3.33	3.06	-0.00393

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-83.696404	4	175.392808
A2	-73.511830	6	159.023660
A3	-73.530233	5	157.060467
fitted	-75.868688	4	159.737377
R	-90.294746	2	184.589492

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	33.5658	4	<.0001
Test 2	20.3691	2	<.0001
Test 3	0.0368066	1	0.8479
Test 4	4.67691	1	0.03057

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation

Specified effect = 1

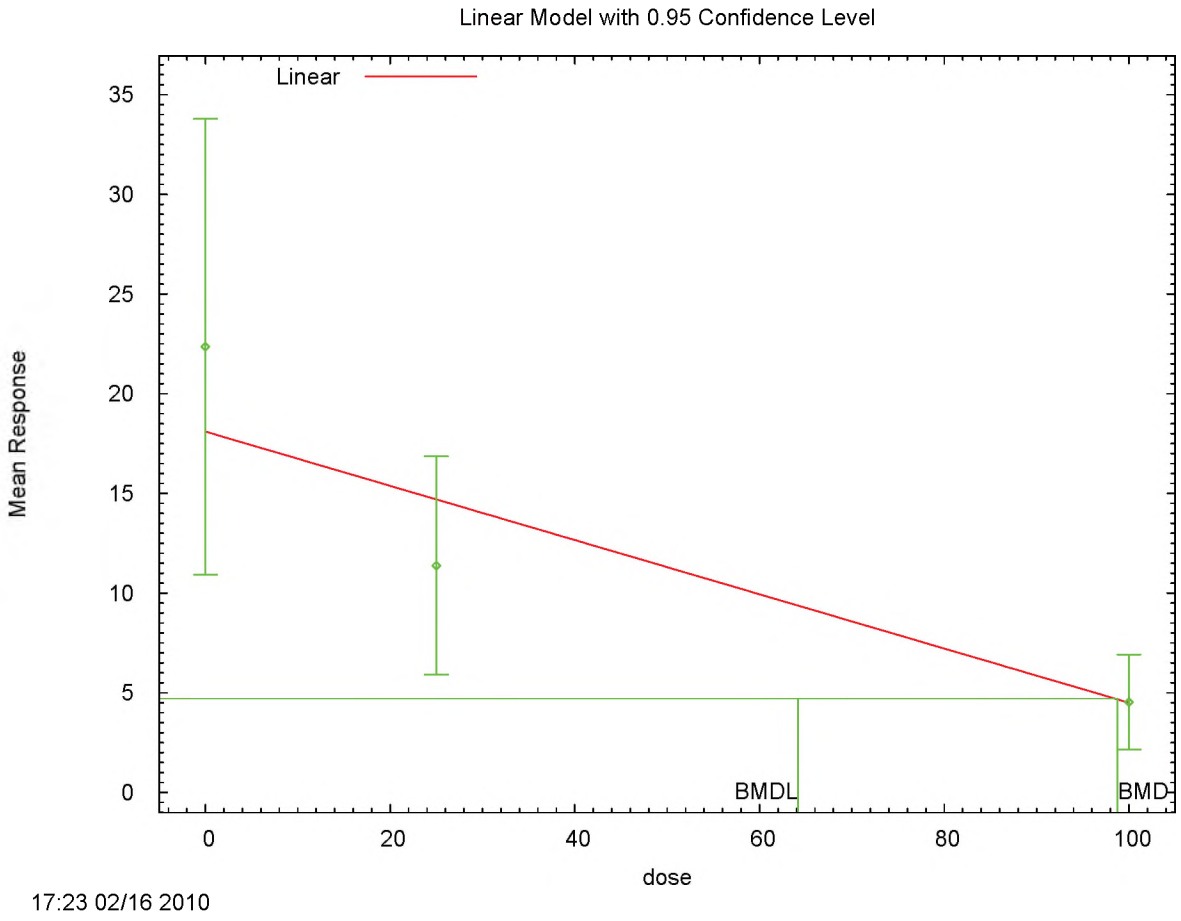
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 98.7409

BMDL = 64.169

E.3.3.3. *Figure for Selected Model: Linear*



E.3.3.4. *Output for Additional Model Presented: Power, Unrestricted*

Amin et al., 2000: 0.50% Saccharin Consumed, Female

```
================================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\1\3_Amin_2000_50_SC_Pwr_U_1.(d)
        Gnuplot Plotting File:  C:\1\3_Amin_2000_50_SC_Pwr_U_1.plt
                                          Tue Feb 16 17:23:15 2010
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  The form of the response function is:

  Y[dose] = control + slope * dose^power


  Dependent variable = Mean
  Independent variable = Dose
  The power is not restricted
  The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
```

```
Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         lalpha =      4.68512
                            rho =            0
                        control =      22.3564
                          slope =     -3.55874
                          power =     0.349799


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho      control        slope        power

  lalpha              1        -0.96         0.34        -0.26        -0.15

     rho          -0.96            1        -0.47          0.3         0.15

 control           0.34        -0.47            1        -0.73        -0.52

   slope          -0.26          0.3        -0.73            1         0.96

   power          -0.15         0.15        -0.52         0.96            1


                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
        lalpha        -0.708629            1.298          -3.25267             1.83541
           rho          1.96142         0.529653           0.923323             2.99953
       control          22.6293          4.48416            13.8405             31.4181
         slope         -4.03215          3.21302           -10.3296             2.26526
         power         0.325414         0.138761           0.053447            0.597381


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

   0    10       22.4         22.6           16           15        -0.0577
  25    10       11.4         11.1         7.66         7.46          0.105
 100    10       4.54         4.58         3.33         3.12        -0.0475

Warning: Likelihood for fitted model larger than the Likelihood for model A3.


Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user
```

```
Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1          -83.696404           4     175.392808
            A2          -73.511830           6     159.023660
            A3          -73.530233           5     157.060467
         fitted         -73.530233           5     157.060467
             R          -90.294746           2     184.589492


                  Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              33.5658          4          <.0001
   Test 2              20.3691          2          <.0001
   Test 3            0.0368066          1          0.8479
   Test 4        -2.84217e-014          0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


               Benchmark Dose Computation

Specified effect =            1

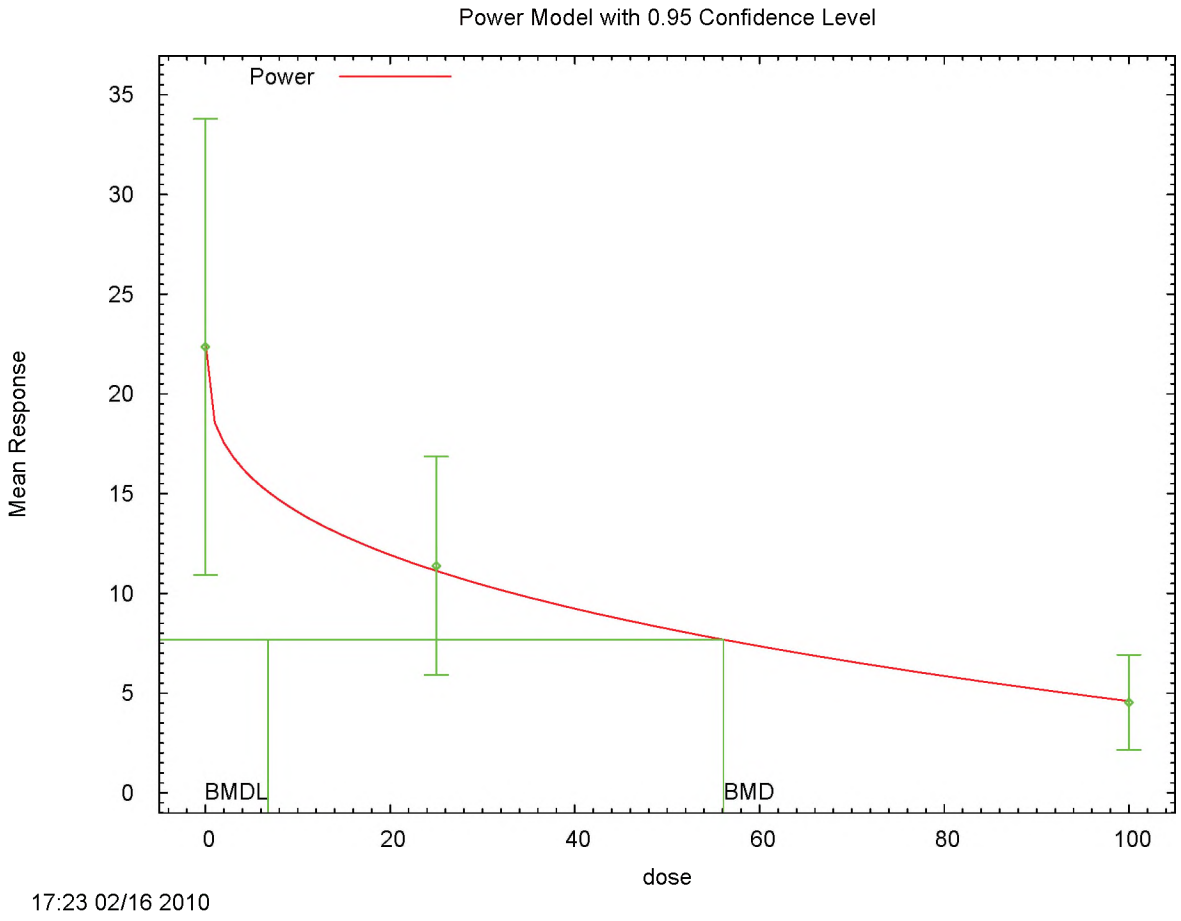
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 56.0967


            BMDL = 6.78112
```

E.3.3.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.3.4. Amin et al., 2000: 0.50% Saccharin Preference Ratio, Female

E.3.4.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
linear [b]	**1**	**0.088**	**234.936**	**8.278E+01**	**5.100E+01**	
polynomial, 2-degree	1	0.088	234.936	8.278E+01	5.100E+01	
power	1	0.088	234.936	8.278E+01	5.100E+01	power bound hit (power = 1)
power, unrestricted [c]	0	N/A	234.020	1.817E+01	1.000E-13	unrestricted (power = 0.232)

[a] Constant variance model selected ($p = 0.5593$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.4.2. *Output for Selected Model: Linear*

Amin et al., 2000: 0.50% Saccharin Preference Ratio, Female

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\4_Amin_2000_50_SP_LinearCV_1.(d)
         Gnuplot Plotting File:  C:\1\4_Amin_2000_50_SP_LinearCV_1.plt
                                                Tue Feb 16 17:23:43 2010
 ================================================================

 -
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                         alpha =      764.602
                           rho =            0   Specified
                        beta_0 =      64.1858
                        beta_1 =    -0.332668
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	2e-008	1.4e-009
beta_0	2e-008	1	-0.7
beta_1	1.4e-009	-0.7	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	758.396	195.817	374.602	1142.19
beta_0	64.1858	7.04184	50.3841	77.9876
beta_1	-0.332668	0.118327	-0.564584	-0.100752

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	72.7	64.2	24.6	27.5	0.981
25	10	44.5	55.9	32.9	27.5	-1.31
100	10	33.8	30.9	24.6	27.5	0.327

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-113.009921	4	234.019841
A2	-112.428886	6	236.857773
A3	-113.009921	4	234.019841
fitted	-114.468091	3	234.936183
R	-117.976057	2	239.952114

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)

```
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              11.0943          4         0.02552
   Test 2              1.16207          2          0.5593
   Test 3              1.16207          2          0.5593
   Test 4              2.91634          1         0.08769

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


             Benchmark Dose Computation

Specified effect =             1

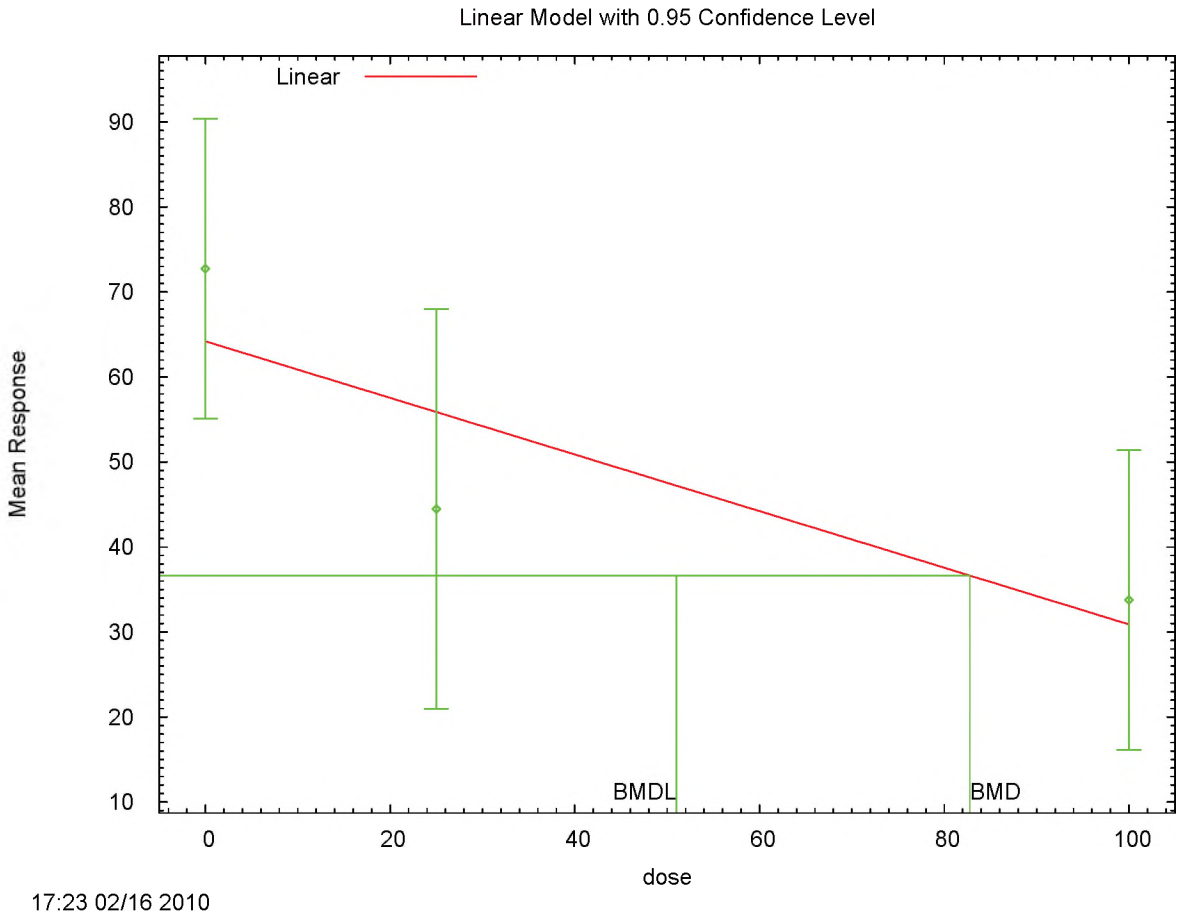
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD =        82.7823


             BMDL =        50.9971
```

E.3.4.3. *Figure for Selected Model: Linear*



E.3.4.4. *Output for Additional Model Presented: Power, Unrestricted*

Amin et al., 2000: 0.50% Saccharin Preference Ratio, Female

```
================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\1\4_Amin_2000_50_SP_PwrCV_U_1.(d)
        Gnuplot Plotting File:  C:\1\4_Amin_2000_50_SP_PwrCV_U_1.plt
                                        Tue Feb 16 17:23:44 2010
================================================================

-
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = control + slope * dose^power


  Dependent variable = Mean
  Independent variable = Dose
  rho is set to 0
  The power is not restricted
  A constant variance model is fit
```

```
   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                         alpha =      764.602
                           rho =            0   Specified
                       control =      72.7273
                         slope =      -13.387
                         power =     0.231973


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha      control        slope        power

     alpha            1    -1.3e-008     5.9e-009     2.5e-009

   control    -1.3e-008            1         -0.4        -0.22

     slope     5.9e-009         -0.4            1         0.97

     power     2.5e-009        -0.22         0.97            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha          688.142          177.677             339.9             1036.38
        control          72.7273          8.29543           56.4686              88.986
          slope          -13.387          15.9957           -44.738             17.9639
          power         0.231973         0.268067         -0.293429            0.757376


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    10       72.7         72.7         24.6         26.2      5.16e-008
   25    10       44.5         44.5         32.9         26.2     -1.27e-008
  100    10       33.8         33.8         24.6         26.2        -2e-008

Degrees of freedom for Test A3 vs fitted <= 0


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
```

```
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -113.009921            4     234.019841
              A2         -112.428886            6     236.857773
              A3         -113.009921            4     234.019841
          fitted         -113.009921            4     234.019841
               R         -117.976057            2     239.952114


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              11.0943          4         0.02552
   Test 2              1.16207          2          0.5593
   Test 3              1.16207          2          0.5593
   Test 4                    0          0             NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


               Benchmark Dose Computation

Specified effect =             1

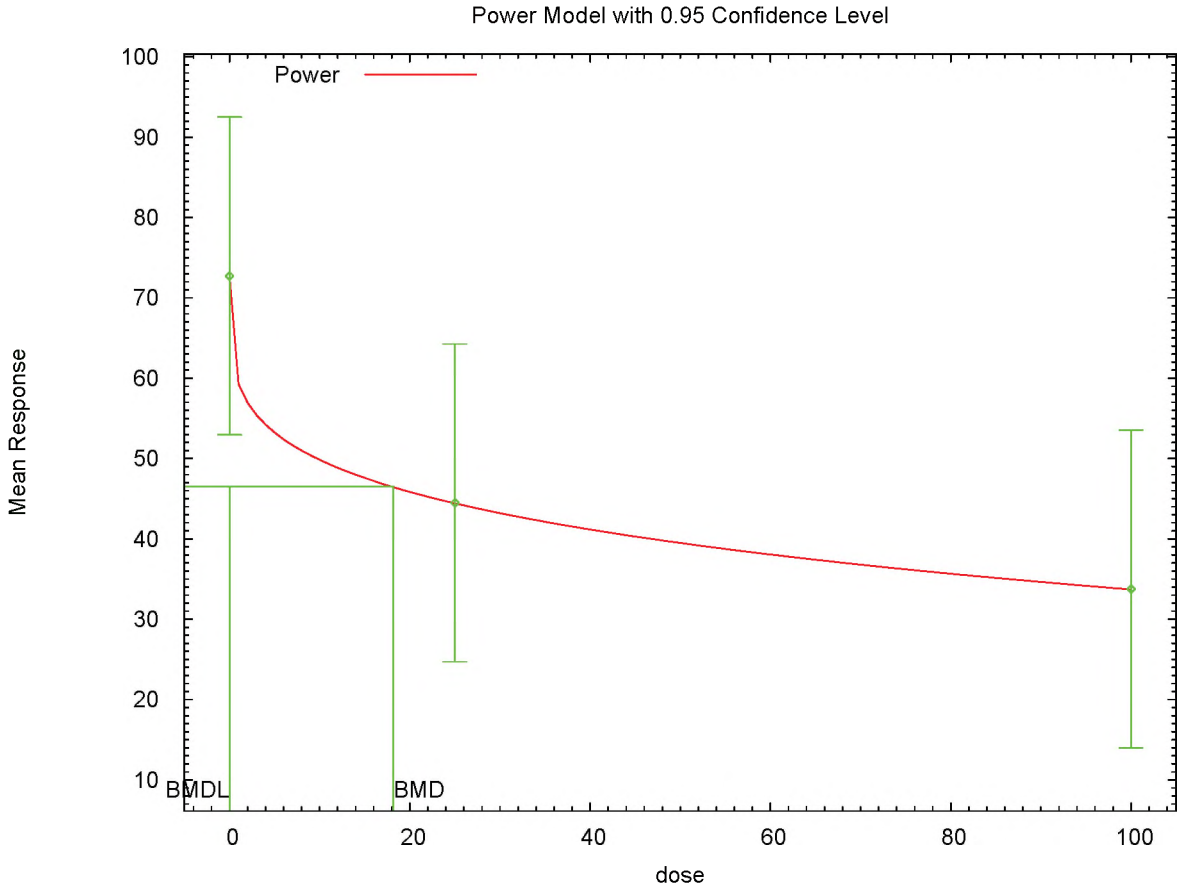
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 18.1732


            BMDL = 1e-013
```

E.3.4.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.3.5. Bell et al., 2007a: Balano-Preputial Separation, Postnatal Day 49

E.3.5.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	2	0.369	113.514	7.332E+00	4.687E+00	power bound hit (power = 1)
logistic	2	0.237	114.853	1.501E+01	1.137E+01	negative intercept (intercept = -2.07)
log-logistic [a]	**2**	**0.456**	**112.952**	**5.209E+00**	**2.870E+00**	**slope bound hit (slope = 1)**
log-probit	2	0.178	115.488	1.428E+01	9.138E+00	slope bound hit (slope = 1)
multistage, 3-degree	2	0.369	113.514	7.332E+00	4.687E+00	final ß = 0
probit	2	0.248	114.723	1.399E+01	1.061E+01	negative intercept (intercept = -1.23)
Weibull	2	0.369	113.514	7.332E+00	4.687E+00	power bound hit (power = 1)
gamma, unrestricted	1	0.566	113.746	1.894E+00	7.609E-02	unrestricted (power = 0.506)
log-logistic, unrestricted [b]	1	0.484	113.908	2.127E+00	1.363E-01	unrestricted (slope = 0.67)
log-probit, unrestricted	1	0.439	114.021	2.179E+00	1.671E-01	unrestricted (slope = 0.389)
Weibull, unrestricted	1	0.534	113.802	2.007E+00	1.075E-01	unrestricted (power = 0.574)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.3.5.2.

E.3.5.3. *Output for Selected Model: Log-Logistic*

Bell et al., 2007a: Balano-Preputial Separation, Postnatal Day 49

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\5_Bell_2007_BPS_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\5_Bell_2007_BPS_LogLogistic_1.plt
                                              Tue Feb 16 17:24:10 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1
```

```
   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                  Default Initial Parameter Values
                     background =    0.0333333
                      intercept =     -3.75371
                          slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             background    intercept

background            1        -0.58

 intercept        -0.58            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0635251            *               *                   *
      intercept         -3.84765            *               *                   *
          slope                1            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -53.7077         4
   Fitted model          -54.476         2       1.53661      2          0.4638
  Reduced model         -63.9797         1        20.544      3       0.0001309

           AIC:          112.952


                                  Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
    0.0000     0.0635         1.906     1.000          30       -0.678
    2.4000     0.1091         3.274     5.000          30        1.011
    8.0000     0.2000         6.001     6.000          30       -0.000
   46.0000     0.5273        15.819    15.000          30       -0.300

 Chi^2 = 1.57      d.f. = 2        P-value = 0.4559


   Benchmark Dose Computation

Specified effect =            0.1
```

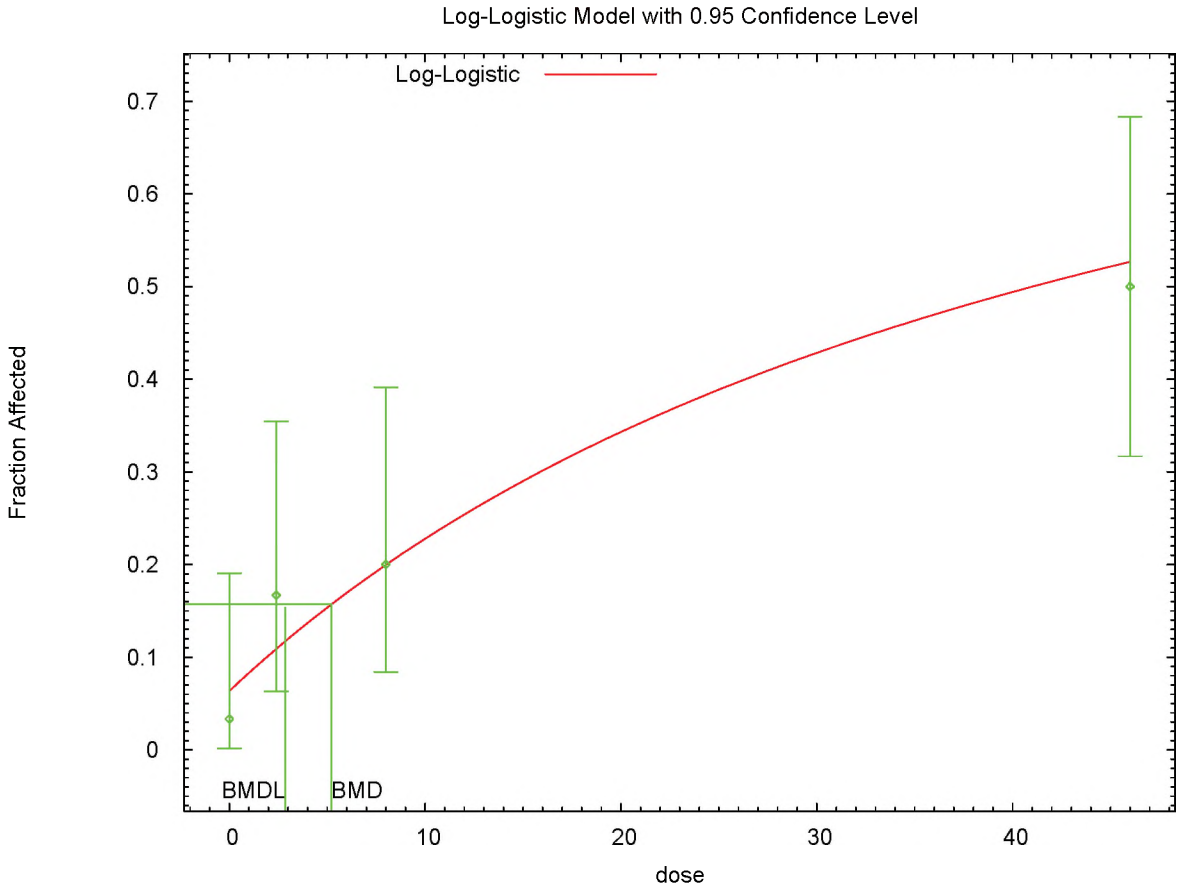
```
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        5.20918

            BMDL =        2.86991
```

E.3.5.4. *Figure for Selected Model: Log-Logistic*



E.3.5.5. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

Bell et al., 2007a: Balano-Preputial Separation, Postnatal Day 49

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\5_Bell_2007_BPS_LogLogistic_U_1.(d)
         Gnuplot Plotting File:  C:\1\5_Bell_2007_BPS_LogLogistic_U_1.plt
                                              Tue Feb 16 17:24:10 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0333333
                     intercept =     -2.54947
                         slope =     0.615936


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.49         0.35

 intercept        -0.49            1        -0.93

     slope         0.35        -0.93            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0354714            *                *                   *
      intercept         -2.70296            *                *                   *
          slope         0.670238            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
      Full model        -53.7077         4
```

Fitted model	-53.9541	3	0.492844	1	0.4827
Reduced model	-63.9797	1	20.544	3	0.0001309

AIC: 113.908

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0355	1.064	1.000	30	-0.063
2.4000	0.1392	4.176	5.000	30	0.435
8.0000	0.2405	7.216	6.000	30	-0.520
46.0000	0.4848	14.544	15.000	30	0.167

Chi^2 = 0.49 d.f. = 1 P-value = 0.4836

Benchmark Dose Computation

Specified effect = 0.1

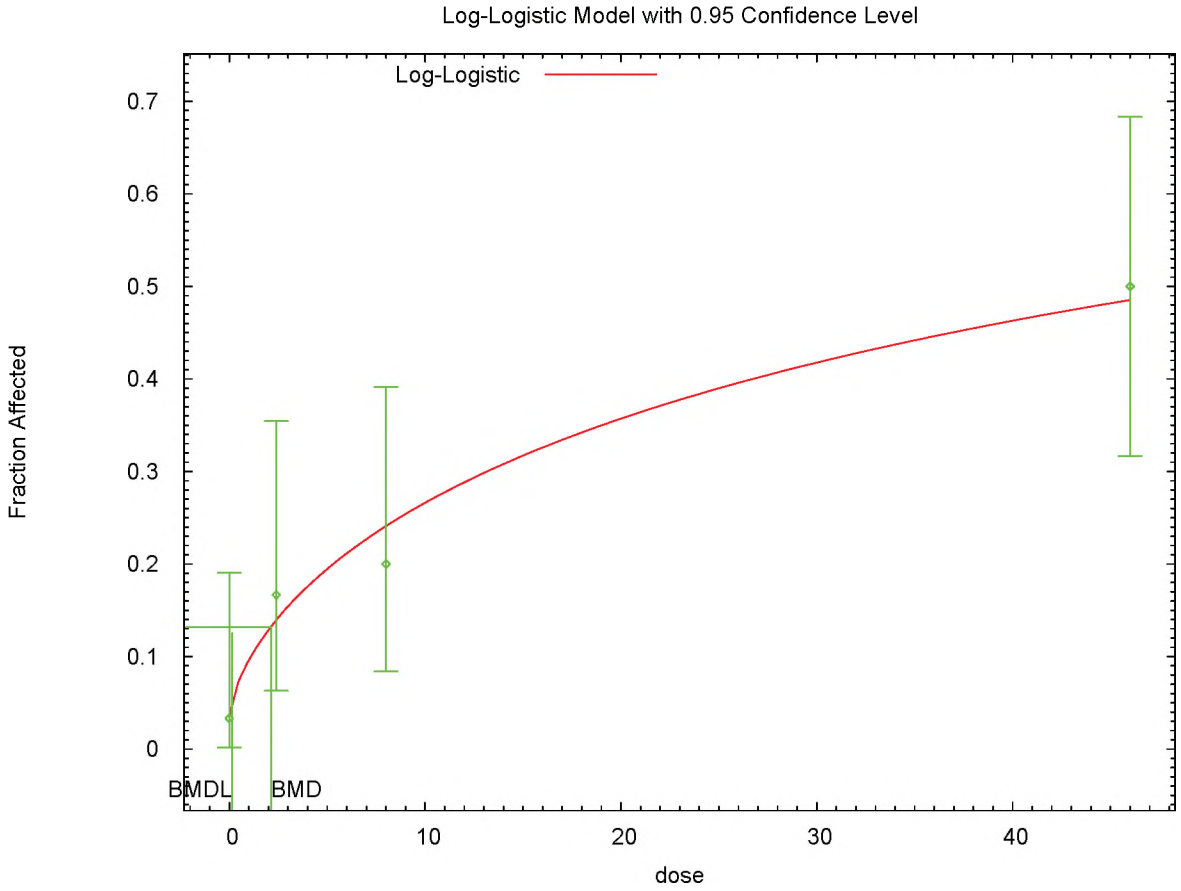
Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.12667

BMDL = 0.13633

E.3.5.6. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.3.6. Cantoni et al., 1981: Urinary Coproporhyrins, 3 Months

E.3.6.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	0.002	33.792	1.101E+02	5.318E+01	
exponential (M3)	2	0.002	33.792	1.101E+02	5.318E+01	power hit bound (d = 1)
exponential (M4) [b]	**1**	**0.341**	**23.881**	**3.741E-01**	**1.253E-01**	
exponential (M5)	1	0.341	23.881	3.741E-01	1.253E-01	power hit bound (d = 1)
Hill	1	0.535	23.359	3.273E-01	error	n lower bound hit (n = 1)
linear	2	0.002	33.301	7.734E+01	1.975E+01	
polynomial, 3-degree	2	0.002	33.301	7.734E+01	1.975E+01	
power	2	0.002	33.301	7.734E+01	1.975E+01	power bound hit (power = 1)
power, unrestricted [c]	1	0.665	23.162	4.637E-03	8.796E-08	unrestricted (power = 0.22)
Hill, unrestricted	0	N/A	24.974	7.264E-02	1.656E-04	unrestricted (n = 0.48)

[a] Non-constant variance model selected ($p = 0.0039$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.6.2. *Output for Selected Model: Exponential (M4)*

Cantoni et al., 1981: Urinary Coproporhyrins, 3 Months

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\6_Cantoni_1981_UriCopro_Exp_1.(d)
         Gnuplot Plotting File:
                                                Tue Feb 16 17:24:39 2010
 ================================================================

 Figure1-UrinaryCoproporphyrin_3months
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
```

```
      sign = -1 for decreasing trend.

   Model 2 is nested within Models 3 and 4.
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


              Initial Parameter Values

              Variable          Model 4
              --------          --------
                lnalpha             -1.50063
                    rho              2.60979
                      a             0.704303
                      b            0.0205927
                      c              4.47268
                      d                    1



                Parameter Estimates

               Variable          Model 4
               --------          -------
                lnalpha            -1.74154
                    rho             2.66803
                      a            0.755982
                      b              0.3715
                      c             3.93845
                      d                   1


        Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
     0      4        0.7414       0.3475
  1.43      4         1.807       0.8341
  14.3      4         2.734        1.506
   143      4             3          2.6


              Estimated Values of Interest

  Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
     0         0.756       0.2882          -0.1014
  1.43         1.671       0.8307           0.3265
  14.3         2.966        1.786          -0.2607
   143         2.977        1.794          0.02532


Other models for which likelihoods are calculated:
```

```
  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                 Model      Log(likelihood)      DF         AIC
                -------    -----------------    ----   ------------
                    A1       -12.90166           5      35.80333
                    A2       -6.203643           8      28.40729
                    A3       -6.487204           6      24.97441
                     R       -15.73713           2      35.47427
                     4       -6.940389           5      23.88078


Additive constant for all log-likelihoods =      -14.7.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                          Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                        19.07           6            0.004052
  Test 2                         13.4           3            0.003854
  Test 3                       0.5671           2              0.7531
 Test 6a                       0.9064           1              0.3411


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

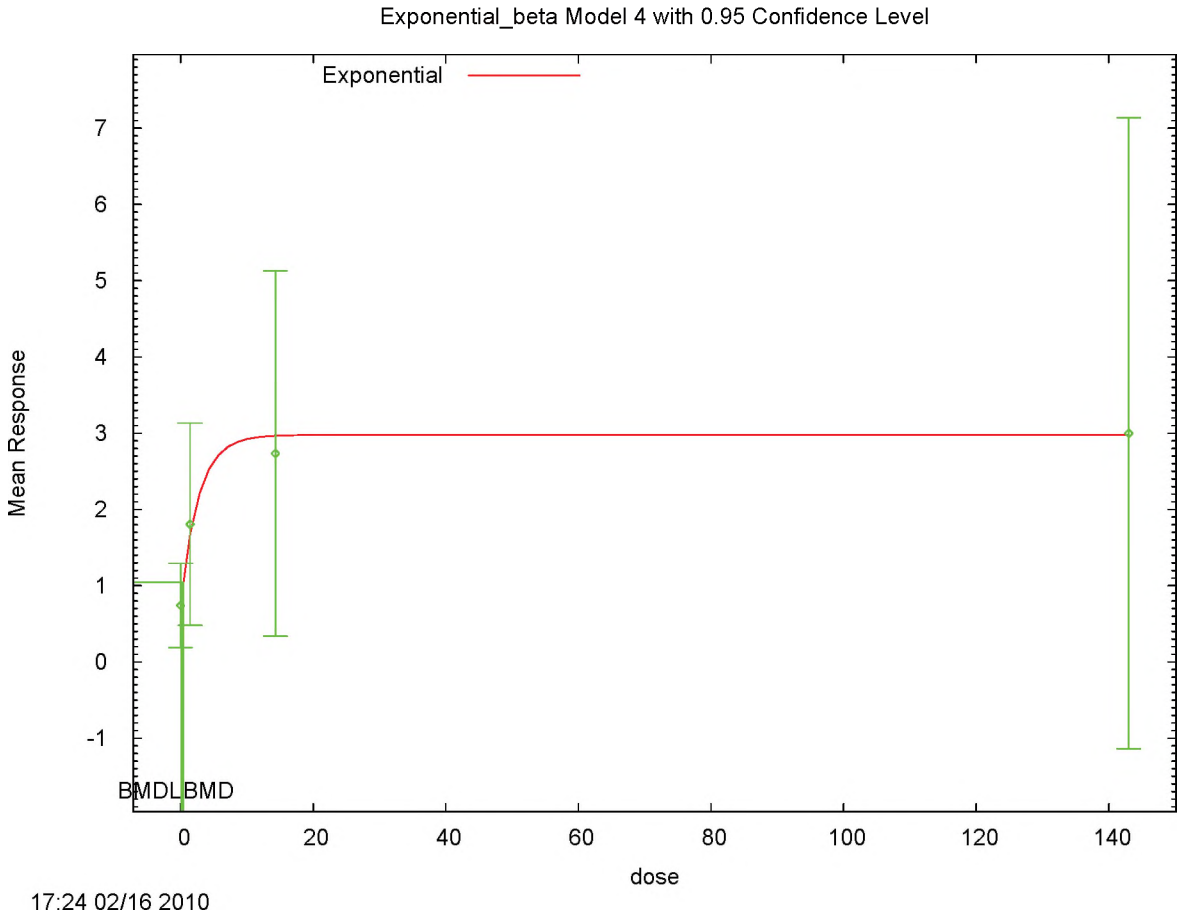
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000
```

```
BMD =      0.374114

BMDL =     0.125287
```

E.3.6.3. *Figure for Selected Model: Exponential (M4)*



E.3.6.4. *Output for Additional Model Presented: Power, Unrestricted*

Cantoni et al., 1981: Urinary Coproporhyrins, 3 Months

```
================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\1\6_Cantoni_1981_UriCopro_Pwr_U_1.(d)
        Gnuplot Plotting File:  C:\1\6_Cantoni_1981_UriCopro_Pwr_U_1.plt
                                          Tue Feb 16 17:24:41 2010
================================================================

Figure1-UrinaryCoproporphyrin_3months
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = control + slope * dose^power
```

Dependent variable = Mean
Independent variable = Dose
The power is not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	0.90039
rho =	0
control =	0.741372
slope =	1.00533
power =	0.163111

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.62	-0.53	-0.038	0.027
rho	-0.62	1	0.43	-0.24	-0.16
control	-0.53	0.43	1	-0.3	0.09
slope	-0.038	-0.24	-0.3	1	-0.72
power	0.027	-0.16	0.09	-0.72	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
lalpha	-1.78404	0.61698	-2.9933	-0.57478
rho	2.6428	0.74449	1.18363	4.10197
control	0.757242	0.139966	0.482915	1.03157
slope	0.927009	0.325923	0.288212	1.56581
power	0.220276	0.0964599	0.031218	0.409334

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	4	0.741	0.757	0.348	0.284	-0.112
1.43	4	1.81	1.76	0.834	0.865	0.108
14.3	4	2.73	2.42	1.51	1.32	0.471
143	4	3	3.52	2.6	2.16	-0.483

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

```
Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                        Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -12.901663            5      35.803325
             A2           -6.203643            8      28.407287
             A3           -6.487204            6      24.974409
         fitted           -6.580755            5      23.161510
              R          -15.737135            2      35.474269


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1               19.067          6        0.004052
   Test 2               13.396          3        0.003854
   Test 3             0.567122          2          0.7531
   Test 4             0.187101          1          0.6653

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


               Benchmark Dose Computation

Specified effect =             1

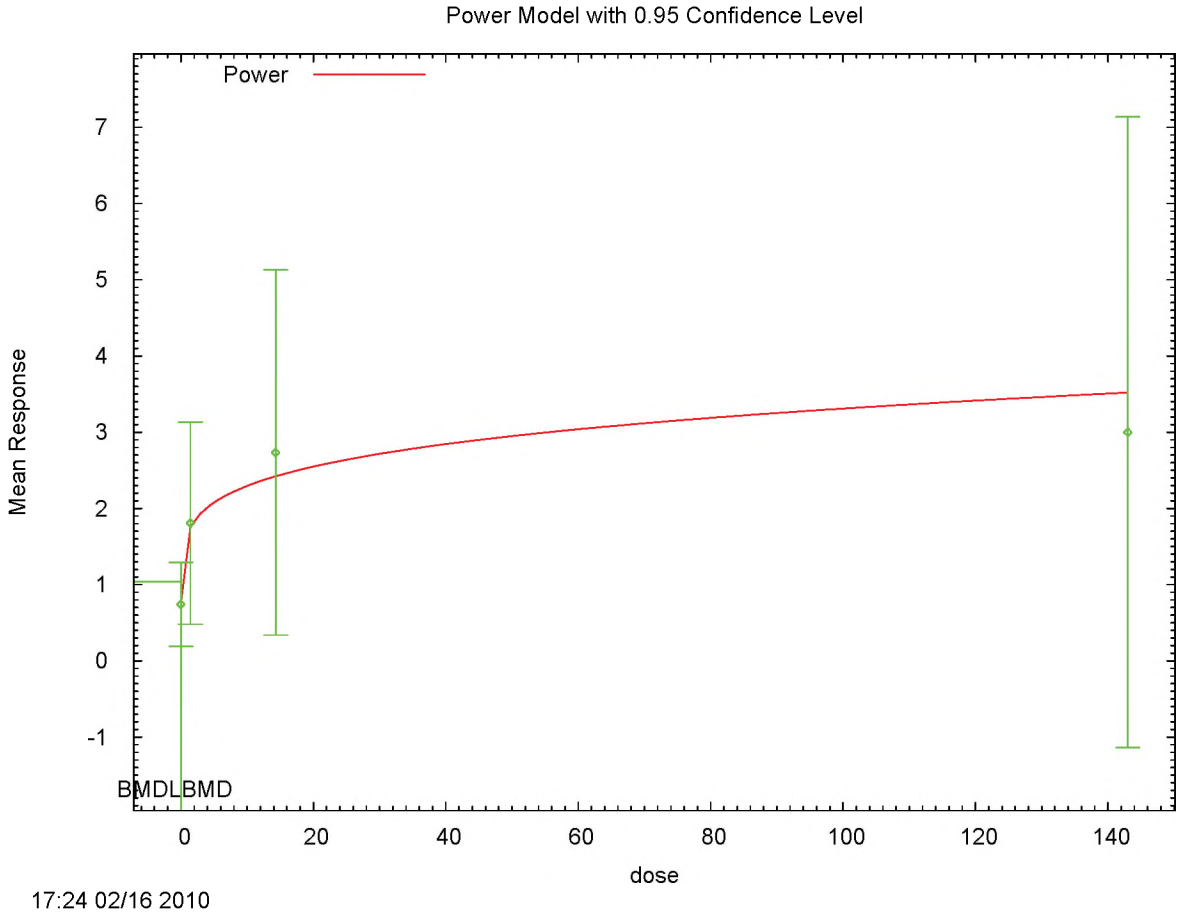
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 0.00463746


            BMDL = 8.79634e-008
```

E.3.6.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.3.7. Cantoni et al., 1981: Urinary Porphyrins

E.3.7.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2) [b]	**2**	**<0.0001**	**58.753**	**1.223E+01**	**9.037E+00**	
exponential (M3)	2	<0.0001	58.753	1.223E+01	9.037E+00	power hit bound (d = 1)
exponential (M4)	1	<0.0001	63.138	2.227E-01	1.137E-01	
exponential (M5)	1	<0.0001	63.138	2.227E-01	1.137E-01	power hit bound (d = 1)
Hill	0	N/A	62.356	9.363E+00	4.664E+00	
linear	2	<0.0001	62.487	7.732E-01	2.816E-01	
polynomial, 3-degree	1	<0.0001	10.000	error	error	
power	2	<0.0001	62.487	7.732E-01	2.816E-01	power bound hit (power = 1)
power, unrestricted	1	<0.0001	59.914	1.025E-01	2.389E-02	unrestricted (power = 0.746)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

E.3.7.2. *Output for Selected Model: Exponential (M2)*

Cantoni et al., 1981: Urinary Porphyrins

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\7_Cantoni_1981_UriPor_Exp_1.(d)
         Gnuplot Plotting File:
                                                 Tue Feb 16 17:25:14 2010
 ================================================================

 Table 1, dose converted to ng per kg per day
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2
lnalpha	-3.57509
rho	2.23456
a	3.83141
b	0.0277822
c	0
d	1

Parameter Estimates

Variable	Model 2
lnalpha	-1.55886
rho	1.77962
a	4.17268
b	0.0270415
c	0
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	4	2.27	0.49
1.43	4	5.55	0.85
14.3	3	7.62	1.79
143	3	196.9	63.14

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	4.173	1.635	-2.327
1.43	4.337	1.692	1.433
14.3	6.143	2.307	1.109
143	199.4	51.04	-0.08645

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

```
  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                   Model      Log(likelihood)      DF         AIC
                  -------    -----------------    ----   ------------
                      A1       -51.42175            5      112.8435
                      A2       -15.31211            8      46.62422
                      A3       -15.66963            6      43.33925
                       R       -68.75058            2      141.5012
                       2       -25.37651            4      58.75302


Additive constant for all log-likelihoods =     -12.87.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                                Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does Model 2 fit the data? (A3 vs. 2)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                      106.9             6           < 0.0001
  Test 2                      72.22             3           < 0.0001
  Test 3                      0.715             2             0.6994
  Test 4                      19.41             2           < 0.0001


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 4 is less than .1.  Model 2 may not adequately
  describe the data; you may want to consider another model.


Benchmark Dose Computations:

  Specified Effect = 1.000000

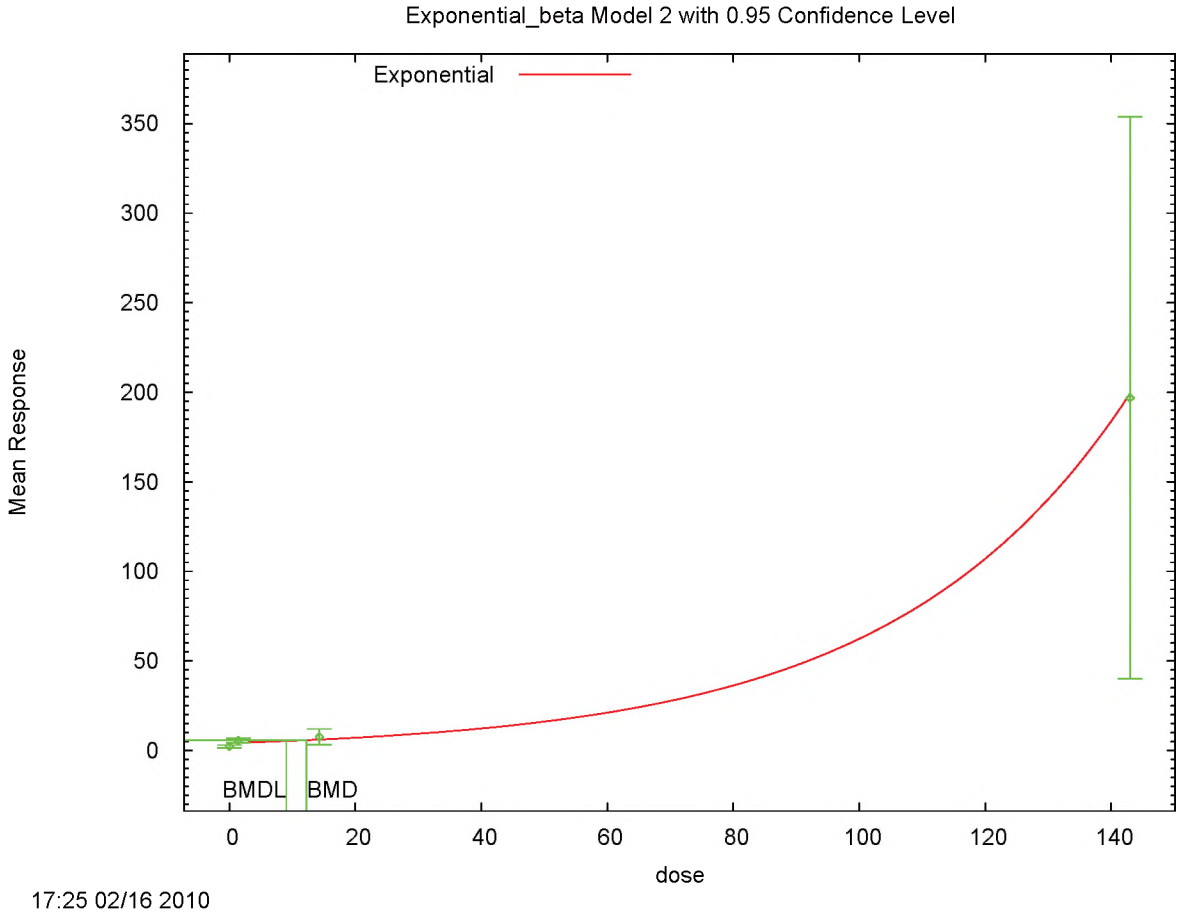
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      12.2272

              BMDL =      9.03732
```

E.3.7.3. *Figure for Selected Model: Exponential (M2)*



E.3.8. Crofton et al., 2005: Serum, T4

E.3.8.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	8	<0.0001	518.241	2.136E+03	1.157E+03	
exponential (M3)	8	<0.0001	518.241	2.136E+03	1.157E+03	power hit bound (d = 1)
exponential (M4) [b]	7	**0.957**	**476.204**	**5.633E+01**	**3.006E+01**	
exponential (M5)	7	0.957	476.204	5.633E+01	3.006E+01	power hit bound (d = 1)
Hill	6	0.973	477.434	5.564E+01	2.590E+01	
linear	8	<0.0001	523.518	4.246E+03	3.086E+03	
polynomial, 8-degree	8	<0.0001	523.518	4.246E+03	3.086E+03	
power	8	<0.0001	523.518	4.246E+03	3.086E+03	power bound hit (power = 1)
power, unrestricted	7	0.030	489.670	2.179E+01	2.271E+00	unrestricted (power = 0.217)

[a] Constant variance model selected ($p = 0.7647$)

[b] Best-fitting model, BMDS output presented in this appendix

E.3.8.2. *Output for Selected Model: Exponential (M4)*

Crofton et al., 2005: Serum, T4

```
 ================================================================================
          Exponential Model. (Version: 1.61;  Date: 7/24/2009)
          Input Data File: C:\1\8_Crofton_2005_T4_ExpCV_1.(d)
          Gnuplot Plotting File:
                                                    Tue Feb 16 17:26:01 2010
 ================================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the response function by Model:
       Model 2:     Y[dose] = a * exp{sign * b * dose}
       Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
       Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
       Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

      Note: Y[dose] is the median response for exposure = dose;
            sign = +1 for increasing trend in data;
            sign = -1 for decreasing trend.

       Model 2 is nested within Models 3 and 4.
       Model 3 is nested within Model 5.
       Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 10
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	5.47437
rho(S)	0
a	104.999
b	0.000371694
c	0.445764
d	1

(S) = Specified

Parameter Estimates

Variable	Model 4
lnalpha	5.50283
rho	0
a	99.776
b	0.00728387
c	0.533516
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	14	100	15.44
0.1	6	96.27	14.98
3	12	98.57	18.11
10	6	99.76	19.04
30	6	93.32	12.11
100	6	70.94	12.74
300	6	62.52	14.75
1000	6	52.68	22.73
3000	6	54.66	19.71
1e+004	4	49.15	11.15

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	99.78	15.66	0.05325
0.1	99.74	15.66	-0.5434
3	98.77	15.66	-0.04357
10	96.51	15.66	0.5085
30	90.64	15.66	0.4195

100	75.7	15.66	-0.744
300	58.47	15.66	0.6334
1000	53.26	15.66	-0.09133
3000	53.23	15.66	0.2237
1e+004	53.23	15.66	-0.5218

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-233.0774	11	488.1549
A2	-230.2028	20	500.4056
A3	-233.0774	11	488.1549
R	-268.4038	2	540.8076
4	-234.1019	4	476.2038

Additive constant for all log-likelihoods = -66.16. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	76.4	18	< 0.0001
Test 2	5.749	9	0.7647
Test 3	5.749	9	0.7647
Test 6a	2.049	7	0.9571

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

```
Benchmark Dose Computations:

  Specified Effect = 1.000000

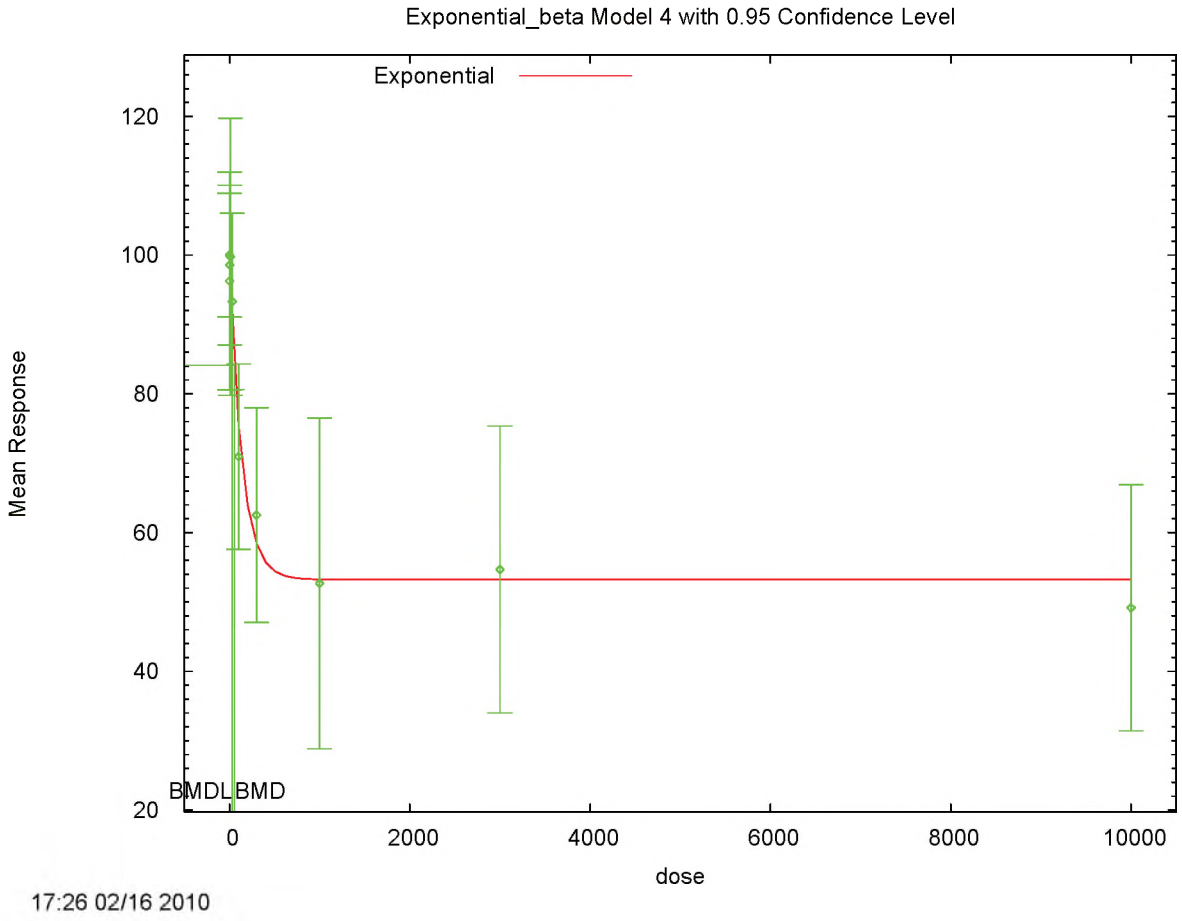
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      56.3321

              BMDL =      30.0635
```

E.3.8.3. *Figure for Selected Model: Exponential (M4)*



E.3.9. Franc et al., 2001: S-D Rats, Relative Liver Weight

E.3.9.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Hill	1	0.797	236.371	1.826E+01	5.463E+00	n lower bound hit (n = 1)
exponential (M2)	2	0.935	234.440	2.262E+01	1.757E+01	
exponential (M3)	2	0.935	234.440	2.262E+01	1.757E+01	power hit bound (d = 1)
exponential (M4)	1	0.797	236.371	1.827E+01	6.112E+00	
exponential (M5)	1	0.797	236.371	1.827E+01	6.112E+00	power hit bound (d = 1)
linear	2	0.967	234.372	1.861E+01	1.339E+01	
polynomial, 3-degree	2	0.967	234.372	1.861E+01	1.339E+01	
power [b]	2	0.967	234.372	1.861E+01	1.339E+01	power bound hit (power = 1)
Hill, unrestricted	0	N/A	238.366	1.726E+01	2.022E+00	unrestricted (n = 0.965)
power, unrestricted [c]	1	0.805	236.365	1.725E+01	2.003E+00	unrestricted (power = 0.962)

[a] Constant variance model selected ($p = 0.107$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.9.2. *Output for Selected Model: Power*

Franc et al., 2001: S-D Rats, Relative Liver Weight

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\1\88_Franc_2001_SD_RelLivWt_PowerCV_1.(d)
         Gnuplot Plotting File:  C:\1\88_Franc_2001_SD_RelLivWt_PowerCV_1.plt
                                              Fri Apr 16 16:28:45 2010
 ================================================================

 Figure 5, SD rats, relative liver weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
```

```
   The power is restricted to be greater than or equal to 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                        alpha =      527.447
                          rho =            0   Specified
                      control =          100
                        slope =      1.15946
                        power =     0.839423


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho    -power
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha      control        slope

     alpha            1     1.3e-012    -6.2e-013

   control     1.3e-012            1        -0.67

     slope    -6.2e-013        -0.67            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha          462.485          115.621             235.872             689.099
        control          101.047          5.10511             91.0415             111.053
          slope         0.542984        0.0973507            0.352181            0.733788
          power                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     8        100          101           14         21.5         -0.138
   10     8        108          106         16.9         21.5          0.208
   30     8        117          117         25.9         21.5        -0.0702
  100     8        155          155         30.9         21.5       0.000298


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2
```

```
Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                        Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1         -114.152281            5     238.304562
              A2         -111.103649            8     238.207299
              A3         -114.152281            5     238.304562
          fitted         -114.185827            3     234.371654
               R         -125.052064            2     254.104127


                    Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              27.8968          6          <.0001
  Test 2              6.09726          3           0.107
  Test 3              6.09726          3           0.107
  Test 4            0.0670927          2           0.967

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


                Benchmark Dose Computation

Specified effect =           0.1

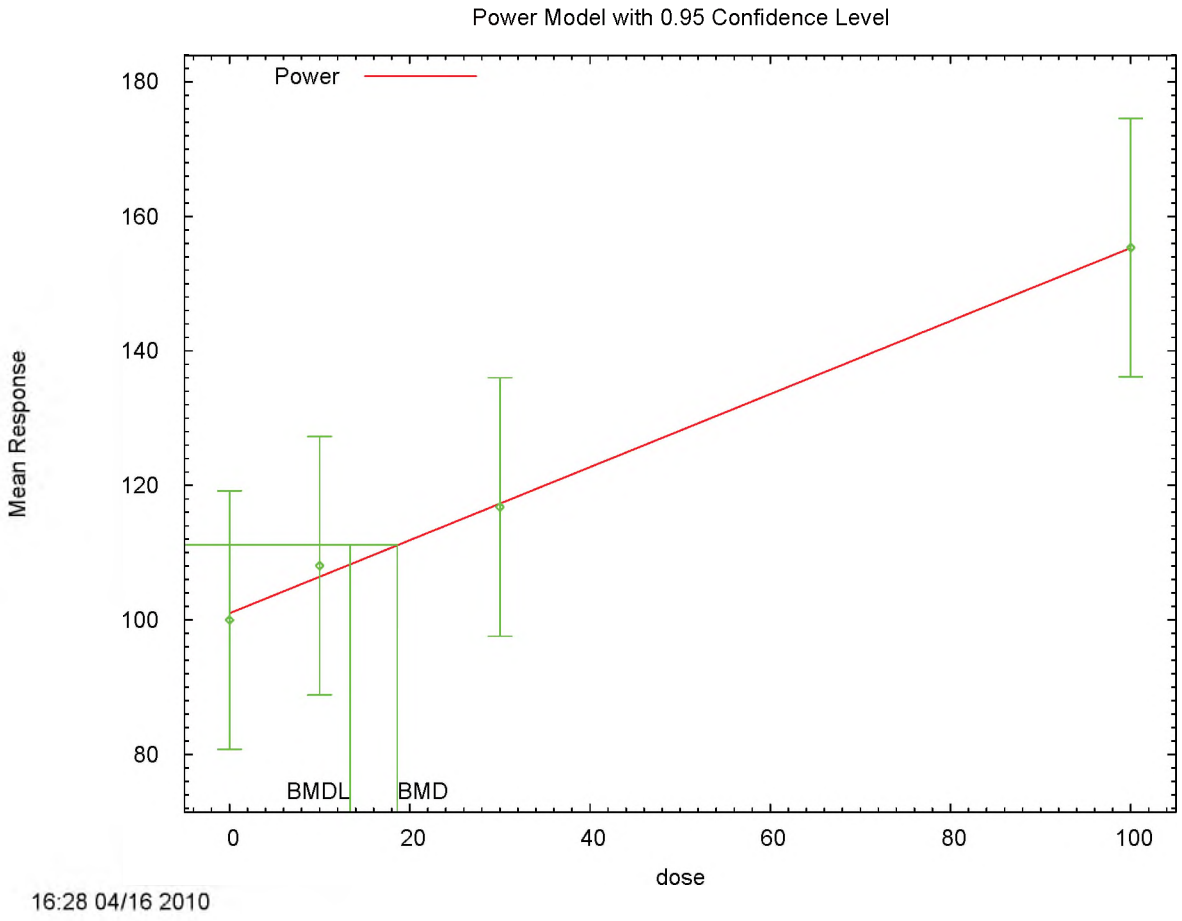
Risk Type        =     Relative risk

Confidence level =          0.95

             BMD = 18.6096


            BMDL = 13.3879
```

E.3.9.3. *Figure for Selected Model: Power*



E.3.9.4. *Output for Additional Model Presented: Power, Unrestricted*

Franc et al., 2001: S-D Rats, Relative Liver Weight

```
  ================================================================
          Power Model. (Version: 2.15;  Date: 04/07/2008)
          Input Data File: C:\1\88_Franc_2001_SD_RelLivWt_PowerCV_U_1.(d)
          Gnuplot Plotting File:  C:\1\88_Franc_2001_SD_RelLivWt_PowerCV_U_1.plt
                                                Fri Apr 16 16:28:46 2010
  ================================================================

 Figure 5, SD rats, relative liver weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the response function is:

    Y[dose] = control + slope * dose^power


    Dependent variable = Mean
    Independent variable = Dose
    rho is set to 0
    The power is not restricted
    A constant variance model is fit
```

```
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =      527.447
                            rho =            0   Specified
                        control =          100
                          slope =      1.15946
                          power =     0.839423


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                    alpha      control        slope        power

      alpha             1       1e-009    -6.2e-010     4.7e-010

    control       1e-009            1        -0.74         0.71

      slope    -6.2e-010        -0.74            1           -1

      power     4.7e-010         0.71           -1            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha          462.394          115.598           235.825             688.963
        control          100.636          7.29156           86.3448             114.927
          slope         0.650456          1.43713          -2.16627             3.46718
          power         0.961853         0.465182         0.0501134             1.87359


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     8        100          101           14         21.5        -0.0836
   10     8        108          107         16.9         21.5          0.192
   30     8        117          118         25.9         21.5         -0.128
  100     8        155          155         30.9         21.5         0.0192


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user
```

```
Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1         -114.152281            5     238.304562
            A2         -111.103649            8     238.207299
            A3         -114.152281            5     238.304562
         fitted        -114.182670            4     236.365340
             R         -125.052064            2     254.104127


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              27.8968          6          <.0001
   Test 2              6.09726          3           0.107
   Test 3              6.09726          3           0.107
   Test 4            0.0607785          1          0.8053

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


               Benchmark Dose Computation

Specified effect =           0.1

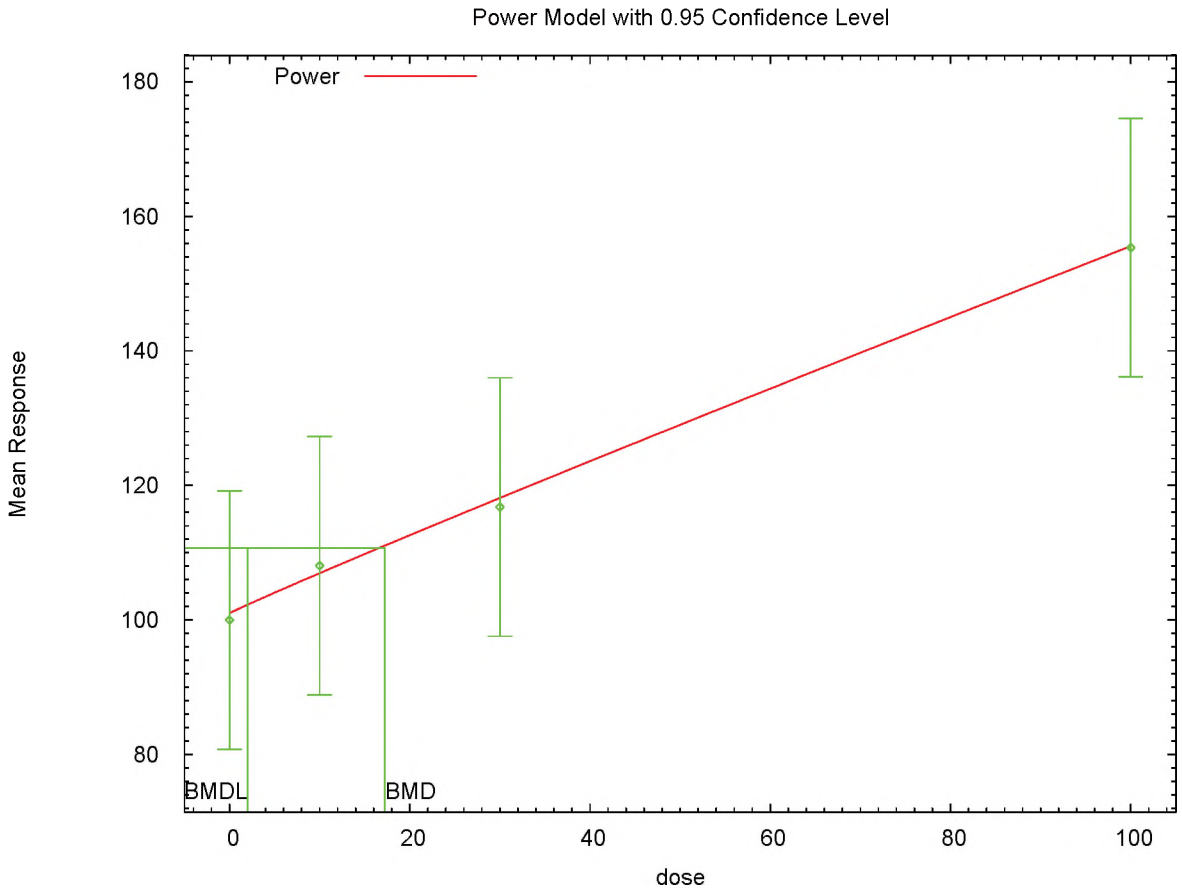
Risk Type        =     Relative risk

Confidence level =          0.95

             BMD = 17.2469


            BMDL = 2.00336
```

E.3.9.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.3.10. Franc et al., 2001: L-E Rats, Relative Liver Weight

E.3.10.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	0.245	210.148	5.143E+01	3.188E+01	
exponential (M3)	2	0.245	210.148	5.143E+01	3.188E+01	power hit bound (d = 1)
exponential (M4)	1	0.607	209.599	1.476E+01	3.702E+00	
exponential (M5)	1	0.607	209.599	1.476E+01	3.702E+00	power hit bound (d = 1)
Hill [b]	1	0.703	209.480	1.321E+01	1.591E+00	n lower bound hit (n = 1)
linear	2	0.273	209.933	4.753E+01	2.788E+01	
polynomial, 3-degree	1	<0.0001	10.000	1.505E+01	error	
power	2	0.273	209.933	4.753E+01	2.788E+01	power bound hit (power = 1)
Hill, unrestricted [c]	0	N/A	211.341	1.163E+01	9.756E-01	unrestricted (n = 0.418)
power, unrestricted	1	0.940	209.340	1.155E+01	1.513E-02	unrestricted (power = 0.394)

[a] Non-constant variance model selected ($p = 0.0632$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.10.2. *Output for Selected Model: Hill*

Franc et al., 2001: L-E Rats, Relative Liver Weight

```
 ======================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\89_Franc_2001_LE_RelLivWt_Hill_1.(d)
         Gnuplot Plotting File:  C:\1\89_Franc_2001_LE_RelLivWt_Hill_1.plt
                                              Fri Apr 16 16:29:20 2010
 ======================================================================

 Figure 5, L-E rats, relative liver weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
```

The variance is to be modeled as Var(i) = exp(lalpha + rho * ln(mean(i)))

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	5.41581
rho =	0
intercept =	100
v =	22.225
n =	0.329526
k =	40.8403

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	intercept	v	k
lalpha	1	-1	-0.18	0.38	0.2
rho	-1	1	0.17	-0.38	-0.2
intercept	-0.18	0.17	1	-0.13	0.39
v	0.38	-0.38	-0.13	1	0.77
k	0.2	-0.2	0.39	0.77	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
lalpha	-15.3958	17.0376	-48.7889	17.9973
rho	4.38043	3.61867	-2.71204	11.4729
intercept	99.5667	3.7178	92.28	106.853
v	28.8965	12.6477	4.10739	53.6856
n	1	NA		
k	25.1273	30.138	-33.9421	84.1966

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	100	99.6	10	10.8	0.114
10	8	106	108	17.9	12.8	-0.329
30	8	117	115	8.97	14.9	0.288
100	8	122	123	19.9	17	-0.0723

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1         -100.516456            5     211.032912
            A2          -96.870820            8     209.741641
            A3          -99.666984            6     211.333969
        fitted          -99.739888            5     209.479776
             R         -105.717087            2     215.434174


                  Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              17.6925          6        0.007048
  Test 2              7.29127          3         0.06317
  Test 3              5.59233          2         0.06104
  Test 4             0.145807          1          0.7026

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =           0.1

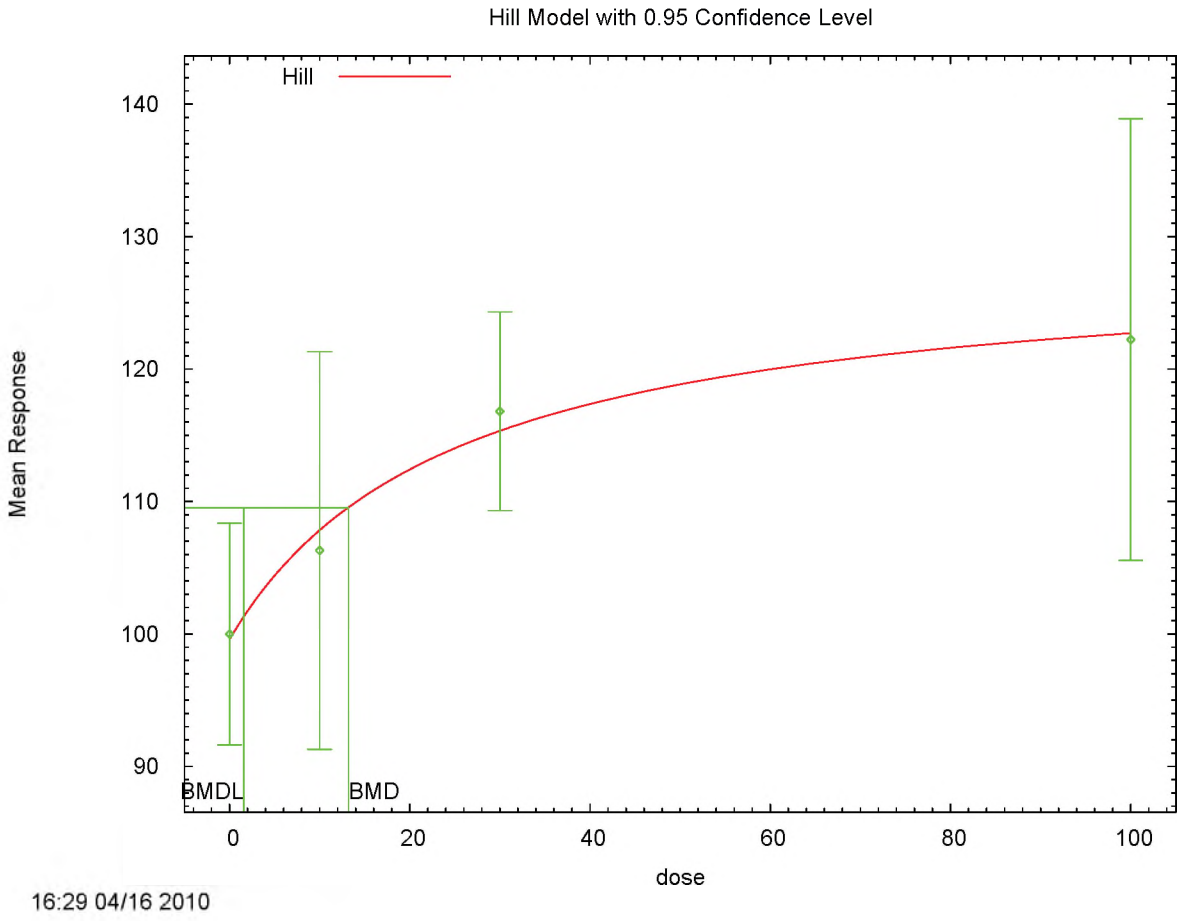
Risk Type        =     Relative risk

Confidence level =           0.95

             BMD =        13.2094

            BMDL =       1.59127
```

E.3.10.3. *Figure for Selected Model: Hill*



E.3.10.4. *Output for Additional Model Presented: Hill, Unrestricted*

Franc et al., 2001: L-E Rats, Relative Liver Weight

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\89_Franc_2001_LE_RelLivWt_Hill_U_1.(d)
         Gnuplot Plotting File:  C:\1\89_Franc_2001_LE_RelLivWt_Hill_U_1.plt
                                          Fri Apr 16 16:29:27 2010
 ================================================================

 Figure 5, L-E rats, relative liver weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 4
```

```
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         lalpha =      5.41581
                            rho =            0
                      intercept =          100
                              v =       22.225
                              n =     0.329526
                              k =      40.8403


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho    intercept            v            n            k

    lalpha            1           -1        -0.21       -0.099         0.23        -0.13

       rho           -1            1         0.21        0.099        -0.23         0.13

 intercept        -0.21         0.21            1        0.023         0.14        0.011

         v       -0.099        0.099        0.023            1        -0.84            1

         n         0.23        -0.23         0.14        -0.84            1        -0.88

         k        -0.13         0.13        0.011            1        -0.88            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha         -18.8355         18.0637           -54.2397             16.5688
            rho           5.1098         3.83743           -2.41144              12.631
      intercept            99.526         3.53402            92.5994             106.453
              v          286.422          4487.2           -8508.33             9081.17
              n         0.418159         0.457476          -0.478477             1.31479
              k          32981.9     1.52481e+006       -2.95559e+006        3.02155e+006


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     8        100          99.5           10         10.3           0.13
   10     8        106           109         17.9           13         -0.563
   30     8        117           114         8.97         14.6          0.529
  100     8        122           123         19.9         17.7        -0.0942

Degrees of freedom for Test A3 vs fitted <= 0


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2
```

```
Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -100.516456            5     211.032912
             A2          -96.870820            8     209.741641
             A3          -99.666984            6     211.333969
          fitted         -99.670736            6     211.341472
              R         -105.717087            2     215.434174


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              17.6925          6        0.007048
  Test 2              7.29127          3         0.06317
  Test 3              5.59233          2         0.06104
  Test 4           0.00750301          0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


        Benchmark Dose Computation

Specified effect =           0.1

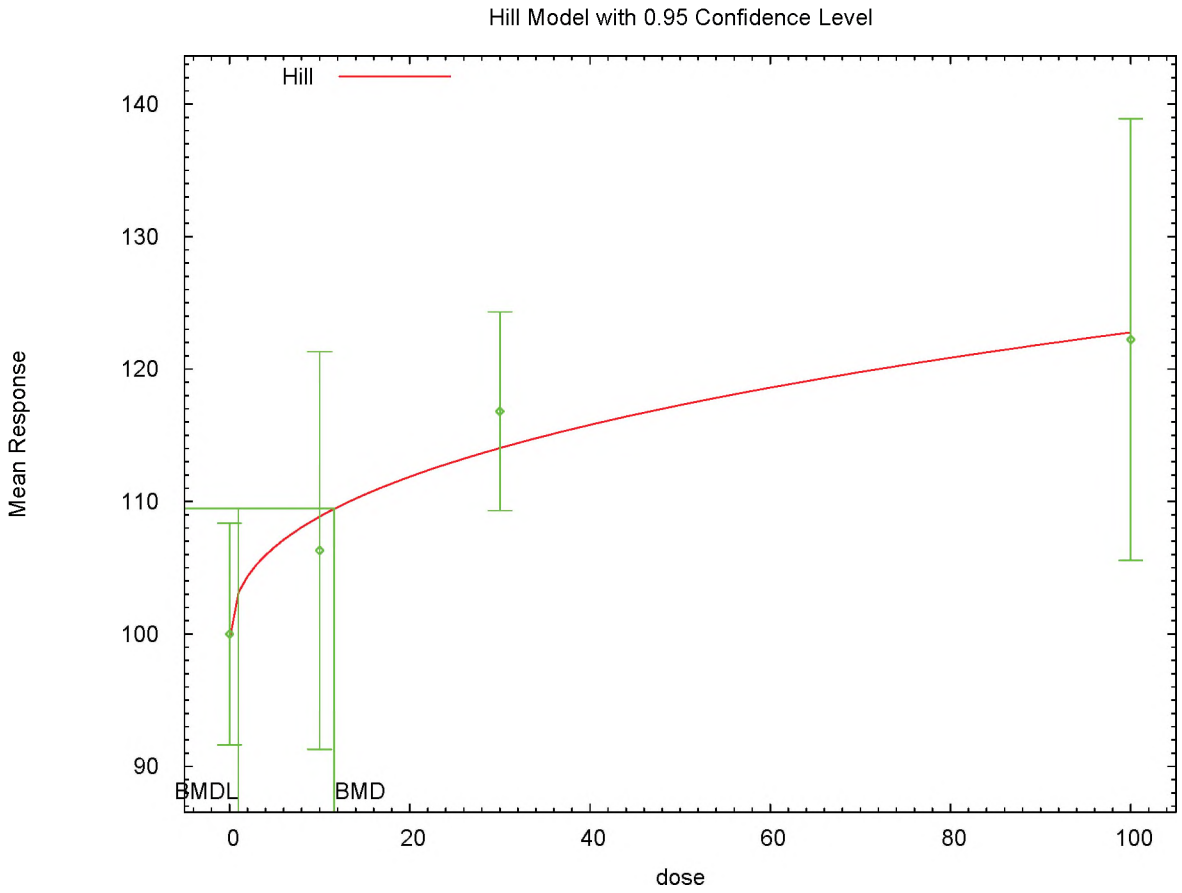
Risk Type        =     Relative risk

Confidence level =           0.95

             BMD =        11.6342

            BMDL =      0.975601
```

E.3.10.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.3.11. Franc et al., 2001: S-D Rats, Relative Thymus Weight

E.3.11.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	0.551	285.890	6.730E+00	3.627E+00	
exponential (M3)	1	<0.0001	303.995	3.858E+02	6.615E-01	
exponential (M4) [b]	1	0.972	286.698	3.559E+00	1.714E+00	
exponential (M5)	0	N/A	288.696	3.796E+00	1.714E+00	
Hill	0	N/A	288.696	4.299E+00	9.311E-01	
linear	2	0.252	287.456	1.330E+01	1.062E+01	
polynomial, 3-degree [c]	2	0.252	287.456	1.330E+01	1.062E+01	
power	2	0.252	287.456	1.330E+01	1.062E+01	power bound hit (power = 1)
power, unrestricted	1	0.510	287.131	5.049E-01	4.411E-04	unrestricted (power = 0.388)

[a] Non-constant variance model selected ($p = 0.0320$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.11.2. *Output for Selected Model: Exponential (M4)*

Franc et al., 2001: S-D Rats, Relative Thymus Weight

```
 ================================================================
          Exponential Model. (Version: 1.61;  Date: 7/24/2009)
          Input Data File: C:\1\91_Franc_2001_SD_RelThyWt_Exp_1.(d)
          Gnuplot Plotting File:
                                                 Fri Apr 16 16:30:07 2010
 ================================================================

 Figure 5, SD rats, relative thymus weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
```

```
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


                Initial Parameter Values

                Variable          Model 4
                --------          --------
                  lnalpha             3.35464
                      rho             1.08199
                        a                 105
                        b           0.0424361
                        c            0.206726
                        d                   1



                  Parameter Estimates

                 Variable          Model 4
                 --------          -------
                  lnalpha            2.54324
                      rho            1.25901
                        a            108.904
                        b          0.0379343
                        c           0.208146
                        d                  1


         Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
     0      8          100          83.2
    10      8        91.17        47.97
    30      8        51.41        43.48
   100      8        22.79        29.98


                Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
     0         108.9        68.33          -0.3686
    10         81.68        57.01           0.4706
    30          50.3        42.02           0.0748
   100         24.61        26.79           -0.192


Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

```

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                  Model      Log(likelihood)      DF         AIC
                 -------    ------------------    ----   ------------
                     A1       -141.9834            5      293.9669
                     A2       -137.5818            8      291.1637
                     A3       -138.3482            6      288.6964
                      R       -146.9973            2      297.9946
                      4       -138.3488            5      286.6976


Additive constant for all log-likelihoods =     -29.41.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                      18.83             6           0.004459
  Test 2                      8.803             3            0.03203
  Test 3                      1.533             2             0.4647
 Test 6a                   0.001216             1             0.9722


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 0.100000

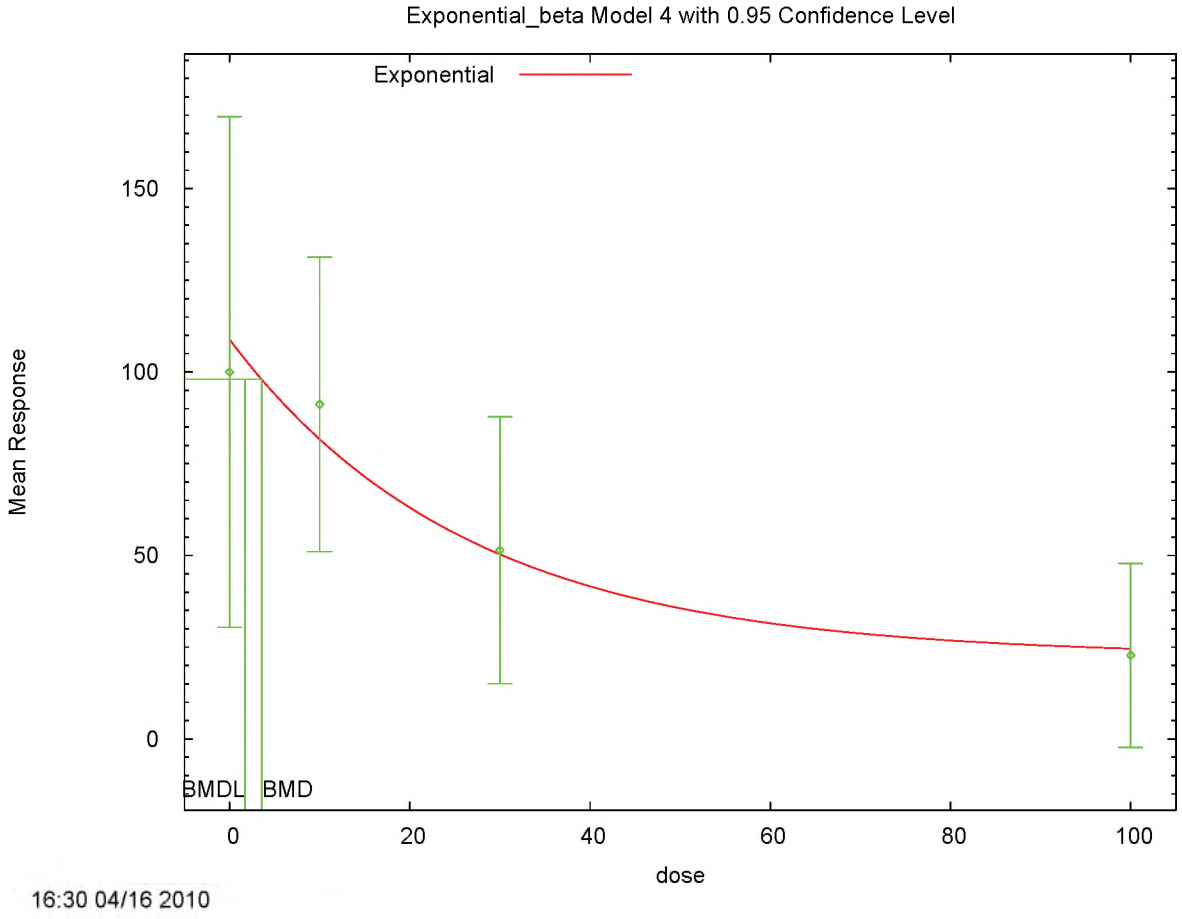
         Risk Type = Relative deviation

  Confidence Level = 0.950000

               BMD =      3.55883

              BMDL =      1.71399
```

E.3.11.3. *Figure for Selected Model: Exponential (M4)*



E.3.11.4. *Output for Additional Model Presented: Polynomial, 3-Degree*

Franc et al., 2001: S-D Rats, Relative Thymus Weight

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\91_Franc_2001_SD_RelThyWt_Poly_1.(d)
         Gnuplot Plotting File:  C:\1\91_Franc_2001_SD_RelThyWt_Poly_1.plt
                                              Fri Apr 16 16:30:11 2010
 ================================================================

 Figure 5, SD rats, relative thymus weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   The polynomial coefficients are restricted to be negative
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
```

```
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         lalpha =       8.0075
                            rho =            0
                         beta_0 =          100
                         beta_1 =    -0.352259
                         beta_2 =   -0.0585481
                         beta_3 =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -beta_2    -beta_3
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 lalpha          rho       beta_0       beta_1

    lalpha            1        -0.99        0.031       -0.016

       rho        -0.99            1       -0.034        0.022

    beta_0        0.031       -0.034            1        -0.84

    beta_1       -0.016        0.022        -0.84            1


                                 Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha          2.92328           1.7394          -0.485884             6.33243
            rho          1.18295         0.423359           0.353177             2.01271
         beta_0           89.841          13.7418            62.9076             116.774
         beta_1        -0.675682         0.175538           -1.01973           -0.331634
         beta_2                0               NA
         beta_3                0               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0       8        100         89.8         83.2         61.7          0.466
   10       8       91.2         83.1           48         58.9          0.388
   30       8       51.4         69.6         43.5           53         -0.968
  100       8       22.8         22.3           30           27         0.0543


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
```

```
            Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
             Var{e(i)} = Sigma^2


                        Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1         -141.983433            5     293.966865
              A2         -137.581833            8     291.163667
              A3         -138.348184            6     288.696368
           fitted        -139.728204            4     287.456407
               R         -146.997301            2     297.994602


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                      Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              18.8309          6        0.004459
   Test 2               8.8032          3         0.03203
   Test 3               1.5327          2          0.4647
   Test 4              2.76004          2          0.2516

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =           0.1

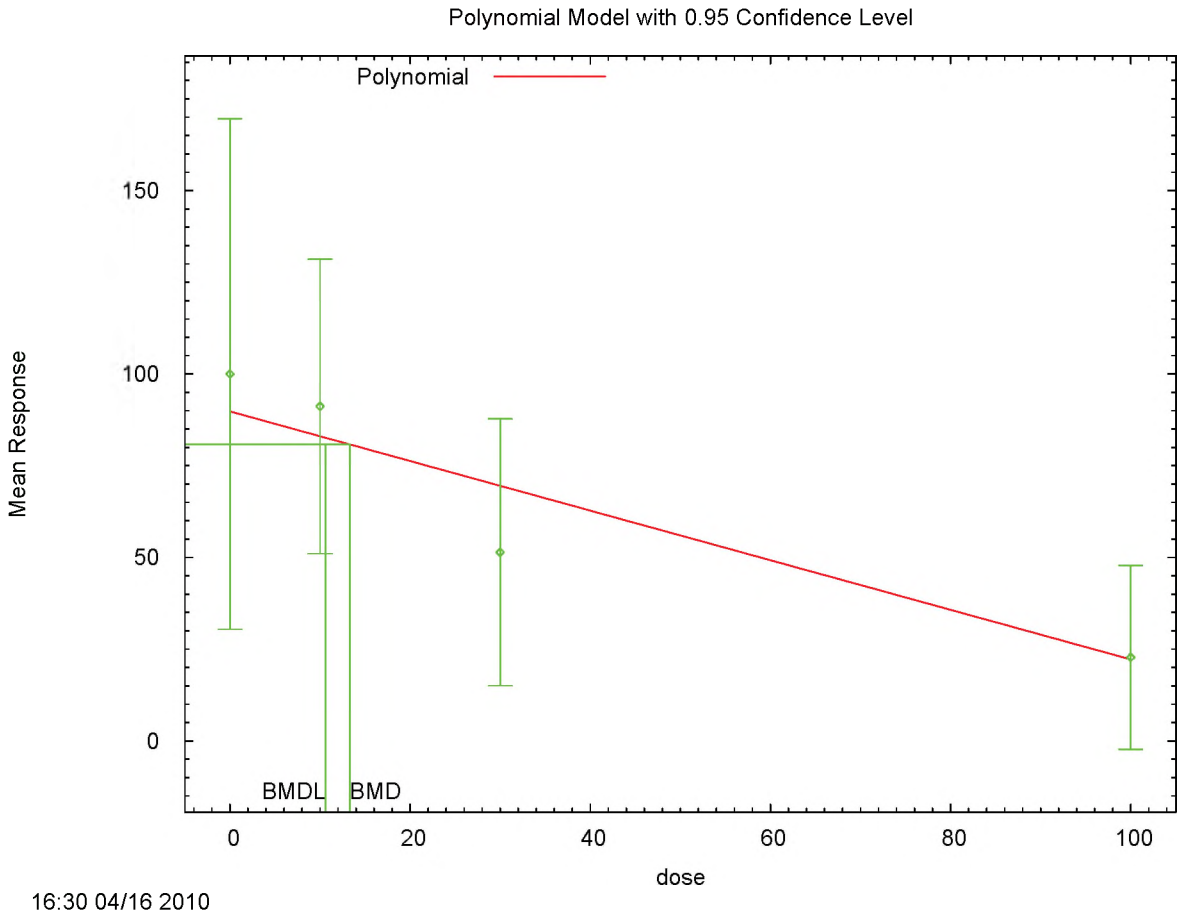
Risk Type        =     Relative risk

Confidence level =          0.95

             BMD =        13.2963


             BMDL =        10.6163
```

E.3.11.5. *Figure for Additional Model Presented: Polynomial, 3-Degree*



E.3.12. Franc et al., 2001: L-E Rats, Relative Thymus Weight

E.3.12.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	0.394	301.666	6.406E+00	2.122E+00	
exponential (M3)	2	0.394	301.666	6.406E+00	2.122E+00	power hit bound (d = 1)
exponential (M4) [b]	1	0.317	302.808	3.520E+00	1.067E+00	
exponential (M5)	0	N/A	303.805	1.280E+01	1.450E+00	
Hill	0	N/A	303.805	1.195E+01	9.965E-01	
linear	2	0.236	302.690	1.429E+01	9.087E+00	
polynomial, 3-degree	2	0.236	302.690	1.429E+01	9.087E+00	
power	2	0.236	302.690	1.429E+01	9.087E+00	power bound hit (power = 1)
power, unrestricted	1	0.175	303.643	1.297E+00	2.703E-08	unrestricted (power = 0.454)

[a] Constant variance model selected ($p = 0.5063$)

[b] Best-fitting model, BMDS output presented in this appendix

E.3.12.2. *Output for Selected Model: Exponential (M4)*

Franc et al., 2001: L-E Rats, Relative Thymus Weight

```
 ========================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\92_Franc_2001_LE_RelThyWt_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                  Fri Apr 16 16:30:58 2010
 ========================================================================

 Figure 5, L-E rats, relative thymus weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	8.1814
rho(S)	0
a	105
b	0.0413945
c	0.3173
d	1

(S) = Specified

Parameter Estimates

Variable	Model 4
lnalpha	8.21275
rho	0
a	106.57
b	0.0425967
c	0.28189
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	8	100	54.72
10	8	95.41	70.46
30	8	38.69	47.97
100	8	34.98	77.96

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	106.6	60.73	-0.306
10	80.03	60.73	0.7164
30	51.36	60.73	-0.5902
100	31.12	60.73	0.1798

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                  Model      Log(likelihood)      DF         AIC
                 -------    -----------------    ----   ------------
                     A1       -146.9024            5      303.8049
                     A2       -145.7361            8      307.4723
                     A3       -146.9024            5      303.8049
                      R       -150.6049            2      305.2098
                      4        -147.404            4      302.8079


Additive constant for all log-likelihoods =     -29.41.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                      9.738             6             0.1362
  Test 2                      2.333             3             0.5063
  Test 3                      2.333             3             0.5063
 Test 6a                      1.003             1             0.3166


  The p-value for Test 1 is greater than .05.  There may not be a
  diffence between responses and/or variances among the dose levels
  Modelling the data with a dose/response curve may not be appropriate.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 0.100000

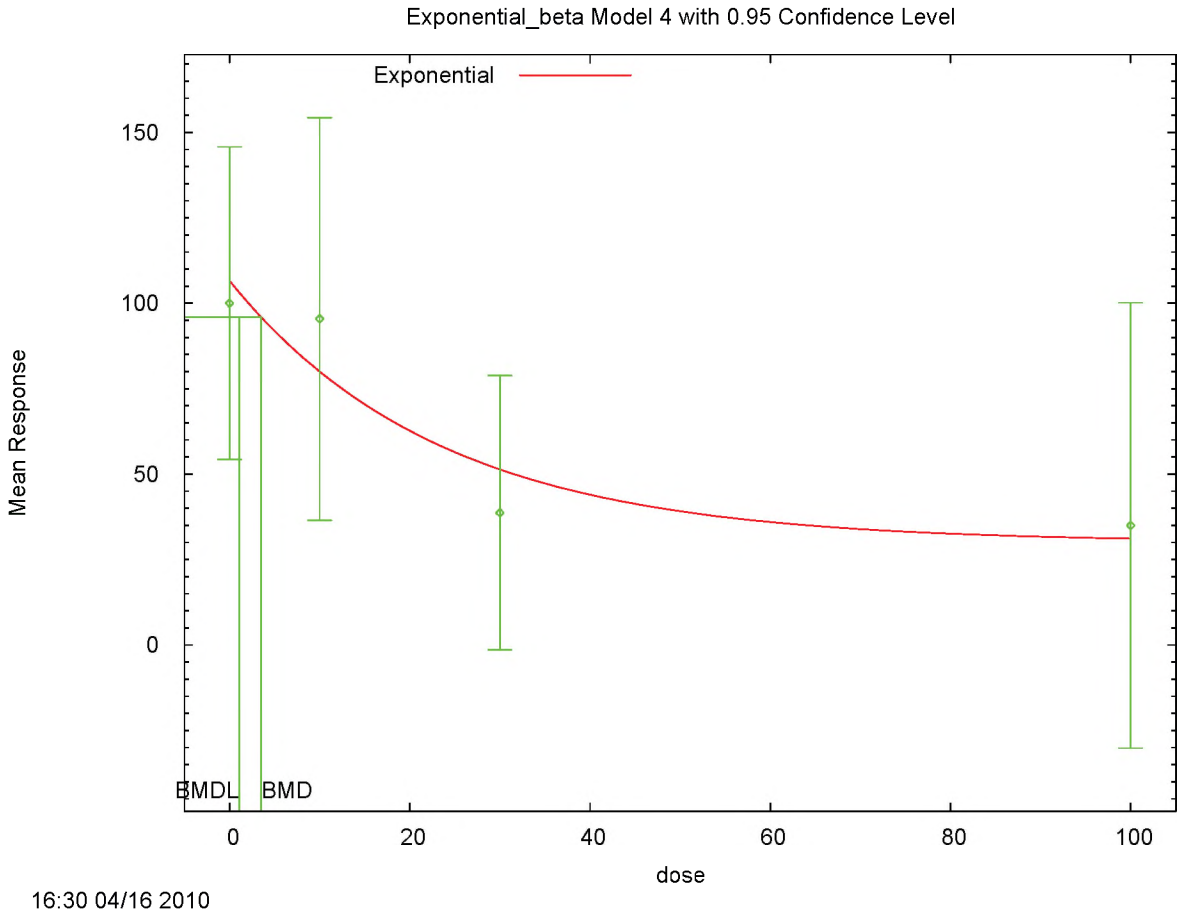
         Risk Type = Relative deviation

  Confidence Level = 0.950000

               BMD =      3.52038
```

BMDL = 1.06729

E.3.12.3. *Figure for Selected Model: Exponential (M4)*



16:30 04/16 2010

E.3.13. Franc et al., 2001: H/W Rats, Relative Thymus Weight

E.3.13.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2) [c]	2	0.682	261.694	1.366E+01	8.014E+00	
exponential (M3)	2	0.682	261.694	1.366E+01	8.014E+00	power hit bound (d = 1)
exponential (M4) [b]	1	0.512	263.358	8.820E+00	3.219E+00	
exponential (M5)	0	N/A	264.927	1.776E+01	3.500E+00	
Hill	0	N/A	264.927	1.701E+01	2.729E+00	
linear	2	0.543	262.148	1.919E+01	1.373E+01	
polynomial, 3-degree	2	0.543	262.148	1.919E+01	1.373E+01	
power	2	0.543	262.148	1.919E+01	1.373E+01	power bound hit (power = 1)
power, unrestricted	1	0.381	263.694	8.127E+00	1.406E-01	unrestricted (power = 0.665)

[a] Constant variance model selected ($p = 0.4331$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.13.2. *Output for Selected Model: Exponential (M2)*

Franc et al., 2001: H/W Rats, Relative Thymus Weight

```
 ================================================================
          Exponential Model. (Version: 1.61;  Date: 7/24/2009)
          Input Data File: C:\1\93_Franc_2001_HW_RelThyWt_ExpCV_1.(d)
          Gnuplot Plotting File:
                                                 Fri Apr 16 16:31:40 2010
 ================================================================

 Figure 5, H/W rats, relative thymus weight
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
```

```
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


               Initial Parameter Values

               Variable          Model 2
               --------          --------
                 lnalpha             6.96647
                     rho(S)                0
                       a              59.5084
                       b            0.00715458
                       c                     0
                       d                     1

  (S) = Specified


                  Parameter Estimates

                Variable          Model 2
                --------          -------
                 lnalpha           6.99043
                     rho                 0
                       a           99.7761
                       b        0.00771341
                       c                 0
                       d                 1


         Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
     0      8          100         35.98
    10      8         97.53        32.98
    30      8         71.02        23.99
   100      8         49.29        43.48


               Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0         99.78        32.96          0.01921
     10         92.37        32.96           0.4426
     30         79.16        32.96          -0.6986
    100         46.14        32.96            0.271


Other models for which likelihoods are calculated:
```

```
  Model A1:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                    Model      Log(likelihood)      DF         AIC
                    -------    -----------------    ----   ------------
                       A1       -127.4636           5      264.9271
                       A2       -126.0925           8       268.185
                       A3       -127.4636           5      264.9271
                        R        -132.935           2        269.87
                        2        -127.8469          3      261.6939


Additive constant for all log-likelihoods =     -29.41.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does Model 2 fit the data? (A3 vs. 2)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                        13.69           6             0.03336
  Test 2                        2.742           3              0.4331
  Test 3                        2.742           3              0.4331
  Test 4                       0.7668           2              0.6815


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 4 is greater than .1.  Model 2 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 0.100000

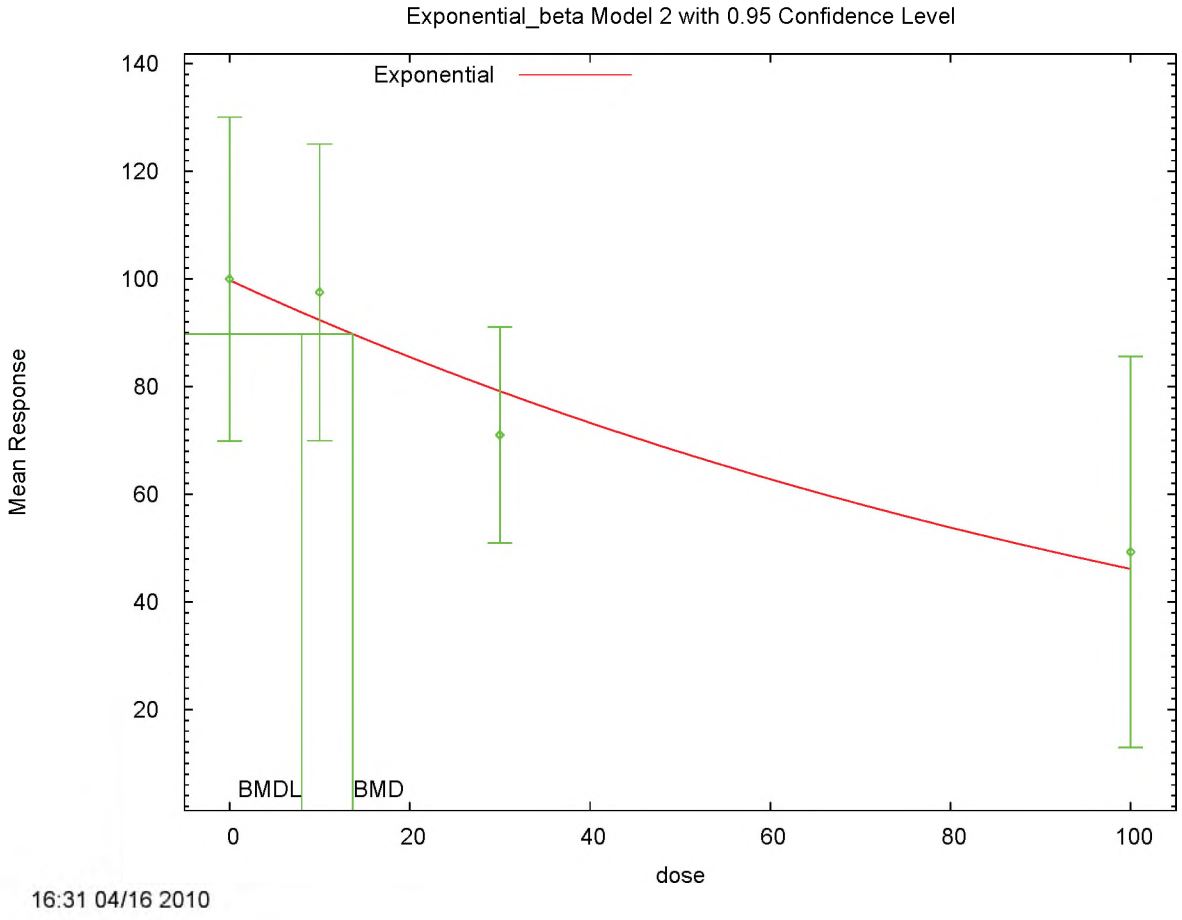
         Risk Type = Relative deviation

  Confidence Level = 0.950000

              BMD =      13.6594
```

```
BMDL =      8.01373
```

E.3.13.3. *Figure for Selected Model: Exponential (M2)*



E.3.13.4. *Output for Additional Model Presented: Exponential (M4)*

Franc et al., 2001: H/W Rats, Relative Thymus Weight

```
==============================================================================
        Exponential Model. (Version: 1.61;  Date: 7/24/2009)
        Input Data File: C:\1\93_Franc_2001_HW_RelThyWt_ExpCV_1.(d)
        Gnuplot Plotting File:
                                                  Fri Apr 16 16:31:40 2010
==============================================================================

 Figure 5, H/W rats, relative thymus weight
------------------------------------------------------------------------------

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.
```

```
   Model 2 is nested within Models 3 and 4.
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


              Initial Parameter Values

              Variable          Model 4
              --------          --------
                lnalpha             6.96647
                    rho(S)                0
                      a                 105
                      b             0.03169
                      c            0.447105
                      d                   1

  (S) = Specified


                Parameter Estimates

               Variable          Model 4
               --------          -------
                lnalpha            6.97993
                    rho                  0
                      a            103.091
                      b            0.02048
                      c           0.394904
                      d                  1


        Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
     0      8          100        35.98
    10      8         97.53       32.98
    30      8         71.02       23.99
   100      8         49.29       43.48


              Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
     0         103.1        32.78          -0.2667
    10         91.54        32.78           0.5166
    30         74.46        32.78          -0.2961
   100         48.76        32.78          0.04621
```

Other models for which likelihoods are calculated:

```
  Model A1:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-127.4636	5	264.9271
A2	-126.0925	8	268.185
A3	-127.4636	5	264.9271
R	-132.935	2	269.87
4	-127.6789	4	263.3577

Additive constant for all log-likelihoods = -29.41. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	13.69	6	0.03336
Test 2	2.742	3	0.4331
Test 3	2.742	3	0.4331
Test 6a	0.4306	1	0.5117

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Benchmark Dose Computations:

Specified Effect = 0.100000

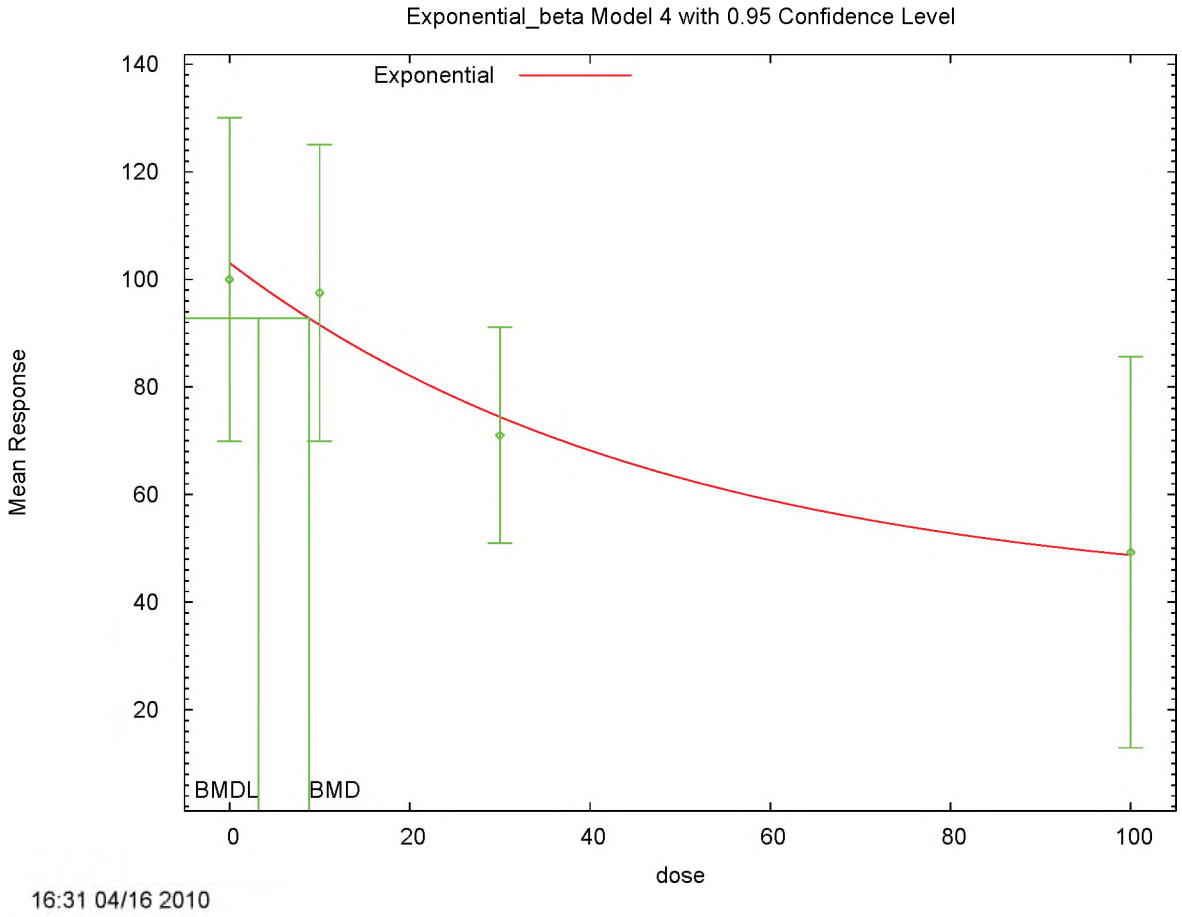
Risk Type = Relative deviation

```
Confidence Level = 0.950000

BMD =      8.82023

BMDL =      3.21928
```

E.3.13.5. *Figure for Additional Model Presented: Exponential (M4)*



E.3.14. Hojo et al., 2002: DRL Reinforce Per Minute

E.3.14.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Hill	0	N/A	6.465	2.060E+01	1.713E-05	
linear [b]	**2**	**0.008**	**9.552**	**2.677E+02**	**1.100E+02**	
polynomial, 3-degree	2	0.008	9.552	2.677E+02	1.100E+02	
power	2	0.008	9.552	2.677E+02	1.100E+02	power bound hit (power = 1)
power, unrestricted	1	0.025	6.780	2.187E+00	4.612E-08	unrestricted (power = 0.089)
exponential (M2)	2	0.006	9.894	3.043E+02	1.505E+02	
exponential (M3)	2	0.006	9.894	3.043E+02	1.505E+02	power hit bound (d = 1)
exponential (M4) [c]	1	0.062	5.241	1.734E+01	3.827E-02	
exponential (M5)	0	N/A	6.465	2.140E+01	1.240E-05	

[a] Constant variance model selected ($p = 0.4321$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.14.2. *Output for Selected Model: Linear*

Hojo et al., 2002: DRL Reinforce Per Minute

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\20_Hojo_2002_DRLrein_LinearCV_1.(d)
         Gnuplot Plotting File:  C:\1\20_Hojo_2002_DRLrein_LinearCV_1.plt
                                              Tue Feb 16 17:29:42 2010
 ================================================================

 Table 5
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 4
```

```
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =     0.337763
                            rho =            0   Specified
                         beta_0 =       -0.404
                         beta_1 =   0.00249615


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                    alpha       beta_0       beta_1

      alpha             1    -1.4e-008     2.2e-008

     beta_0    -1.4e-008            1        -0.69

     beta_1     2.2e-008        -0.69            1


                                Parameter Estimates

                                                                95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha         0.435671         0.134451           0.172152             0.69919
          beta_0        -0.372098         0.198702          -0.761547            0.017352
          beta_1       0.00246548       0.00211361         -0.00167711          0.00660807


       Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

   0        5     -0.814       -0.372        0.448         0.66           -1.5
  20        5     -0.364       -0.323        0.821         0.66          -0.14
  60        6      0.374       -0.224         0.54         0.66           2.22
 180        5     -0.163       0.0717        0.443         0.66         -0.795


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	3.115550	5	3.768900
A2	4.489557	8	7.020886
A3	3.115550	5	3.768900
fitted	-1.775882	3	9.551763
R	-2.435087	2	8.870174

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	13.8493	6	0.03137
Test 2	2.74801	3	0.4321
Test 3	2.74801	3	0.4321
Test 4	9.78286	2	0.007511

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different
model

Benchmark Dose Computation

Specified effect = 1

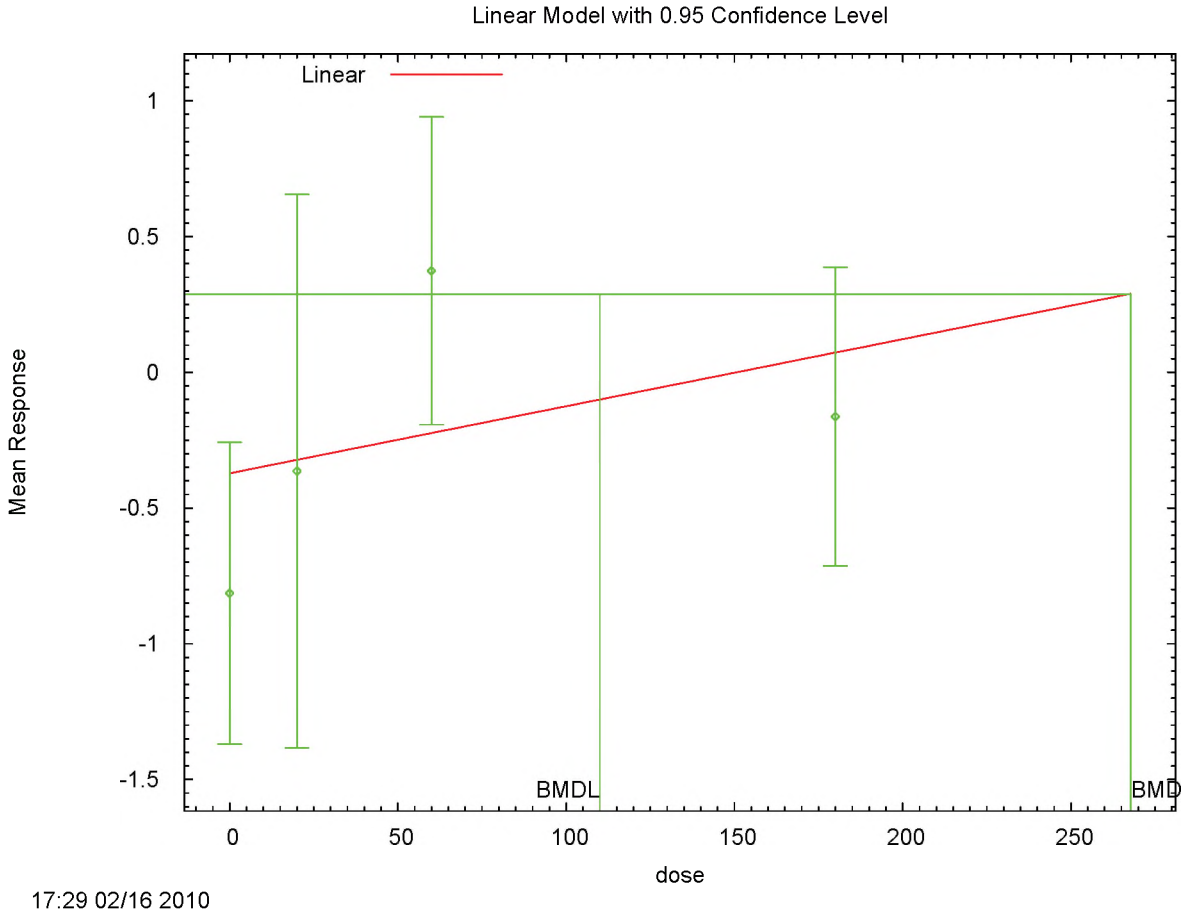
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 267.718

BMDL = 110.032

E.3.14.3. *Figure for Selected Model: Linear*



E.3.14.4. *Output for Additional Model Presented: Exponential (M4)*

Hojo et al., 2002: DRL Reinforce Per Minute

```
 ======================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\21_Hojo_2002_DRLrein_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                Tue Feb 16 17:30:21 2010
 ======================================================================

 Table 5, values adjusted by a constant to allow exponential model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.
```

```
   Model 2 is nested within Models 3 and 4.
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


                Initial Parameter Values

                Variable          Model 4
                --------          --------
                  lnalpha             -1.29672
                      rho(S)                 0
                        a                0.0817
                        b             0.00880867
                        c                16.3733
                        d                      1

  (S) = Specified


                   Parameter Estimates

                  Variable          Model 4
                  --------          -------
                   lnalpha            -1.13136
                       rho                   0
                         a           0.0542868
                         b           0.0525016
                         c             18.5072
                         d                   1


          Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
     0       5        0.086        0.448
    20       5        0.536        0.821
    60       6        1.274         0.54
   180       5        0.737        0.443


                Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0       0.05429        0.568           0.1249
     20        0.6721        0.568          -0.5359
     60         0.964        0.568            1.337
    180         1.005        0.568           -1.054


Other models for which likelihoods are calculated:
```

```
  Model A1:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                           Likelihoods of Interest

                 Model      Log(likelihood)      DF         AIC
                -------    -----------------    ----   ------------
                    A1         3.11555            5        3.7689
                    A2        4.489557            8       7.020886
                    A3         3.11555            5        3.7689
                     R        -2.435087           2       8.870174
                     4         1.379312           4       5.241376


Additive constant for all log-likelihoods =      -19.3.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                      13.85             6             0.03137
  Test 2                      2.748             3              0.4321
  Test 3                      2.748             3              0.4321
 Test 6a                      3.472             1              0.0624


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is less than .1.  Model 4 may not adequately
  describe the data; you may want to consider another model.


Benchmark Dose Computations:

  Specified Effect = 1.000000

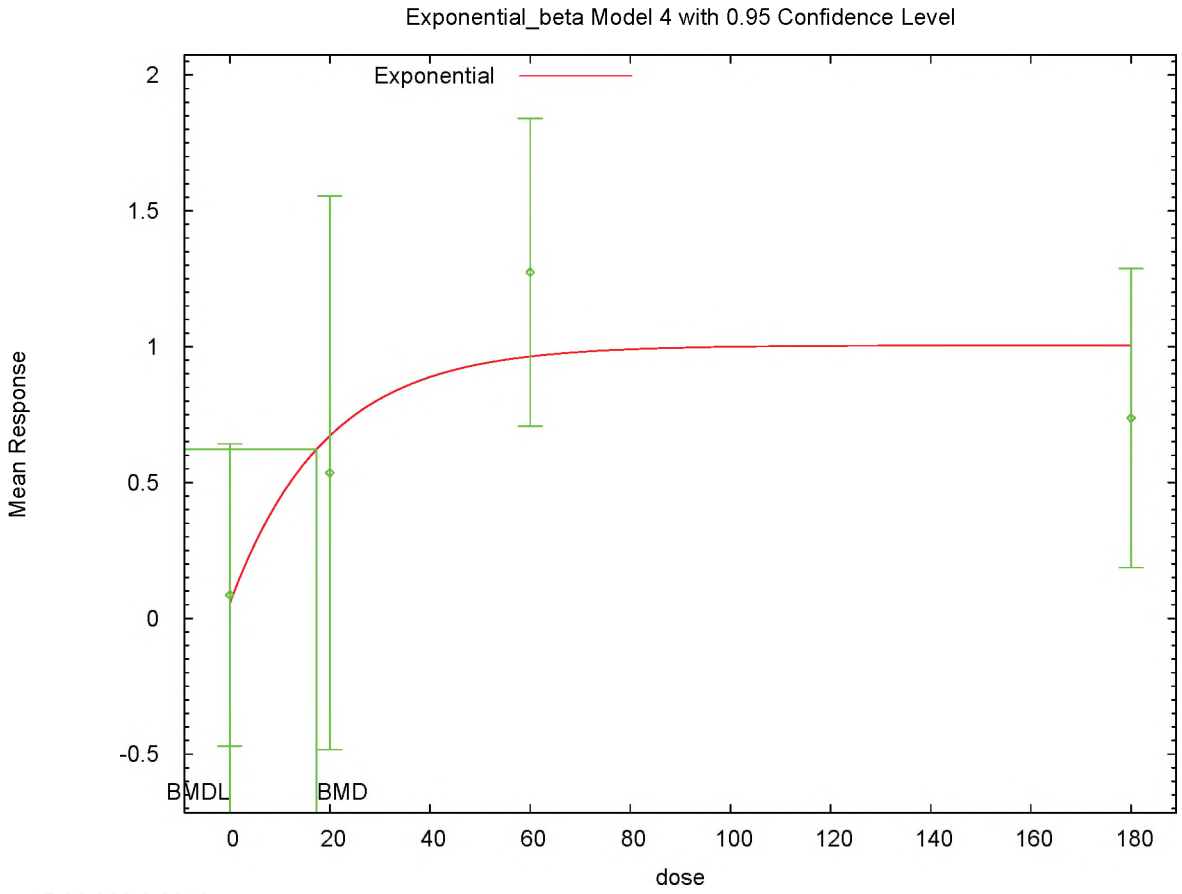
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000
```

```
BMD =      17.3391

BMDL =     0.0382689
```

E.3.14.5. *Figure for Additional Model Presented: Exponential (M4)*



E.3.15. Hojo et al., 2002: DRL Response Per Minute

E.3.15.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Hill	0	N/A	126.353	1.646E+01	1.800E-13	
linear	2	0.004	132.825	2.067E+02	9.757E+01	
polynomial, 3-degree	2	0.004	132.825	2.067E+02	9.757E+01	
power	2	0.004	132.825	2.067E+02	9.757E+01	power bound hit (power = 1)
power, unrestricted	2	0.741	122.455	1.800E+04	error	unrestricted (power = 0)
exponential (M2)	2	0.568	122.985	6.184E+00	error	
exponential (M3)	2	0.568	122.985	6.184E+00	error	power hit bound (d = 1)
exponential (M4) [b]	**1**	**0.479**	**124.356**	**4.775E+00**	**2.704E-01**	
exponential (M5)	0	N/A	126.353	1.118E+01	2.127E-01	

[a] Constant variance model selected ($p = 0.3004$)

[b] Best-fitting model, BMDS output presented in this appendix

E.3.15.2. *Output for Selected Model: Exponential (M4)*

Hojo et al., 2002: DRL Response Per Minute

```
 ================================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\23_Hojo_2002_DRLresp_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                  Tue Feb 16 17:31:24 2010
 ================================================================================

 Table 5, values adjusted by a constant to allow exponential model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	4.51689
rho(S)	0
a	24.6362
b	0.0212679
c	0.0184785
d	1

(S) = Specified

Parameter Estimates

Variable	Model 4
lnalpha	4.54075
rho	0
a	23.465
b	0.12859
c	0.100615
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	5	23.46	7.986
20	5	4.013	10.96
60	6	0.478	7.194
180	5	4.594	15.23

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	23.47	9.683	-0.0004677
20	3.973	9.683	0.009182
60	2.37	9.683	-0.4787
180	2.361	9.683	0.5157

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                 Model      Log(likelihood)      DF         AIC
                -------    -----------------    ----   ------------
                    A1       -57.92733           5      125.8547
                    A2       -56.09669           8      128.1934
                    A3       -57.92733           5      125.8547
                     R       -64.49611           2      132.9922
                     4       -58.17787           4      124.3557


Additive constant for all log-likelihoods =      -19.3.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                      16.8              6             0.01005
  Test 2                     3.661              3              0.3004
  Test 3                     3.661              3              0.3004
 Test 6a                    0.5011              1               0.479


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

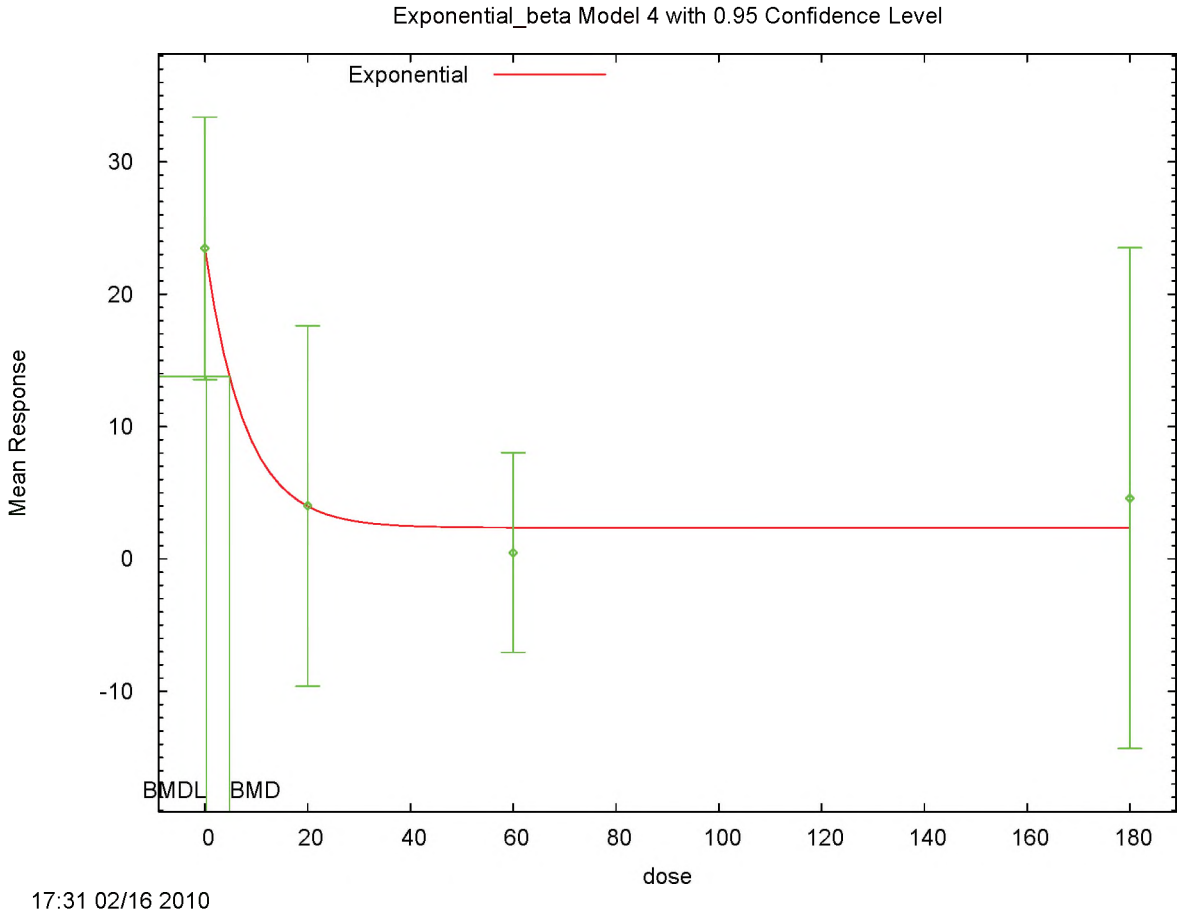
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      4.77493
```

BMDL = 0.270447

E.3.15.3. *Figure for Selected Model: Exponential (M4)*



E.3.16. Kattainen et al., 2001: 3rd Molar Eruption, Female

E.3.16.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
logistic	3	0.292	89.060	1.941E+02	1.390E+02	negative intercept (intercept = -1.508)
log-logistic [a]	**3**	**0.923**	**85.535**	**4.763E+01**	**2.481E+01**	**slope bound hit (slope = 1)**
log-probit	3	0.390	88.231	1.574E+02	9.512E+01	slope bound hit (slope = 1)
probit	3	0.306	88.919	1.858E+02	1.370E+02	negative intercept (intercept = -0.927)
multistage, 4-degree	3	0.641	86.798	8.677E+01	5.520E+01	final ß = 0
log-logistic, unrestricted [b]	2	0.952	87.157	2.599E+01	1.730E+00	unrestricted (slope = 0.794)
log-probit, unrestricted	2	0.941	87.179	2.813E+01	2.334E+00	unrestricted (slope = 0.478)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.3.16.2. *Output for Selected Model: Log-Logistic*

Kattainen et al., 2001: 3rd Molar Eruption, Female

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\24_Katt_2001_Erup_LogLogistic_BMR1.(d)
         Gnuplot Plotting File:  C:\1\24_Katt_2001_Erup_LogLogistic_BMR1.plt
                                              Tue Feb 16 17:31:52 2010
 ================================================================

 Figure 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model
```

```
                    Default Initial Parameter Values
                       background =       0.0625
                        intercept =       -6.063
                            slope =            1


             Asymptotic Correlation Matrix of Parameter Estimates

             ( *** The model parameter(s)  -slope
                   have been estimated at a boundary point, or have been specified by the user,
                   and do not appear in the correlation matrix )

               background    intercept

background            1        -0.56

 intercept        -0.56            1


                                  Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0846785            *               *                   *
      intercept         -6.06063            *               *                   *
          slope                1            *               *                   *

* - Indicates that this value is not calculated.


                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -40.5286         5
    Fitted model       -40.7674         2      0.477533      3          0.9238
   Reduced model       -50.7341         1        20.411      4        0.0004142

            AIC:         85.5347


                                  Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ---------------------------------------------------------------------
     0.0000     0.0847         1.355     1.000          16       -0.319
    30.0000     0.1445         2.457     3.000          17        0.374
   100.0000     0.2578         3.867     4.000          15        0.078
   300.0000     0.4615         5.538     6.000          12        0.267
  1000.0000     0.7254        13.782    13.000          19       -0.402

 Chi^2 = 0.48      d.f. = 3        P-value = 0.9231


    Benchmark Dose Computation

Specified effect =           0.1

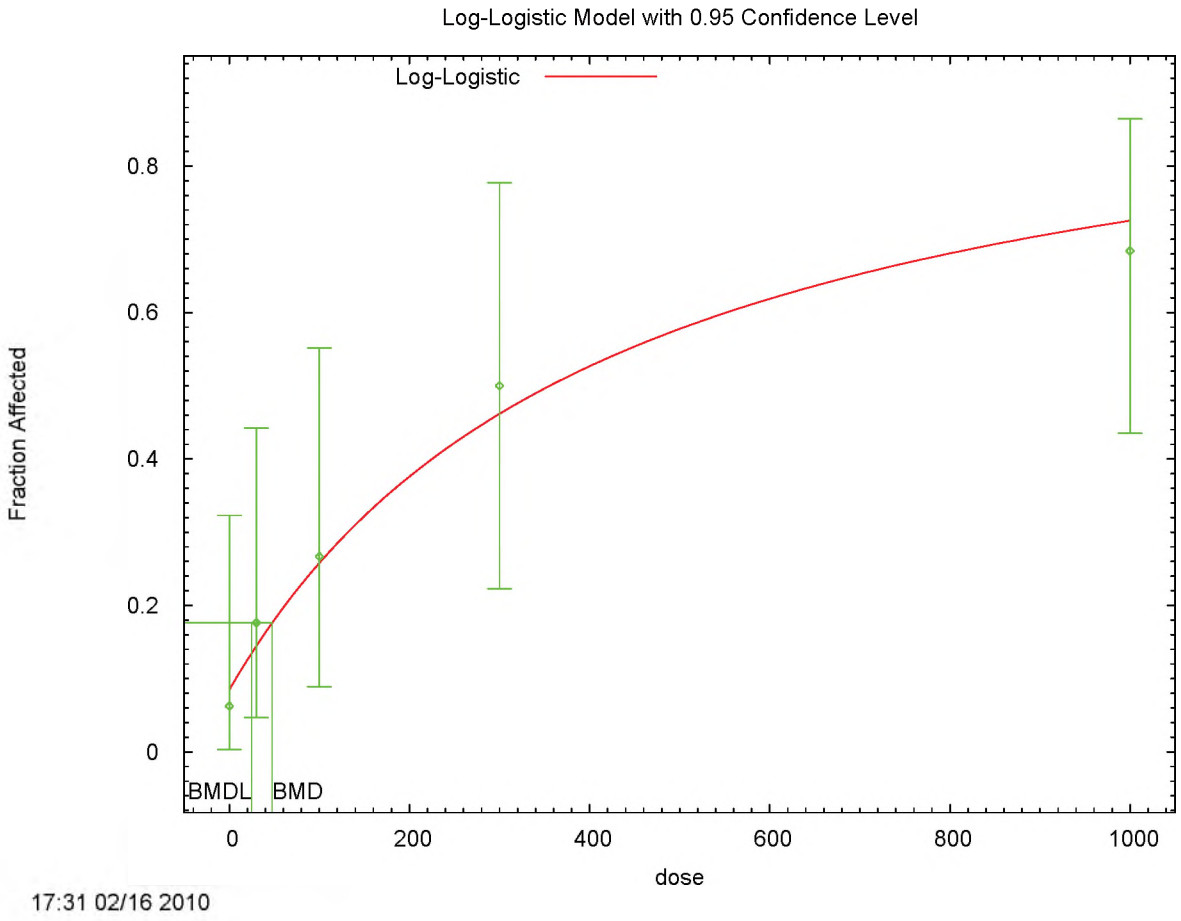
Risk Type        =      Extra risk

Confidence level =          0.95

              BMD =        47.6274

             BMDL =        24.8121
```

E.3.16.3. *Figure for Selected Model: Log-Logistic*



E.3.16.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

Kattainen et al., 2001: 3rd Molar Eruption, Female

```
================================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\1\24_Katt_2001_Erup_LogLogistic_U_BMR1.(d)
        Gnuplot Plotting File:  C:\1\24_Katt_2001_Erup_LogLogistic_U_BMR1.plt
                                                Tue Feb 16 17:31:53 2010
================================================================================

Figure 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


  Dependent variable = DichEff
  Independent variable = Dose
  Slope parameter is not restricted
```

```
   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model

                 Default Initial Parameter Values
                    background =       0.0625
                     intercept =     -4.71231
                         slope =     0.782659


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.48         0.39

 intercept        -0.48            1        -0.98

     slope         0.39        -0.98            1


                               Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0633217            *               *                   *
      intercept         -4.78282            *               *                   *
          slope         0.793723            *               *                   *

* - Indicates that this value is not calculated.


                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -40.5286         5
   Fitted model        -40.5783         3     0.0994416      2          0.9515
  Reduced model        -50.7341         1        20.411      4       0.0004142

           AIC:         87.1566


                                 Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ---------------------------------------------------------------------
     0.0000     0.0633         1.013     1.000          16       -0.013
    30.0000     0.1670         2.840     3.000          17        0.104
   100.0000     0.2924         4.387     4.000          15       -0.219
   300.0000     0.4721         5.666     6.000          12        0.193
  1000.0000     0.6892        13.095    13.000          19       -0.047

 Chi^2 = 0.10      d.f. = 2        P-value = 0.9518


   Benchmark Dose Computation

Specified effect =            0.1

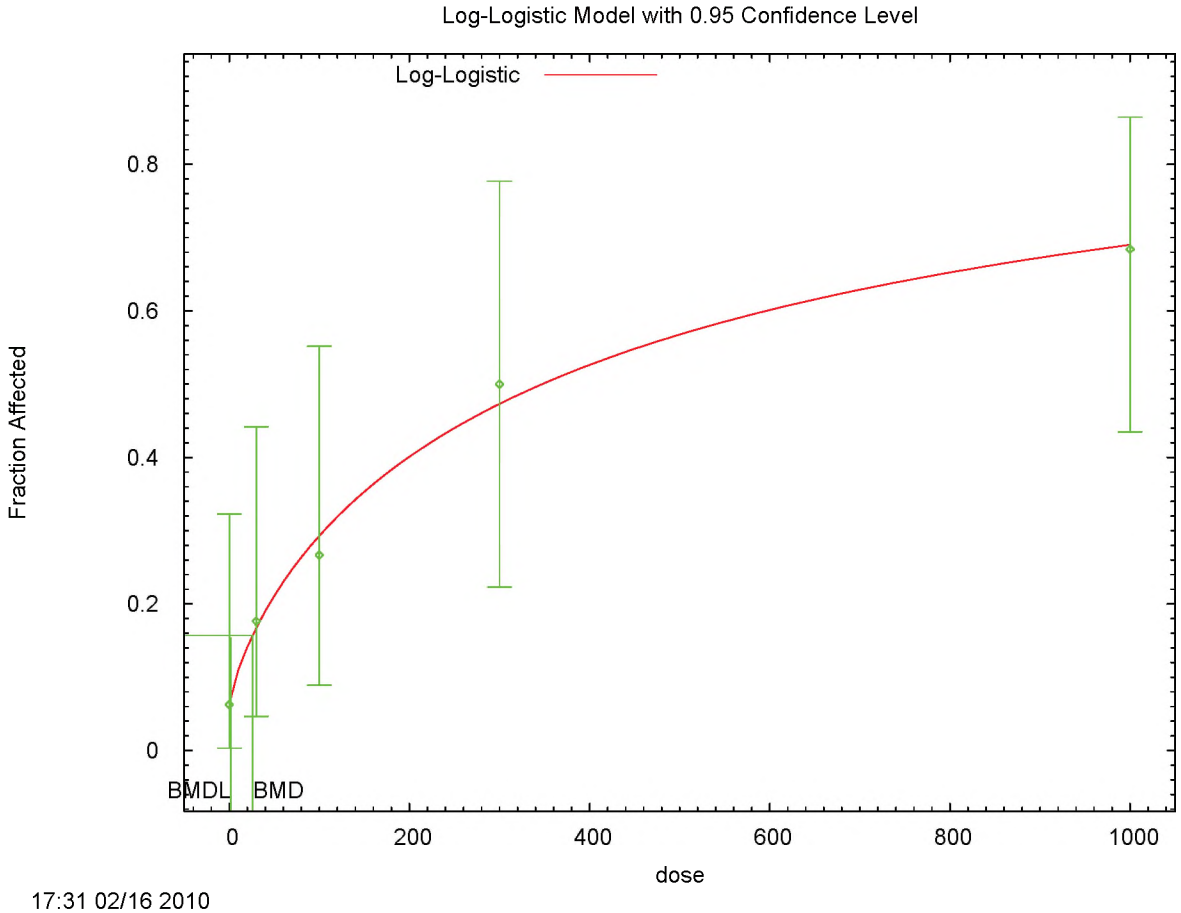
Risk Type        =      Extra risk
```

```
Confidence level =          0.95

              BMD =        25.986

             BMDL =       1.73001
```

E.3.16.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.3.17. Kattainen et al., 2001: 3rd Molar Length, Female

E.3.17.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	3	<0.0001	-122.954	4.027E+02	2.366E+02	
exponential (M3)	3	<0.0001	-122.954	4.027E+02	2.366E+02	power hit bound (d = 1)
exponential (M4)	2	<0.0001	-80.747	error	error	
exponential (M5)	1	<0.0001	-78.747	error	error	
Hill [b]	**2**	**0.013**	**-151.152**	**4.052E+00**	**2.144E+00**	**n lower bound hit (n = 1)**
linear	3	<0.0001	-122.325	4.659E+02	2.963E+02	
polynomial, 4-degree	3	<0.0001	-122.325	4.659E+02	2.963E+02	
power	3	<0.0001	-122.325	4.659E+02	2.963E+02	power bound hit (power = 1)
Hill, unrestricted [c]	1	0.087	-154.939	1.913E-02	1.928E-04	unrestricted (n = 0.197)
power, unrestricted	2	0.250	-157.093	9.098E-03	9.097E-03	unrestricted (power = 0.169)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.17.2. *Output for Selected Model: Hill*

Kattainen et al., 2001: 3rd Molar Length, Female

```
 ======================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\25_Katt_2001_Length_Hill_1.(d)
          Gnuplot Plotting File:  C:\1\25_Katt_2001_Length_Hill_1.plt
                                                Tue Feb 16 17:32:21 2010
 ======================================================================

 Figure 3 female only
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))
```

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	-2.37155
rho =	0
intercept =	1.85591
v =	-0.507874
n =	0.826204
k =	27.3305

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	intercept	v	k
lalpha	1	-0.98	-0.16	0.84	-0.37
rho	-0.98	1	0.2	-0.79	0.39
intercept	-0.16	0.2	1	-0.31	-0.11
v	0.84	-0.79	-0.31	1	-0.48
k	-0.37	0.39	-0.11	-0.48	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	3.34561	1.40443	0.592981	6.09824
rho	-14.3325	2.62129	-19.4701	-9.19484
intercept	1.8548	0.0159017	1.82364	1.88597
v	-0.441166	0.058852	-0.556513	-0.325818
n	1	NA		
k	24.0343	7.84495	8.65852	39.4101

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	16	1.86	1.85	0.0661	0.0637	0.0692
30	17	1.58	1.61	0.185	0.176	-0.768
100	15	1.6	1.5	0.265	0.293	1.28
300	12	1.5	1.45	0.221	0.378	0.527
1000	19	1.35	1.42	0.515	0.423	-0.783

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1           56.758717            6    -101.517434
              A2           85.856450           10    -151.712901
              A3           84.934314            7    -155.868628
          fitted           80.575940            5    -151.151880
               R           45.373551            2     -86.747101


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              80.9658          8          <.0001
  Test 2              58.1955          4          <.0001
  Test 3              1.84427          3          0.6053
  Test 4              8.71675          2          0.0128

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =             1

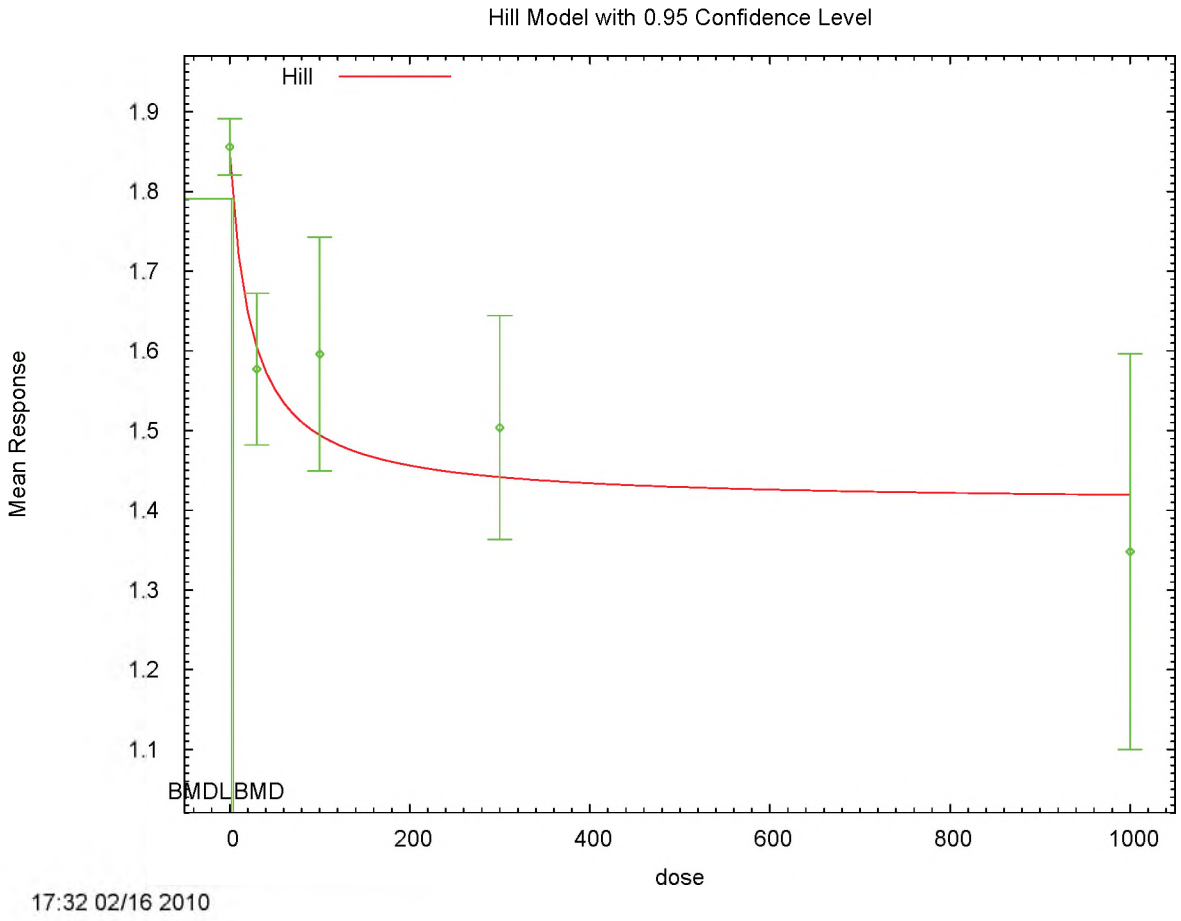
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        4.05231

            BMDL =       2.14357
```

E.3.17.3. *Figure for Selected Model: Hill*



E.3.17.4. *Output for Additional Model Presented: Hill, Unrestricted*

Kattainen et al., 2001: 3rd Molar Length, Female

```
 ================================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\25_Katt_2001_Length_Hill_U_1.(d)
          Gnuplot Plotting File:  C:\1\25_Katt_2001_Length_Hill_U_1.plt
                                                 Tue Feb 16 17:32:21 2010
 ================================================================================

 Figure 3 female only
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))
```

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	-2.37155
rho =	0
intercept =	1.85591
v =	-0.507874
n =	0.826204
k =	27.3305

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	intercept	v	n	k
lalpha	1	-0.98	-0.18	0.18	-0.28	-0.011
rho	-0.98	1	0.22	-0.18	0.29	0.011
intercept	-0.18	0.22	1	-0.025	-0.059	0.0019
v	0.18	-0.18	-0.025	1	0.51	-0.96
n	-0.28	0.29	-0.059	0.51	1	-0.71
k	-0.011	0.011	0.0019	-0.96	-0.71	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	3.21882	1.4221	0.431563	6.00607
rho	-14.0862	2.68292	-19.3446	-8.82777
intercept	1.85564	0.0160224	1.82424	1.88704
v	-2.48572	2.89658	-8.16291	3.19148
n	0.196925	0.0499318	0.0990606	0.29479
k	1.92967e+006	1.60869e+007	-2.96e+007	3.34593e+007

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	16	1.86	1.86	0.0661	0.0643	0.0164
30	17	1.58	1.6	0.185	0.18	-0.598
100	15	1.6	1.54	0.265	0.234	0.857
300	12	1.5	1.48	0.221	0.316	0.259
1000	19	1.35	1.4	0.515	0.471	-0.466

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

```
Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1           56.758717            6    -101.517434
              A2           85.856450           10    -151.712901
              A3           84.934314            7    -155.868628
          fitted           83.469680            6    -154.939361
               R           45.373551            2     -86.747101


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

    Test 1              80.9658          8          <.0001
    Test 2              58.1955          4          <.0001
    Test 3              1.84427          3          0.6053
    Test 4              2.92927          1         0.08699

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =             1

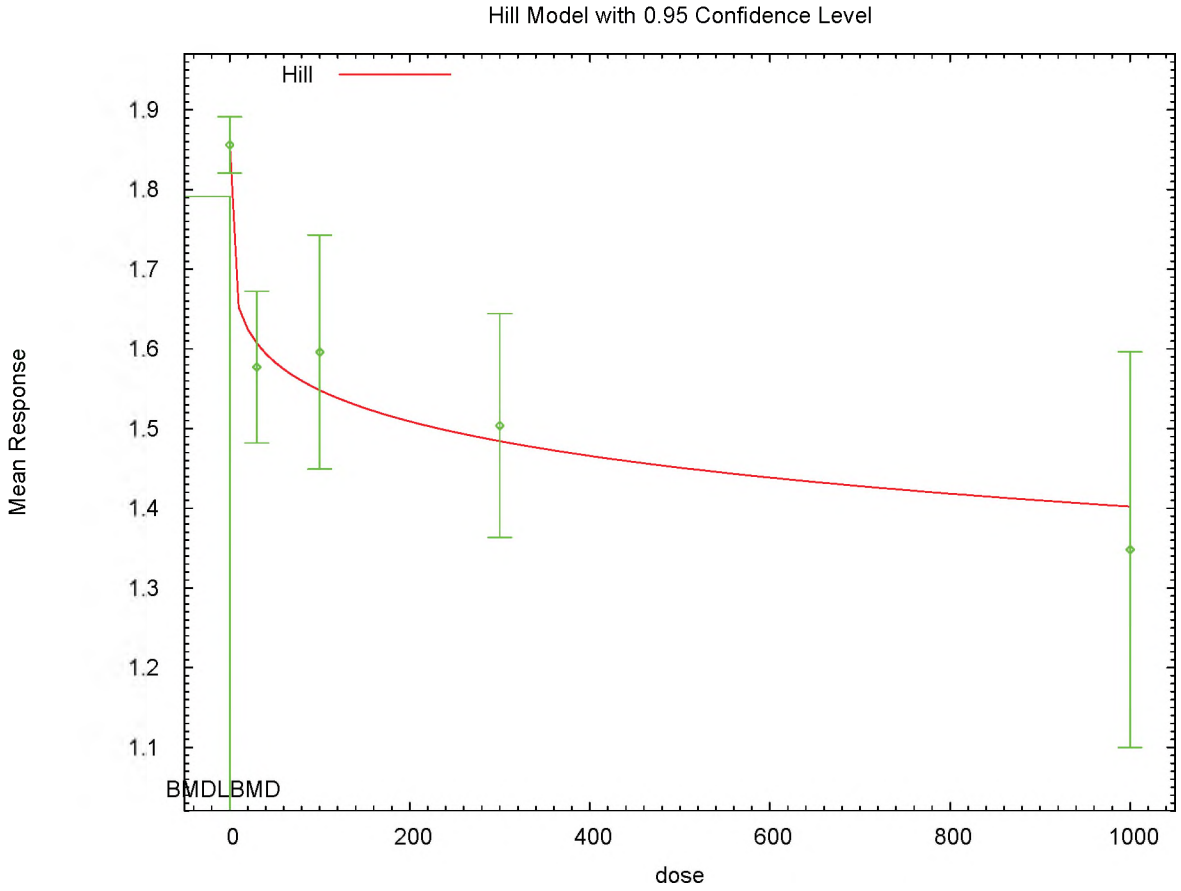
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =      0.0191282

            BMDL =     0.0001928
```

E.3.17.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.3.18. Keller et al., 2007: Missing Mandibular Molars, CBA J

E.3.18.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	1	0.105	52.490	7.293E+01	2.027E+01	
logistic	2	0.320	50.095	7.168E+01	5.142E+01	negative intercept (intercept = -3.372)
log-logistic	1	0.105	52.524	9.278E+01	5.273E+01	
log-probit	1	0.105	52.524	8.849E+01	5.297E+01	
multistage, 1-degree [a]	**3**	**0.276**	**49.409**	**2.778E+01**	**1.884E+01**	
multistage, 2-degree	1	0.126	51.515	4.619E+01	2.214E+01	
multistage, 3-degree	1	0.141	51.222	4.253E+01	2.212E+01	
probit	2	0.325	50.032	6.848E+01	4.775E+01	negative intercept (intercept = -1.851)
Weibull	1	0.108	52.216	6.079E+01	2.078E+01	

[a] Best-fitting model, BMDS output presented in this appendix

E.3.18.2. *Output for Selected Model: Multistage, 1-Degree*

Keller et al., 2007: Missing Mandibular Molars, CBA J

```
 ======================================================================
         Multistage Model. (Version: 3.0;  Date: 05/16/2008)
         Input Data File: C:\1\26_Keller_2007_Molars_Multi1_1.(d)
         Gnuplot Plotting File:  C:\1\26_Keller_2007_Molars_Multi1_1.plt
                                                Tue Feb 16 17:32:56 2010
 ======================================================================

 Table 1 using mandibular molars only
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
```

```
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                    Background =           0
                       Beta(1) = 1.02909e+017


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                Beta(1)

  Beta(1)           1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background                0            *               *                   *
        Beta(1)       0.00379264            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -21.5798         4
    Fitted model        -23.7044         1       4.24924      3          0.2358
  Reduced model          -71.326         1       99.4926      3         <.0001

           AIC:          49.4088


                                 Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
     0.0000     0.0000         0.000     0.000          29        0.000
    10.0000     0.0372         0.856     2.000          23        1.260
   100.0000     0.3156         9.153     6.000          29       -1.260
  1000.0000     0.9775        29.324    30.000          30        0.832

 Chi^2 = 3.87      d.f. = 3        P-value = 0.2762


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

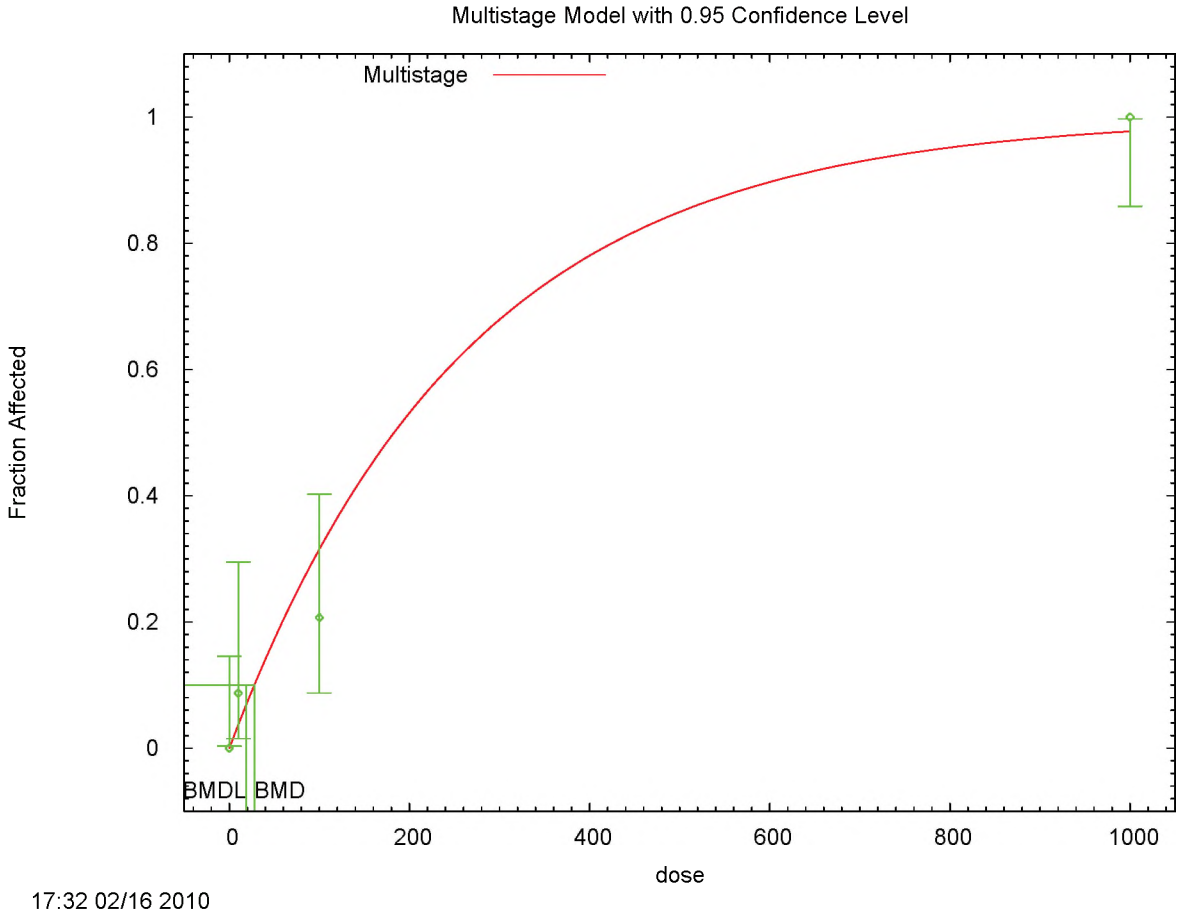
             BMD =        27.7803

            BMDL =        18.8447
```

```
            BMDU =        41.7256

Taken together, (18.8447, 41.7256) is a 90      % two-sided confidence
interval for the BMD
```

E.3.18.3. *Figure for Selected Model: Multistage, 1-Degree*



E.3.19. Kociba et al., 1978: Urinary Coproporphyrin, Females

E.3.19.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	<0.0001	84.006	7.054E+01	4.341E+01	
exponential (M3)	2	<0.0001	84.006	7.054E+01	4.341E+01	power hit bound (d = 1)
exponential (M4) [b]	**1**	**0.040**	**70.556**	**1.625E+00**	**7.300E-01**	
exponential (M5)	0	N/A	69.092	3.128E+00	1.024E+00	
Hill	0	N/A	69.047	6.677E+00	error	
linear	2	<0.0001	83.713	6.195E+01	3.112E+01	
polynomial, 3-degree	2	<0.0001	83.713	6.195E+01	3.112E+01	
power	2	<0.0001	83.713	6.195E+01	3.112E+01	power bound hit (power = 1)
power, unrestricted	1	0.001	78.260	7.808E-01	1.693E-08	unrestricted (power = 0.306)

[a] Non-constant variance model selected ($p = 0.0298$)

[b] Best-fitting model, BMDS output presented in this appendix

E.3.19.2. *Output for Selected Model: Exponential (M4)*

Kociba et al., 1978: Urinary Coproporphyrin, Females

```
 ======================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\29_Kociba_1978_Copro_Exp_1.(d)
         Gnuplot Plotting File:
                                                Tue Feb 16 17:34:45 2010
 ======================================================================

 Table2-UrinaryCoproporphyrin
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	-5.58269
rho	2.98472
a	8.17
b	0.0259469
c	2.23623
d	1

Parameter Estimates

Variable	Model 4
lnalpha	-4.94473
rho	2.76088
a	8.93039
b	0.136554
c	1.9753
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	5	9.8	1.3
1	5	8.6	2
10	5	16.4	4.7
100	5	17.4	4

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	8.93	1.733	1.122
1	10.04	2.038	-1.582
10	15.42	3.683	0.5967
100	17.64	4.436	-0.1211

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

```
  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                 Model      Log(likelihood)      DF         AIC
                -------    -----------------    ----   ------------
                    A1       -31.69739            5      73.39478
                    A2       -27.21541            8      70.43081
                    A3       -28.16434            6      68.32868
                     R       -41.73188            2      87.46376
                     4       -30.27804            5      70.55608


Additive constant for all log-likelihoods =     -18.38.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                              Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                      29.03             6           < 0.0001
  Test 2                      8.964             3            0.02977
  Test 3                      1.898             2             0.3872
 Test 6a                      4.227             1            0.03978


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is less than .1.  Model 4 may not adequately
  describe the data; you may want to consider another model.


Benchmark Dose Computations:

  Specified Effect = 1.000000

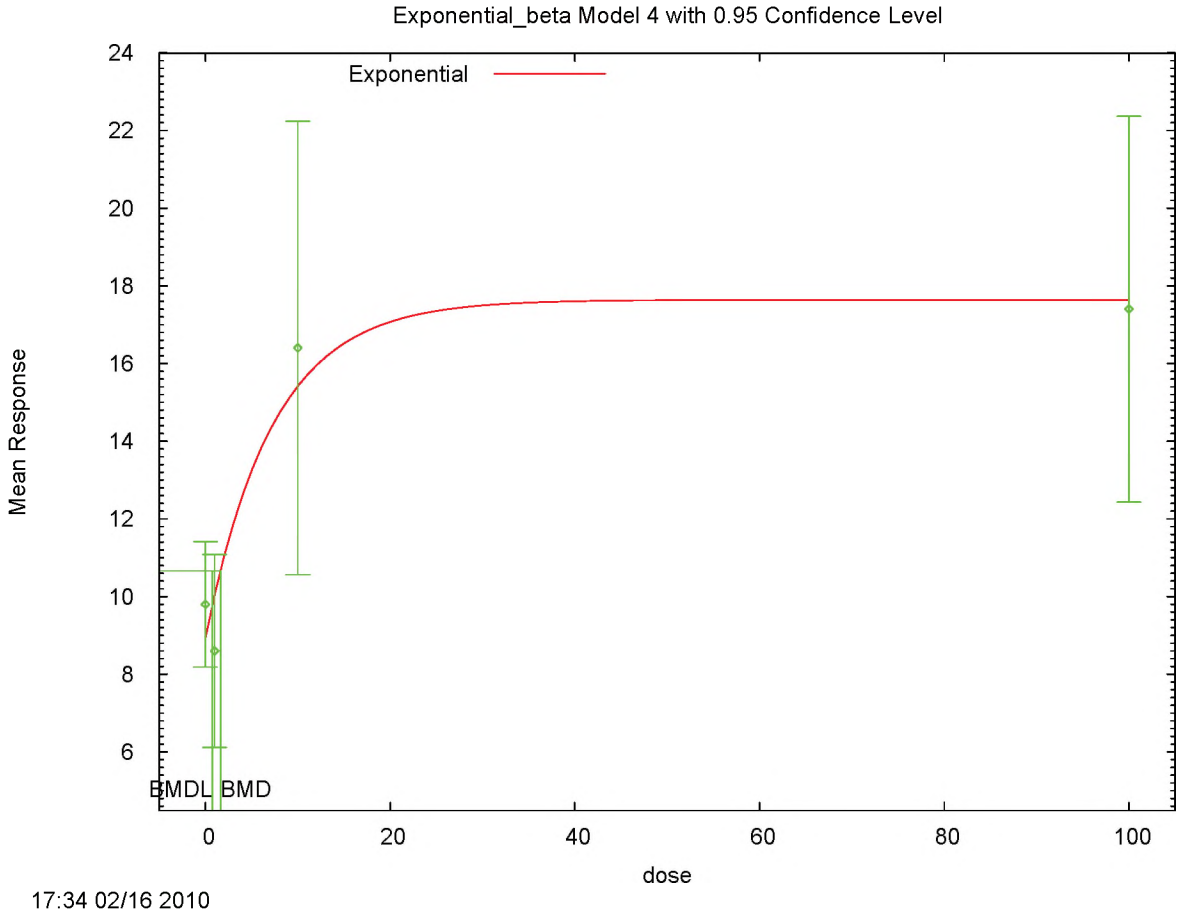
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      1.62505

              BMDL =     0.729987
```

E.3.19.3. *Figure for Selected Model: Exponential (M4)*



E.3.20. Kociba et al., 1978: Uroporphyrin per Creatinine, Female

E.3.20.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	0.661	-93.561	4.357E+01	3.328E+01	
exponential (M3)	2	0.661	-93.561	4.357E+01	3.328E+01	power hit bound (d = 1)
exponential (M4)	1	0.576	-92.078	1.719E+01	5.516E+00	
exponential (M5)	0	N/A	-90.190	1.080E+01	5.613E+00	
Hill	0	N/A	-90.190	1.099E+01	5.088E+00	
linear [b]	**2**	**0.720**	**-93.735**	**3.522E+01**	**2.500E+01**	
polynomial, 3-degree	2	0.720	-93.735	3.522E+01	2.500E+01	
power	2	0.720	-93.735	3.522E+01	2.500E+01	power bound hit (power = 1)
power, unrestricted	1	0.515	-91.967	2.274E+01	3.334E+00	unrestricted (power = 0.731)

[a] Constant variance model selected ($p = 0.4919$)

[b] Best-fitting model, BMDS output presented in this appendix

E.3.20.2. *Output for Selected Model: Linear*

Kociba et al., 1978: Uroporphyrin per Creatinine, Female

```
 ================================================================
          Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
          Input Data File: C:\1\28_Kociba_1978_Uropor_LinearCV_1.(d)
          Gnuplot Plotting File:  C:\1\28_Kociba_1978_Uropor_LinearCV_1.plt
                                                Tue Feb 16 17:34:12 2010
 ================================================================

 Table 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
```

```
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                          alpha =    0.0030385
                            rho =            0   Specified
                         beta_0 =     0.154759
                         beta_1 =    0.0014231

          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha       beta_0       beta_1

     alpha            1    -2.2e-009     3.5e-009

    beta_0    -2.2e-009            1        -0.55

    beta_1     3.5e-009        -0.55            1


                               Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha       0.00251184      0.000794315         0.000955015          0.00406867
         beta_0         0.154759        0.0134422            0.128413            0.181105
         beta_1        0.0014231      0.000267497         0.000898818          0.00194739


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   -----------

    0     5      0.157        0.155         0.05       0.0501            0.1
    1     5      0.143        0.156        0.037       0.0501         -0.588
   10     5      0.181        0.169        0.053       0.0501          0.536
  100     5      0.296        0.297        0.074       0.0501        -0.0477


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                       Likelihoods of Interest
```

Model	Log(likelihood)	# Param's	AIC
A1	50.195349	5	-90.390697
A2	51.400051	8	-86.800103
A3	50.195349	5	-90.390697
fitted	49.867385	3	-93.734769
R	41.049755	2	-78.099510

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	20.7006	6	0.002076
Test 2	2.40941	3	0.4919
Test 3	2.40941	3	0.4919
Test 4	0.655928	2	0.7204

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

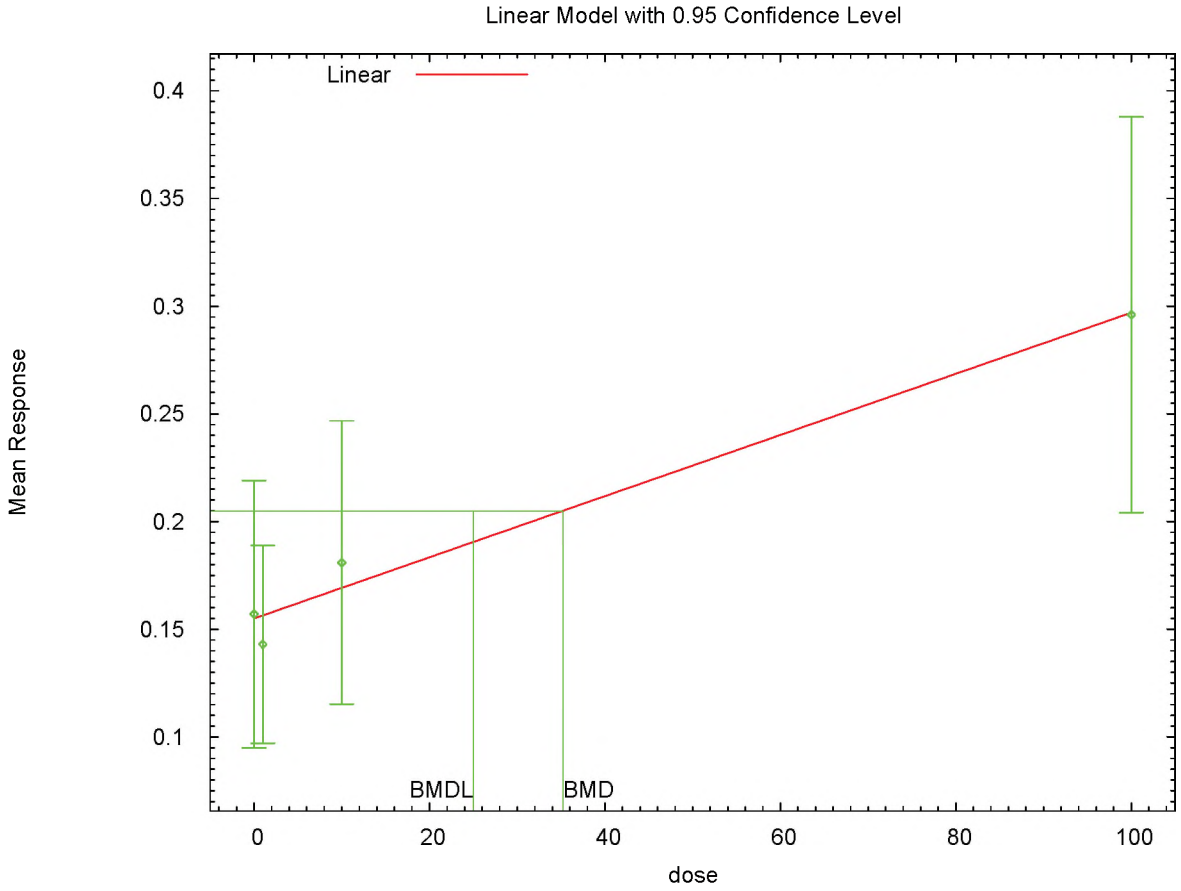
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 35.2176

BMDL = 25.0024

E.3.20.3. *Figure for Selected Model: Linear*



E.3.21. Latchoumycandane and Mathur, 2002: Sperm Production

E.3.21.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	<0.0001	95.106	7.640E+01	3.992E+01	
exponential (M3)	2	<0.0001	95.106	7.640E+01	3.992E+01	power hit bound (d = 1)
exponential (M4)	1	0.699	75.263	2.435E-01	1.016E-01	
exponential (M5)	0	N/A	77.263	3.697E-01	1.016E-01	
Hill [b]	1	0.859	75.144	1.450E-01	1.559E-02	n lower bound hit (n = 1)
linear	2	<0.0001	95.308	8.275E+01	4.852E+01	
polynomial, 3-degree	2	<0.0001	95.308	8.275E+01	4.852E+01	
power	2	<0.0001	95.308	8.275E+01	4.852E+01	power bound hit (power = 1)
Hill, unrestricted [c]	0	N/A	77.113	6.943E-02	2.060E-06	unrestricted (n = 0.709)
power, unrestricted	1	0.499	75.570	2.706E-07	2.706E-07	unrestricted (power = 0.067)

[a] Constant variance model selected ($p = 0.8506$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.21.2. *Output for Selected Model: Hill*

Latchoumycandane and Mathur, 2002: Sperm Production

```
 ======================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\30_Latch_2002_Sperm_HillCV_1.(d)
          Gnuplot Plotting File:  C:\1\30_Latch_2002_Sperm_HillCV_1.plt
                                                Tue Feb 16 18:13:20 2010
 ======================================================================

 (x10^6) Table 1 without Vitamin E
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
```

```
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                          alpha =      7.23328
                            rho =            0   Specified
                      intercept =        22.19
                              v =        -9.09
                              n =      1.80484
                              k =     0.697086


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -n
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                    alpha    intercept            v            k

       alpha            1     6.3e-010       3e-008     8.3e-009

   intercept     6.3e-010            1        -0.78        -0.23

           v       3e-008        -0.78            1        -0.17

           k     8.3e-009        -0.23        -0.17            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
         Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
            alpha          6.03567          1.74235             2.62073             9.45061
        intercept          22.1885          1.00316             20.2223             24.1547
                v         -9.00869          1.26801            -11.4939            -6.52343
                n                1               NA
                k         0.386669         0.265663           -0.134021            0.907359

  NA - Indicates that this parameter has hit a bound
       implied by some inequality constraint and thus
       has no standard error.


       Table of Data and Estimated Values of Interest

   Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
  ------     ---   --------     --------   -----------  -----------   ----------

      0       6       22.2         22.2         2.67         2.46        0.00151
      1       6       15.7         15.7         2.65         2.46        -0.0218
     10       6       13.7         13.5         2.19         2.46          0.134
    100       6       13.1         13.2         3.16         2.46         -0.114


   Model Descriptions for likelihoods calculated


   Model A1:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma^2
```

```
Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                        Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1          -33.556444            5      77.112888
              A2          -33.158811            8      82.317623
              A3          -33.556444            5      77.112888
           fitted         -33.572245            4      75.144490
               R          -47.392394            2      98.784788


                    Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                      Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              28.4672          6          <.0001
  Test 2             0.795266          3          0.8506
  Test 3             0.795266          3          0.8506
  Test 4             0.031602          1          0.8589

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =             1

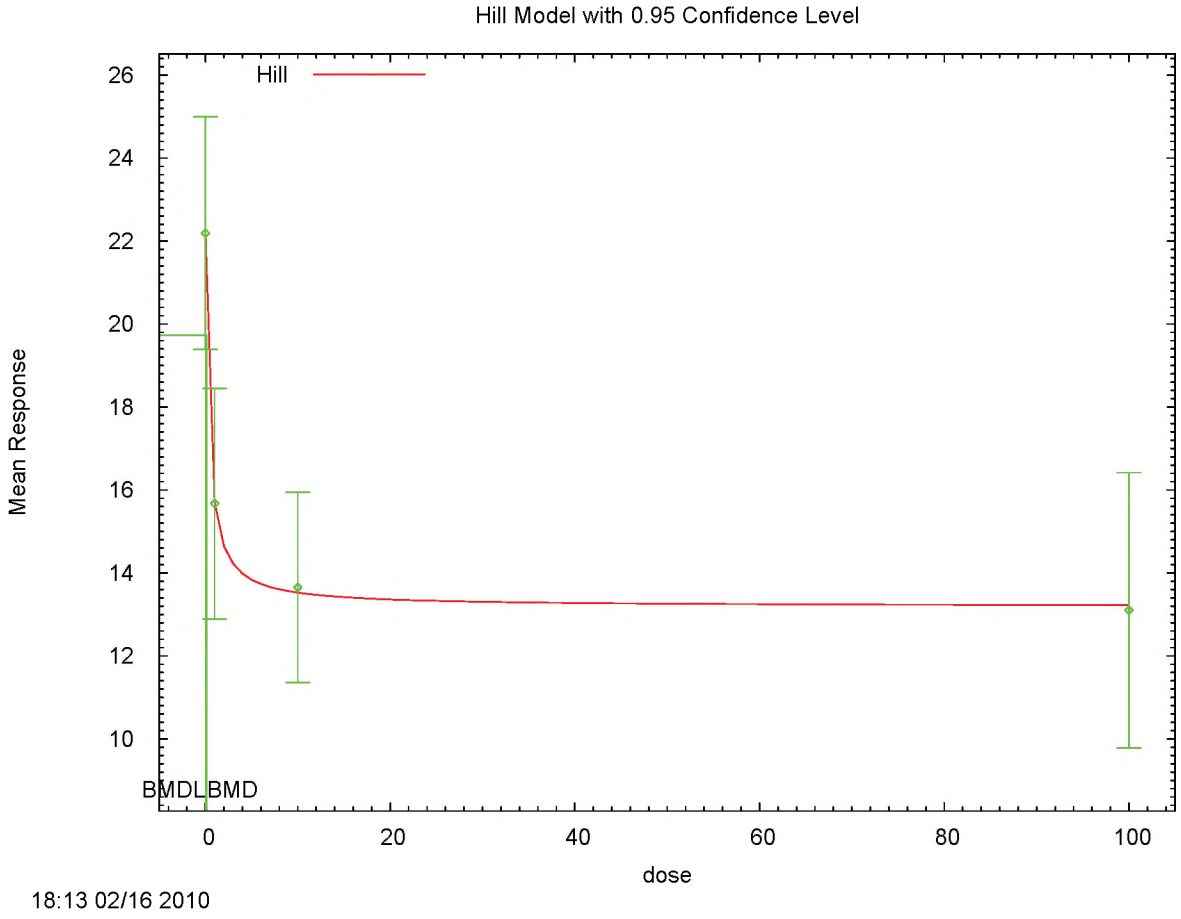
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       0.144988

            BMDL =     0.0155926
```

E.3.21.3. *Figure for Selected Model: Hill*



E.3.21.4. *Output for Additional Model Presented: Hill, Unrestricted*

Latchoumycandane and Mathur, 2002: Sperm Production

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\30_Latch_2002_Sperm_HillCV_U_1.(d)
         Gnuplot Plotting File:  C:\1\30_Latch_2002_Sperm_HillCV_U_1.plt
                                          Tue Feb 16 18:13:21 2010
 ================================================================

 (x10^6) Table 1 without Vitamin E
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter is not restricted
   A constant variance model is fit
```

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	7.23328	
rho =	0	Specified
intercept =	22.19	
v =	-9.09	
n =	1.80484	
k =	0.697086	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	n	k
alpha	1	-7.6e-009	8e-008	5e-008	1.9e-008
intercept	-7.6e-009	1	-0.5	-0.015	-0.13
v	8e-008	-0.5	1	0.75	0.55
n	5e-008	-0.015	0.75	1	0.86
k	1.9e-008	-0.13	0.55	0.86	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	6.02773	1.74006	2.61728	9.43818
intercept	22.19	1.00231	20.2255	24.1545
v	-9.23433	2.02073	-13.1949	-5.27378
n	0.709305	1.28329	-1.8059	3.22451
k	0.290697	0.548737	-0.784807	1.3662

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	6	22.2	22.2	2.67	2.46	2.62e-008
1	6	15.7	15.7	2.65	2.46	-1.5e-008
10	6	13.7	13.7	2.19	2.46	-4.56e-008
100	6	13.1	13.1	3.16	2.46	-3.52e-007

Degrees of freedom for Test A3 vs fitted <= 0

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -33.556444            5      77.112888
             A2          -33.158811            8      82.317623
             A3          -33.556444            5      77.112888
          fitted         -33.556444            5      77.112888
              R          -47.392394            2      98.784788


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              28.4672          6          <.0001
   Test 2             0.795266          3          0.8506
   Test 3             0.795266          3          0.8506
   Test 4          2.84217e-014         0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


        Benchmark Dose Computation

Specified effect =             1

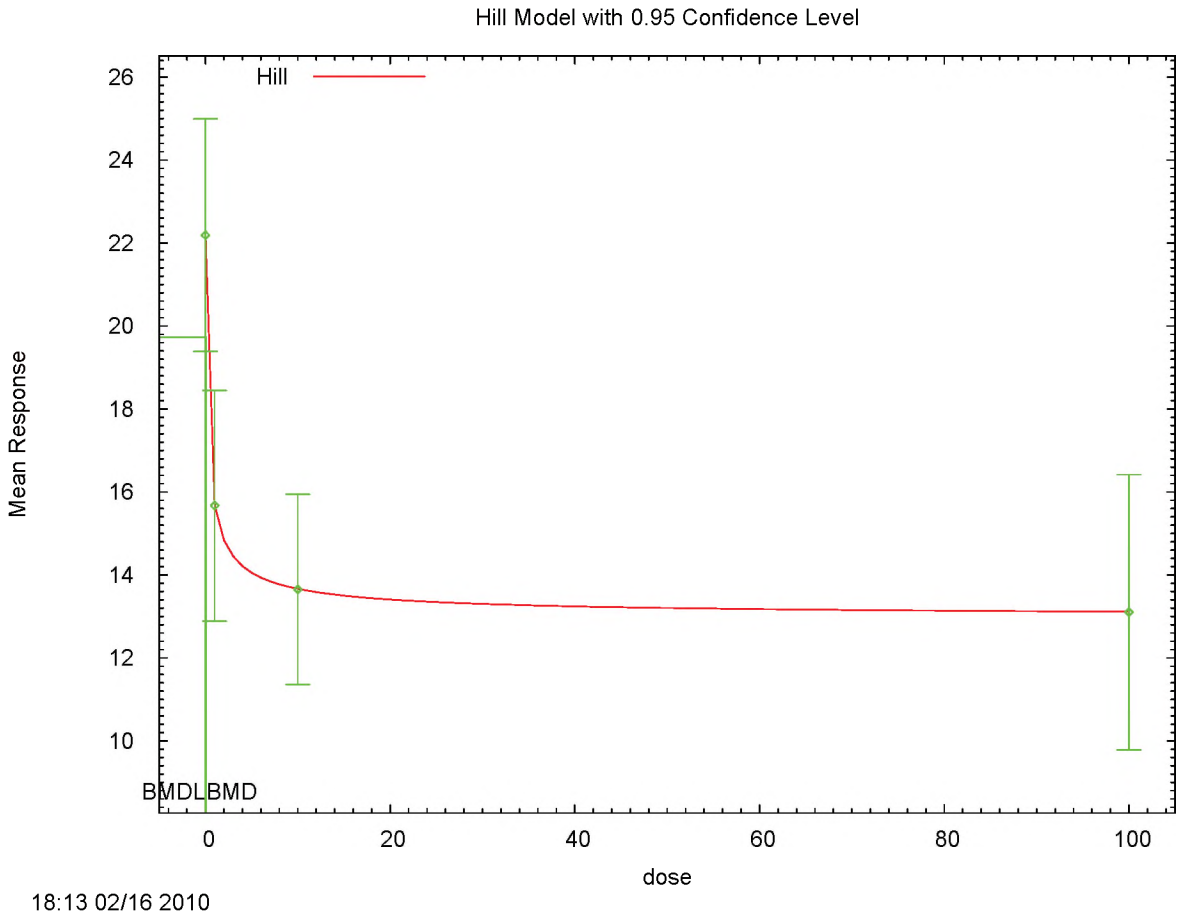
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =      0.0694325

            BMDL =  2.06007e-006
```

E.3.21.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.3.22. Li et al., 1997: FSH

E.3.22.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	8	<0.0001	1095.240	1.340E+04	1.060E+04	
exponential (M3)	8	<0.0001	1095.240	1.340E+04	1.060E+04	power hit bound (d = 1)
exponential (M4)	7	<0.0001	1061.243	1.031E+03	4.015E+02	
exponential (M5)	7	<0.0001	1061.243	1.031E+03	4.015E+02	power hit bound (d = 1)
Hill	7	<0.0001	1059.547	6.645E+02	error	n lower bound hit (n = 1)
linear	8	<0.0001	1078.221	5.287E+03	3.602E+03	
polynomial, 8-degree	9	<0.0001	1155.670	error	error	
power [b]	**8**	**<0.0001**	**1078.221**	**5.287E+03**	**3.602E+03**	**power bound hit (power = 1)**
Hill, unrestricted	6	0.001	1039.902	2.809E+00	6.602E-01	unrestricted (n = 0.291)
power, unrestricted [c]	7	0.002	1037.821	2.508E+00	2.525E-01	unrestricted (power = 0.279)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.22.2. *Output for Selected Model: Power*

Li et al., 1997: FSH

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\1\72_Li_1997_FSH_Pwr_1.(d)
         Gnuplot Plotting File:  C:\1\72_Li_1997_FSH_Pwr_1.plt
                                              Tue Feb 16 20:07:31 2010
 ================================================================

 Figure 3: FSH in female S-D rats 24hr after dosing, 22 day old rats
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   The power is restricted to be greater than or equal to 1
```

The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 10
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	9.8191
rho =	0
control =	22.1591
slope =	26.1213
power =	0.264963

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	control	slope
lalpha	1	-0.99	-0.29	-0.023
rho	-0.99	1	0.2	0.023
control	-0.29	0.2	1	-0.35
slope	-0.023	0.023	-0.35	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	3.5473	1.23656	1.12369	5.9709
rho	1.26137	0.244246	0.782659	1.74009
control	88.9479	12.9114	63.6419	114.254
slope	0.0188972	0.00351723	0.0120035	0.0257908
power	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	23.9	88.9	29.6	99.9	-2.06
3	10	22.2	89	48.5	99.9	-2.12
10	10	85.2	89.1	94.3	100	-0.124
30	10	73.3	89.5	48.5	100	-0.511
100	10	126	90.8	159	101	1.1
300	10	132	94.6	116	104	1.14
1000	10	117	108	51.2	113	0.25
3000	10	304	146	154	136	3.68
1e+004	10	347	278	151	205	1.06
3e+004	10	455	656	286	352	-1.8

```
Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -535.687163          11    1093.374327
              A2         -496.367061          20    1032.734122
              A3         -502.709623          12    1029.419246
          fitted         -535.110448           4    1078.220896
               R         -574.835246           2    1153.670492


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              156.936         18          <.0001
  Test 2              78.6402          9          <.0001
  Test 3              12.6851          8          0.1232
  Test 4              64.8016          8          <.0001

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


               Benchmark Dose Computation

Specified effect =             1

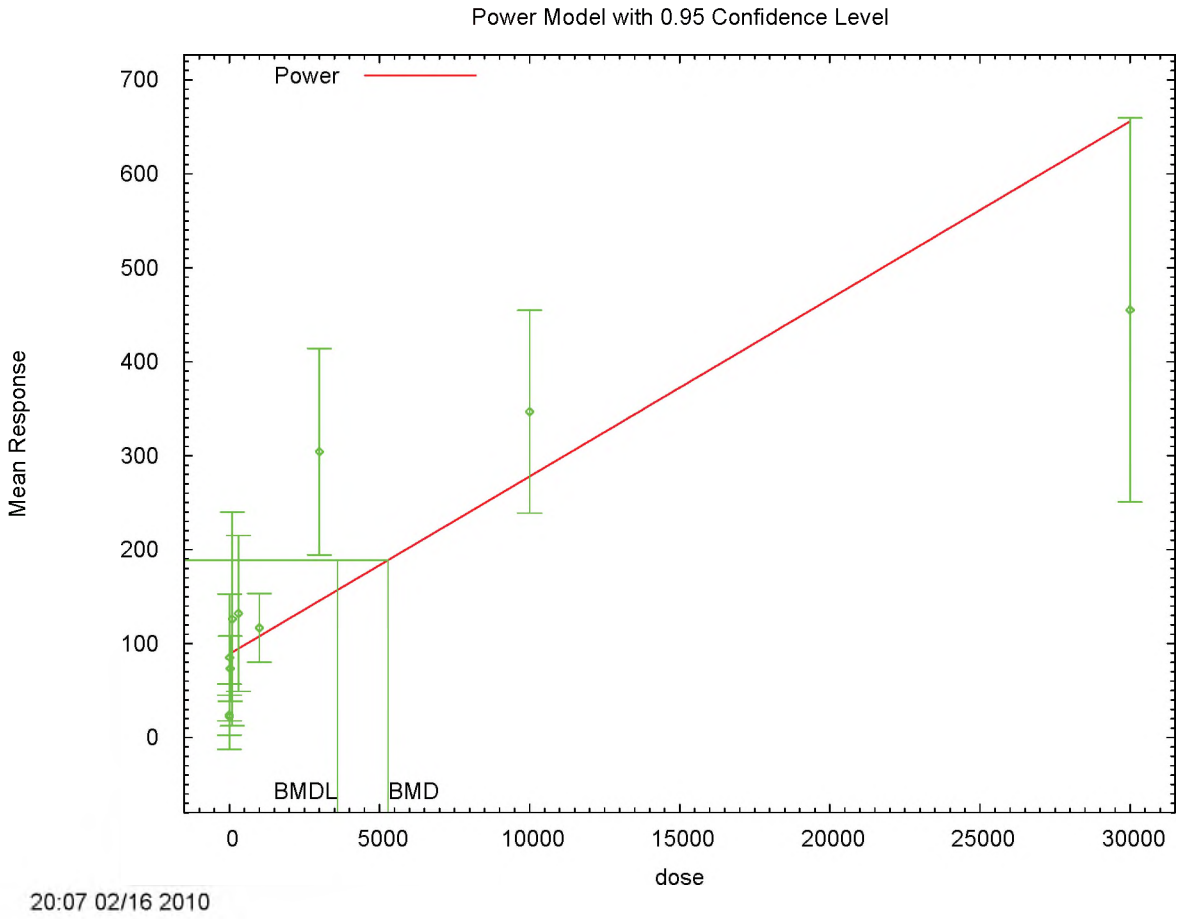
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 5286.67
```

```
BMDL = 3601.91
```

E.3.22.3. *Figure for Selected Model: Power*



E.3.22.4. *Output for Additional Model Presented: Power, Unrestricted*

Li et al., 1997: FSH

```
================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\1\72_Li_1997_FSH_Pwr_U_1.(d)
        Gnuplot Plotting File:  C:\1\72_Li_1997_FSH_Pwr_U_1.plt
                                          Tue Feb 16 20:07:33 2010
================================================================

Figure 3: FSH in female S-D rats 24hr after dosing, 22 day old rats

  The form of the response function is:

  Y[dose] = control + slope * dose^power


  Dependent variable = Mean
```

Independent variable = Dose
The power is not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 10
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	9.8191
rho =	0
control =	22.1591
slope =	26.1213
power =	0.264963

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.99	-0.69	-0.15	0.28
rho	-0.99	1	0.65	0.11	-0.26
control	-0.69	0.65	1	-0.17	0.024
slope	-0.15	0.11	-0.17	1	-0.93
power	0.28	-0.26	0.024	-0.93	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	3.72156	1.13117	1.5045	5.93861
rho	1.17032	0.223249	0.732758	1.60788
control	15.7412	6.97367	2.07307	29.4094
slope	24.963	6.42976	12.3609	37.5651
power	0.278637	0.0312355	0.217417	0.339857

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	23.9	15.7	29.6	32.3	0.796
3	10	22.2	49.6	48.5	63.2	-1.38
10	10	85.2	63.2	94.3	72.7	0.96
30	10	73.3	80.1	48.5	83.6	-0.259
100	10	126	106	159	98.4	0.654
300	10	132	138	116	115	-0.164
1000	10	117	187	51.2	137	-1.62
3000	10	304	248	154	162	1.1
1e+004	10	347	341	151	195	0.0999
3e+004	10	455	457	286	232	-0.0271

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)

```
            Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
        Model A3 uses any fixed variance parameters that
        were specified by the user

 Model  R:         Yi = Mu + e(i)
             Var{e(i)} = Sigma^2


                       Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1         -535.687163          11    1093.374327
              A2         -496.367061          20    1032.734122
              A3         -502.709623          12    1029.419246
           fitted        -513.910636           5    1037.821272
               R         -574.835246           2    1153.670492


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              156.936         18          <.0001
   Test 2              78.6402          9          <.0001
   Test 3              12.6851          8          0.1232
   Test 4               22.402          7        0.002165

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


               Benchmark Dose Computation

Specified effect =             1

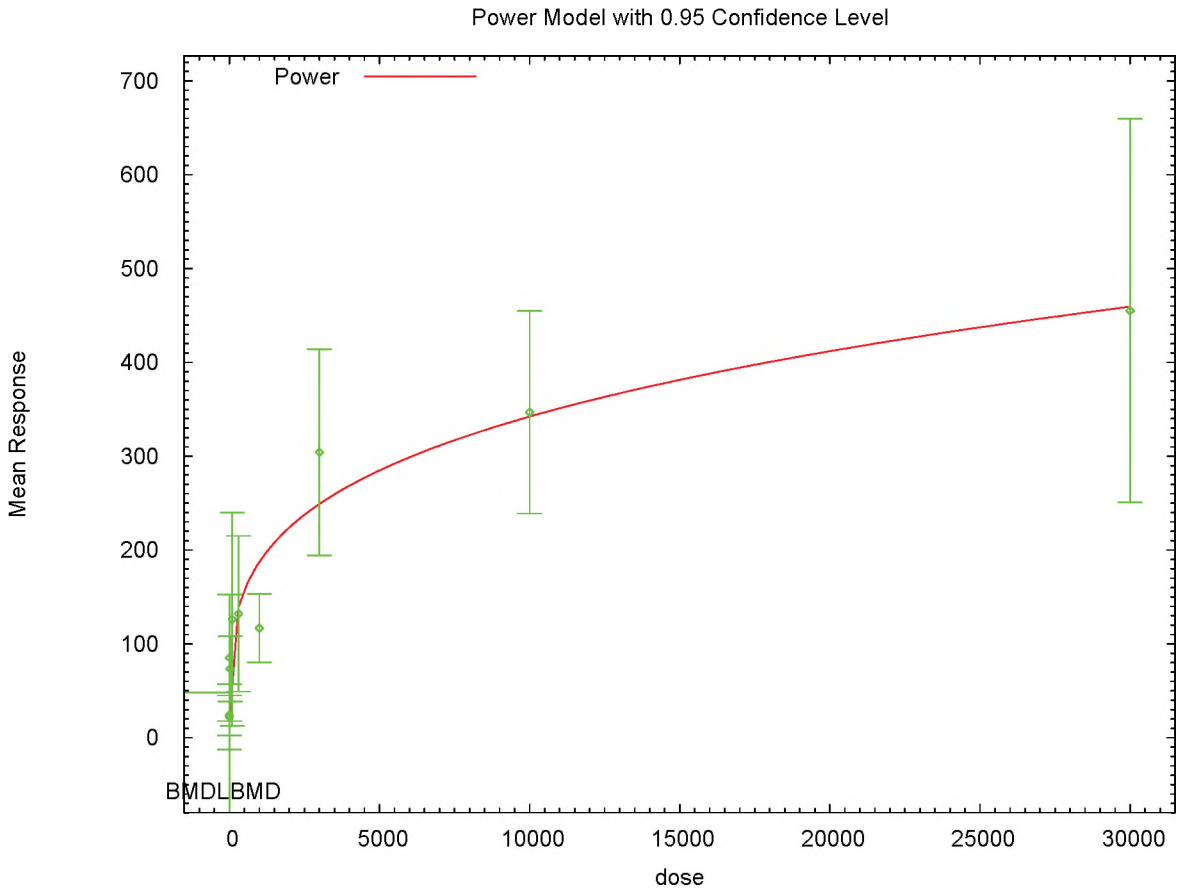
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 2.50839


            BMDL = 0.252541
```

E.3.22.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.3.23. Li et al., 2006: Estradiol, 3-Day

E.3.23.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	0.147	269.146	3.044E+02	1.108E+02	
exponential (M3)	2	0.147	269.146	3.044E+02	1.108E+02	power hit bound (d = 1)
exponential (M4)	1	0.341	268.212	error	error	
exponential (M5)	0	N/A	270.212	error	error	
Hill	0	N/A	270.212	error	error	
linear [b]	**2**	**0.151**	**269.084**	**3.471E+02**	**1.082E+02**	
polynomial, 3-degree	2	0.151	269.084	3.471E+02	1.082E+02	
power	2	0.151	269.084	3.471E+02	1.082E+02	power bound hit (power = 1)
Hill, unrestricted	0	N/A	270.266	1.059E+17	1.059E+17	unrestricted (n = 0.025)
power, unrestricted	1	0.327	268.266	3.727E+14	error	unrestricted (power = 0.012)

[a] Constant variance model selected ($p = 0.4372$)

[b] Best-fitting model, BMDS output presented in this appendix

E.3.23.2. *Output for Selected Model: Linear*

Li et al., 2006: Estradiol, 3-Day

```
 ================================================================
          Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
          Input Data File: C:\1\31_Li_2006_Estra_LinearCV_1.(d)
          Gnuplot Plotting File:  C:\1\31_Li_2006_Estra_LinearCV_1.plt
                                        Tue Feb 16 18:13:56 2010
 ================================================================

 Figure 3, 3-day estradiol
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit
```

```
Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                         alpha =      267.211
                           rho =            0   Specified
                        beta_0 =      16.4428
                        beta_1 =    0.0468351


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha       beta_0       beta_1

      alpha             1    -2.6e-013    -4.5e-015

     beta_0    -2.6e-013            1        -0.68

     beta_1    -4.5e-015        -0.68            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha          264.303             59.1             148.469             380.137
         beta_0          16.4428          3.50431             9.57445             23.3111
         beta_1        0.0468351         0.062677          -0.0760095             0.16968


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    10       10.2         16.4         12.2         16.3          -1.22
    2    10       19.9         16.5           20         16.3         0.656
   50    10       24.7         18.8         14.6         16.3          1.16
  100    10       18.1         21.1         17.6         16.3        -0.591


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-129.653527	5	269.307054
A2	-128.294657	8	272.589314
A3	-129.653527	5	269.307054
fitted	-131.541911	3	269.083823
R	-131.819169	2	267.638338

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	7.04902	6	0.3163
Test 2	2.71774	3	0.4372
Test 3	2.71774	3	0.4372
Test 4	3.77677	2	0.1513

The p-value for Test 1 is greater than .05. There may not be a
diffence between responses and/or variances among the dose levels
Modelling the data with a dose/response curve may not be appropriate

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

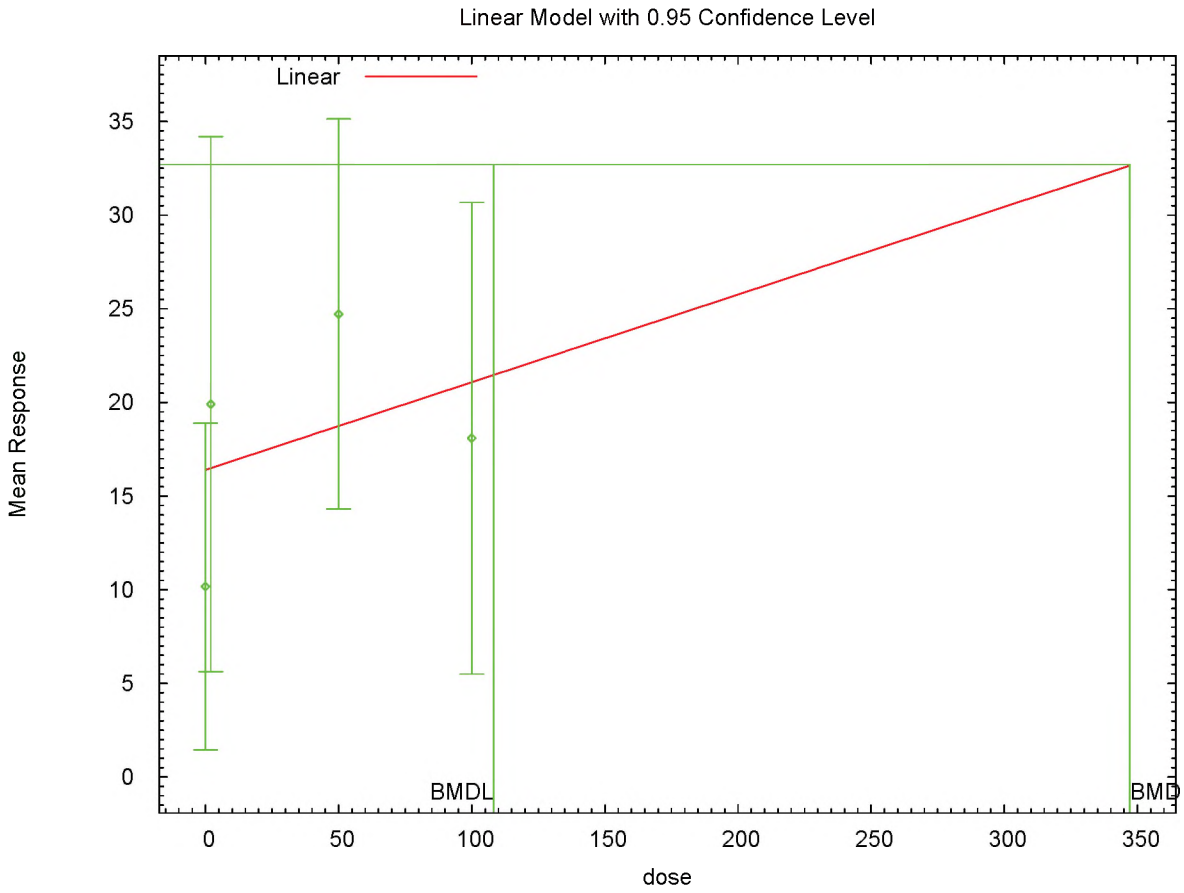
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 347.12

BMDL = 108.173

E.3.23.3. *Figure for Selected Model: Linear*



E.3.24. Li et al., 2006: Progesterone, 3-Day

E.3.24.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	<0.001	330.234	5.252E+01	error	
exponential (M3)	2	<0.001	330.234	5.252E+01	error	power hit bound (d = 1)
exponential (M4) [b]	**1**	**0.384**	**315.734**	**1.353E-01**	**8.351E-02**	
exponential (M5)	0	N/A	317.734	5.225E-01	7.503E-02	
Hill	1	0.386	315.729	1.135E-02	1.161E-05	n lower bound hit (n = 1)
linear	2	<0.001	331.121	7.765E+01	5.264E+01	
polynomial, 3-degree	2	<0.001	331.121	7.765E+01	5.264E+01	
power	2	<0.001	331.121	7.765E+01	5.264E+01	power bound hit (power = 1)
power, unrestricted	1	0.405	315.670	1.066E-63	1.066E-63	unrestricted (power = 0.009)

[a] Non-constant variance model selected ($p = 0.0013$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.24.2. *Output for Selected Model: Exponential (M4)*

Li et al., 2006: Progesterone, 3-Day

```
 ======================================================================
          Exponential Model. (Version: 1.61;  Date: 7/24/2009)
          Input Data File: C:\1\32_Li_2006_Progest_Exp_1.(d)
          Gnuplot Plotting File:
                                                 Tue Feb 16 18:14:31 2010
 ======================================================================

 Figure 4, 3-day progesterone
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
```

```
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


                Initial Parameter Values

                Variable          Model 4
                --------          --------
                  lnalpha             11.3313
                      rho            -1.44835
                        a             64.8274
                        b           0.0456906
                        c            0.166844
                        d                   1



                  Parameter Estimates

                 Variable          Model 4
                 --------          -------
                  lnalpha             14.074
                      rho            -2.27065
                        a             61.7474
                        b             2.13327
                        c            0.318566
                        d                   1


         Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
     0     10         61.74         11.1
     2     10         30.56        40.48
    50     10         16.93         33.3
   100     10         11.36        43.75


                Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
     0         61.75        10.55        -0.002085
     2         20.26        37.38           0.8713
    50         19.67        38.66           -0.224
   100         19.67        38.66          -0.6801


Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
```

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                    Model      Log(likelihood)      DF         AIC
                    -------    -----------------    ----   ------------
                       A1       -159.6327            5      329.2653
                       A2       -151.8128            8      319.6255
                       A3       -152.4882            6      316.9763
                        R       -165.6989            2      335.3978
                        4       -152.8668            5      315.7335


Additive constant for all log-likelihoods =     -36.76.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                       27.77            6          0.0001037
  Test 2                       15.64            3           0.001344
  Test 3                       1.351            2             0.5089
 Test 6a                      0.7572            1             0.3842


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

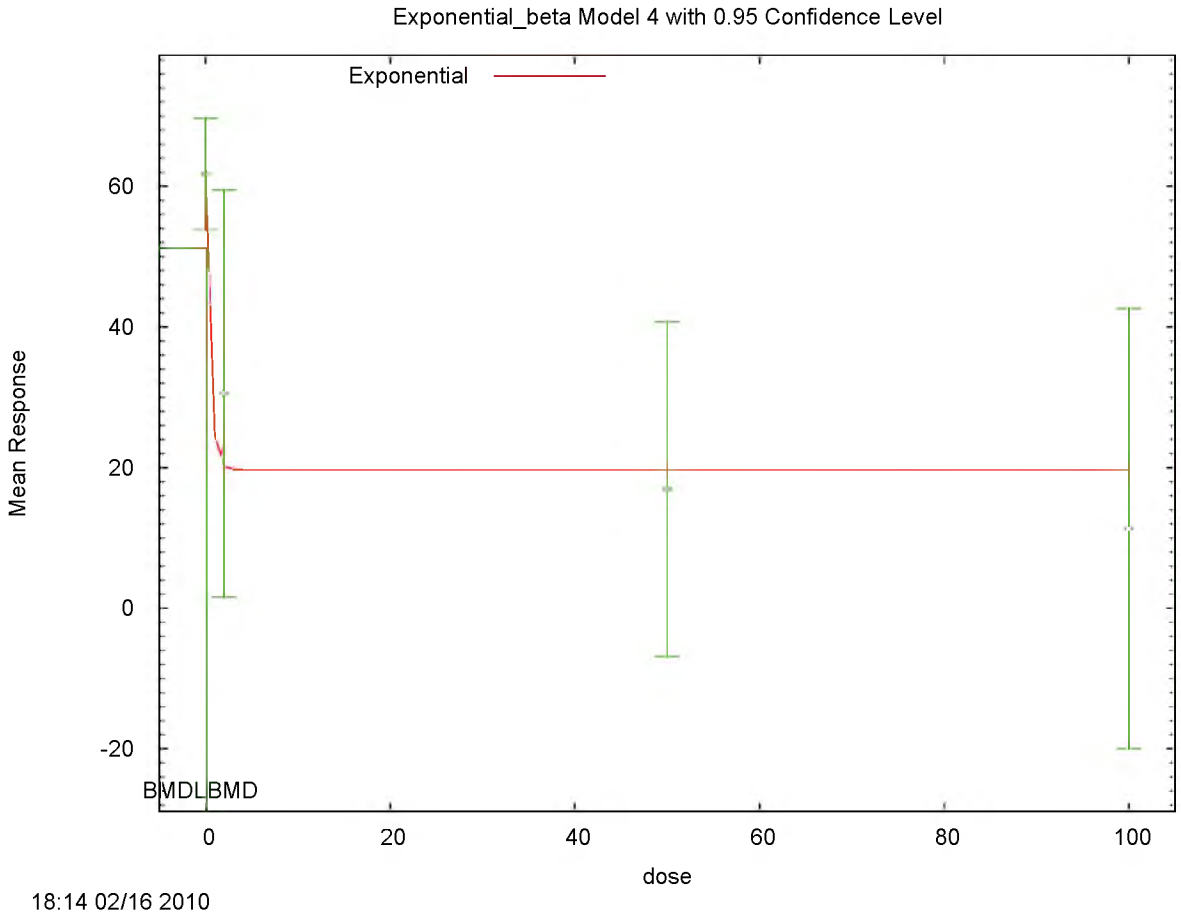
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

              BMD =     0.135296

             BMDL =    0.0835054
```

E.3.24.3. *Figure for Selected Model: Exponential (M4)*



E.3.24.4. *Output for Additional Model Presented: Hill, Unrestricted*

Li et al., 2006: Progesterone, 3-Day

```
 ======================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\32_Li_2006_Progest_Hill_U_1.(d)
         Gnuplot Plotting File:  C:\1\32_Li_2006_Progest_Hill_U_1.plt
                                                Tue Feb 16 18:14:41 2010
 ======================================================================

 Figure 4, 3-day progesterone
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))
```

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	7.08699
rho =	0
intercept =	61.7404
v =	-50.3835
n =	1.43997
k =	1.6159

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -k
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	intercept	v	n
lalpha	1	-0.99	-0.097	0.84	NA
rho	-0.99	1	0.13	-0.81	NA
intercept	-0.097	0.13	1	-0.43	NA
v	0.84	-0.81	-0.43	1	NA
n	NA	NA	NA	NA	NA

NA - This parameter's variance has been estimated as zero or less.
THE MODEL HAS PROBABLY NOT CONVERGED!!!

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	13.9863	NA	NA	NA
rho	-2.25026	NA	NA	NA
intercept	61.7404	NA	NA	NA
v	-42.1239	NA	NA	NA
n	2.02774	NA	NA	NA
k	1e-013	NA		

At least some variance estimates are negative.
THIS USUALLY MEANS THE MODEL HAS NOT CONVERGED!
Try again from another starting point.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	61.7	61.7	11.1	10.5	9.74e-008
2	10	30.6	19.6	40.5	38.3	0.905
50	10	16.9	19.6	33.3	38.3	-0.222
100	10	11.4	19.6	43.7	38.3	-0.683

```
Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -159.632675           5     329.265349
              A2         -151.812765           8     319.625529
              A3         -152.488175           6     316.976349
          fitted         -152.873643           5     315.747285
               R         -165.698875           2     335.397750


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              27.7722          6       0.0001037
  Test 2              15.6398          3        0.001344
  Test 3              1.35082          2          0.5089
  Test 4             0.770936          1          0.3799

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =            1

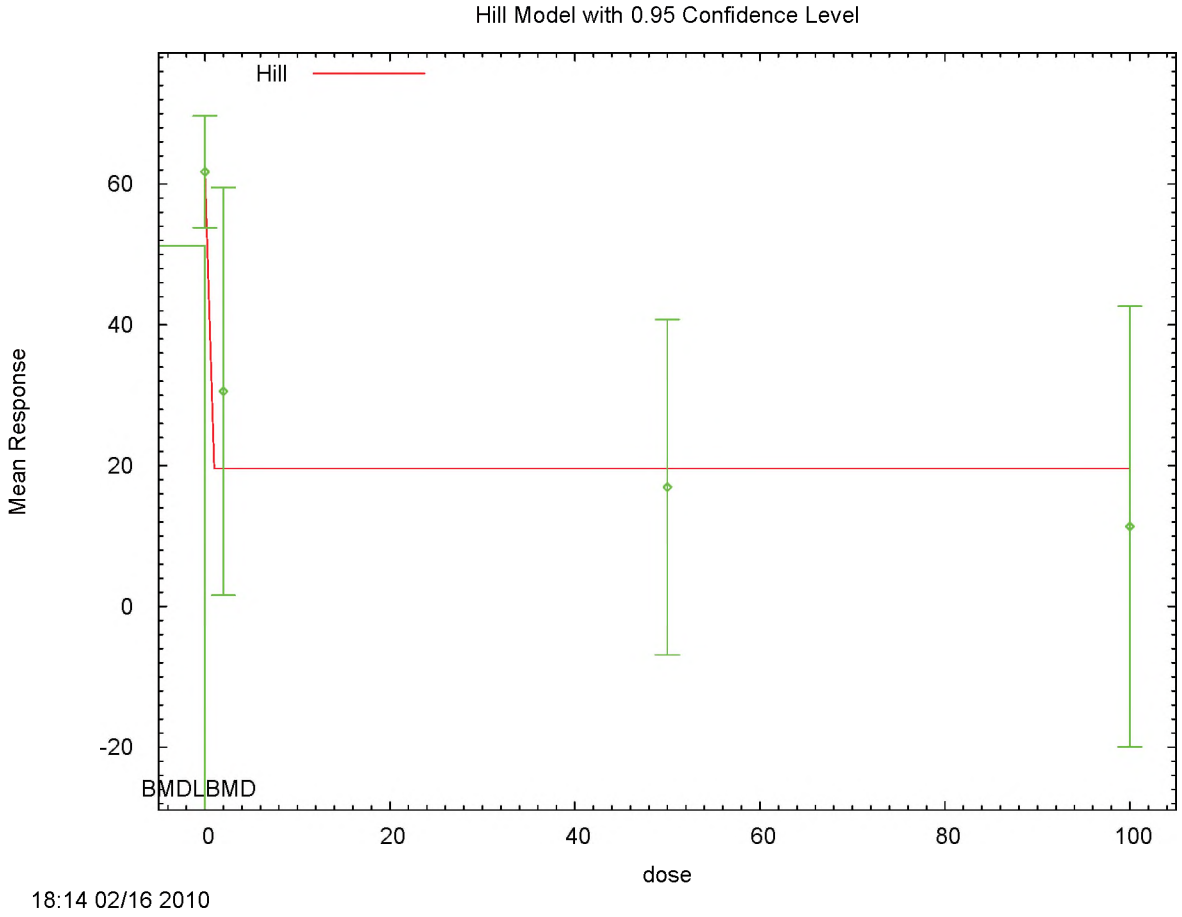
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =   5.81703e-014
```

```
BMDL =  5.81703e-014
```

E.3.24.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.3.25. Markowski et al., 2001: FR10 Run Opportunities

E.3.25.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2) [b]	**2**	**0.248**	**117.557**	**1.653E+02**	**5.025E+01**	
exponential (M3)	2	0.248	117.557	1.653E+02	5.025E+01	power hit bound (d = 1)
exponential (M4)	1	0.412	117.445	4.742E+01	1.729E-01	
exponential (M5)	0	N/A	118.918	3.178E+01	3.967E-05	
Hill	0	N/A	118.918	2.348E+01	6.728E-06	
linear	2	0.190	118.089	2.081E+02	1.051E+02	
polynomial, 3-degree	2	0.190	118.089	2.081E+02	1.051E+02	
power	2	0.190	118.089	2.081E+02	1.051E+02	power bound hit (power = 1)
power, unrestricted	1	0.238	118.164	9.153E+01	5.911E-07	unrestricted (power = 0.237)

[a] Constant variance model selected ($p = 0.1719$)

[b] Best-fitting model, BMDS output presented in this appendix

E.3.25.2. *Output for Selected Model: Exponential (M2)*

Markowski et al., 2001: FR10 Run Opportunities

```
 ======================================================================
          Exponential Model. (Version: 1.61;  Date: 7/24/2009)
          Input Data File: C:\1\33_Mark_2001_FR10opp_ExpCV_1.(d)
          Gnuplot Plotting File:
                                                  Tue Feb 16 18:15:26 2010
 ======================================================================

 Table 3
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the response function by Model:
       Model 2:     Y[dose] = a * exp{sign * b * dose}
       Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
       Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
       Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

      Note: Y[dose] is the median response for exposure = dose;
            sign = +1 for increasing trend in data;
            sign = -1 for decreasing trend.

       Model 2 is nested within Models 3 and 4.
       Model 3 is nested within Model 5.
       Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2
lnalpha	3.5321
rho(S)	0
a	6.98169
b	0.00309891
c	0
d	1

(S) = Specified

Parameter Estimates

Variable	Model 2
lnalpha	3.64823
rho	0
a	11.9443
b	0.0044262
c	0
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	7	13.29	8.65
20	4	11.25	5.56
60	6	5.75	3.53
180	7	7	6.01

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	11.94	6.197	0.5745
20	10.93	6.197	0.1025
60	9.158	6.197	-1.347
180	5.385	6.197	0.6897

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                  Model      Log(likelihood)      DF         AIC
                 -------    -----------------    ----   ------------
                     A1       -54.38526           5      118.7705
                     A2       -51.88568           8      119.7714
                     A3       -54.38526           5      118.7705
                      R       -57.45429           2      118.9086
                      2       -55.77871           3      117.5574


Additive constant for all log-likelihoods =     -22.05.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does Model 2 fit the data? (A3 vs. 2)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                       11.14             6            0.08423
  Test 2                       4.999             3             0.1719
  Test 3                       4.999             3             0.1719
  Test 4                       2.787             2             0.2482


  The p-value for Test 1 is greater than .05.  There may not be a
  diffence between responses and/or variances among the dose levels
  Modelling the data with a dose/response curve may not be appropriate.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 4 is greater than .1.  Model 2 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

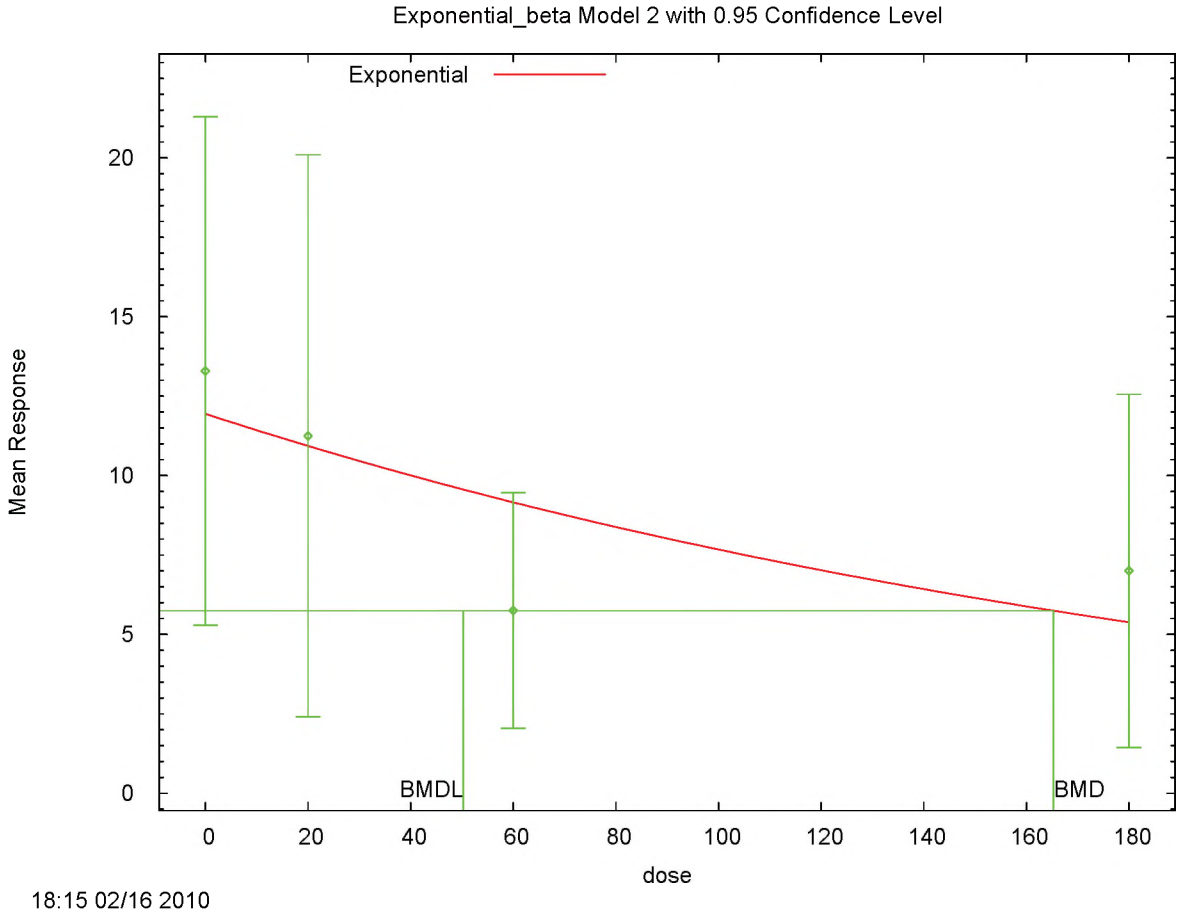
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      165.284

              BMDL =      50.2488
```

E.3.25.3. *Figure for Selected Model: Exponential (M2)*



E.3.26. Markowski et al., 2001: FR2 Revolutions

E.3.26.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	0.192	217.636	1.627E+02	5.807E+01	
exponential (M3)	2	0.192	217.636	1.627E+02	5.807E+01	power hit bound (d = 1)
exponential (M4)	1	0.298	217.415	4.668E+01	1.965E-01	
exponential (M5)	0	N/A	218.532	3.308E+01	1.193E+01	
Hill [b]	**0**	**N/A**	**218.532**	**2.364E+01**	**7.336E+00**	**n upper bound hit (n = 18)**
linear	2	0.150	218.129	1.989E+02	1.025E+02	
polynomial, 3-degree	2	0.150	218.129	1.989E+02	1.025E+02	
power	2	0.150	218.129	1.989E+02	1.025E+02	power bound hit (power = 1)
power, unrestricted [c]	1	0.160	218.302	9.101E+01	1.800E-13	unrestricted (power = 0.272)

[a] Constant variance model selected ($p = 0.1092$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.26.2. *Output for Selected Model: Hill*

Markowski et al., 2001: FR2 Revolutions

```
 ================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\34_Mark_2001_FR2rev_HillCV_1.(d)
          Gnuplot Plotting File:  C:\1\34_Mark_2001_FR2rev_HillCV_1.plt
                                                Tue Feb 16 18:16:03 2010
 ================================================================

 Table 3
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit
```

```
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                          alpha =      2598.74
                            rho =            0   Specified
                      intercept =       119.29
                              v =       -62.79
                              n =      1.80602
                              k =        35.85


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                    alpha    intercept            v            n            k

      alpha             1    -8.1e-009     4.5e-008      -3e-005       3e-005

  intercept    -8.1e-009            1        -0.81     -0.00013      -0.0022

          v     4.5e-008        -0.81            1       0.0002       0.0014

          n      -3e-005     -0.00013       0.0002            1           -1

          k       3e-005      -0.0022       0.0014           -1            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha          2183.85          630.425             948.245             3419.46
       intercept           119.29          17.6629             84.6713             153.909
               v         -56.5223          21.9082            -99.4615            -13.5831
               n               18          8854.08            -17335.7             17371.7
               k          21.6708          855.263            -1654.61             1697.95


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     7        119          119         69.9         46.7      2.74e-008
   20     4        109          108           61         46.7      8.42e-010
   60     6       56.5         62.8         31.2         46.7         -0.329
  180     7       68.1         62.8         33.2         46.7          0.304

Degrees of freedom for Test A3 vs fitted <= 0


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
```

```
            Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2
        Model A3 uses any fixed variance parameters that
        were specified by the user

 Model  R:         Yi = Mu + e(i)
             Var{e(i)} = Sigma^2


                        Likelihoods of Interest

              Model      Log(likelihood)   # Param's      AIC
                A1         -104.165520            5     218.331040
                A2         -101.140174            8     218.280349
                A3         -104.165520            5     218.331040
            fitted         -104.266162            5     218.532324
                 R         -107.599268            2     219.198536


                     Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                       Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              12.9182          6         0.04435
   Test 2              6.05069          3          0.1092
   Test 3              6.05069          3          0.1092
   Test 4             0.201283          0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0.  The Chi-Square
     test for fit is not valid


        Benchmark Dose Computation

Specified effect =            1

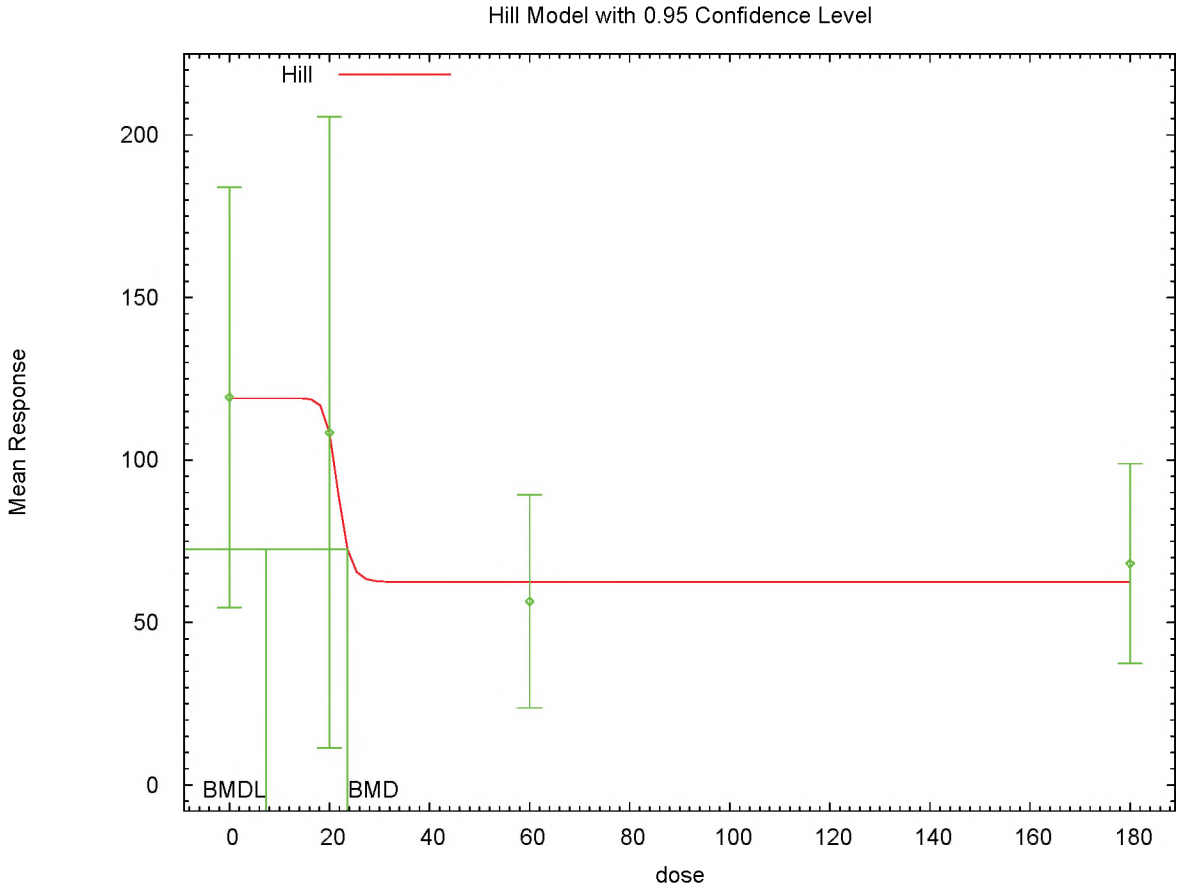
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        23.6366

            BMDL =       7.33648
```

E.3.26.3. *Figure for Selected Model: Hill*



E.3.26.4. *Output for Additional Model Presented: Power, Unrestricted*

Markowski et al., 2001: FR2 Revolutions

```
 ================================================================
          Power Model. (Version: 2.15;  Date: 04/07/2008)
          Input Data File: C:\1\34_Mark_2001_FR2rev_PowerCV_U_1.(d)
          Gnuplot Plotting File:  C:\1\34_Mark_2001_FR2rev_PowerCV_U_1.plt
                                                Tue Feb 16 18:16:04 2010
 ================================================================

 Table 3
 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the response function is:

    Y[dose] = control + slope * dose^power


    Dependent variable = Mean
    Independent variable = Dose
    rho is set to 0
    The power is not restricted
    A constant variance model is fit
```

```
Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                         alpha =      2598.74
                           rho =            0   Specified
                       control =       119.29
                         slope =     -1.79436
                         power =     0.708231


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha      control        slope        power

    alpha             1     9.7e-009    -1.9e-008    -1.6e-008

  control     9.7e-009            1        -0.49        -0.28

    slope    -1.9e-008        -0.49            1         0.96

    power    -1.6e-008        -0.28         0.96            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         alpha             2351          678.674            1020.82             3681.17
       control          120.074          18.0837            84.6305             155.517
         slope         -14.1965          22.2073            -57.722              29.329
         power          0.27229         0.301344          -0.318334            0.862913


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

   0        7        119          120         69.9         48.5        -0.0428
  20        4        109           88           61         48.5          0.846
  60        6       56.5         76.8         31.2         48.5          -1.02
 180        7       68.1         61.7         33.2         48.5          0.352


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
```

were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-104.165520	5	218.331040
A2	-101.140174	8	218.280349
A3	-104.165520	5	218.331040
fitted	-105.151136	4	218.302271
R	-107.599268	2	219.198536

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	12.9182	6	0.04435
Test 2	6.05069	3	0.1092
Test 3	6.05069	3	0.1092
Test 4	1.97123	1	0.1603

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

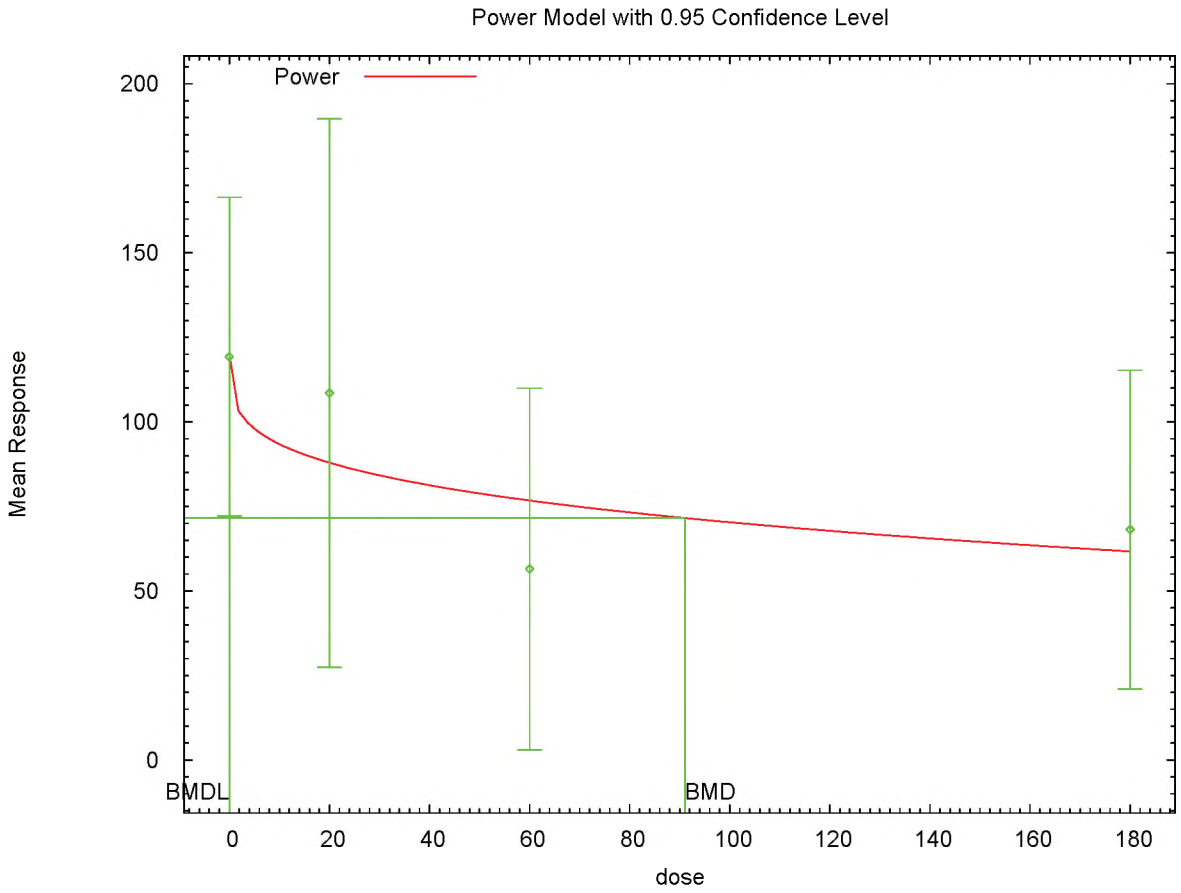
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 91.0145

BMDL = 1.8e-013

E.3.26.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.3.27. Markowski et al., 2001: FR5 Run Opportunities

E.3.27.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	2	0.149	133.830	9.491E+01	4.324E+01	
exponential (M3)	2	0.149	133.830	9.491E+01	4.324E+01	power hit bound (d = 1)
exponential (M4)	1	0.303	133.087	2.961E+01	9.356E+00	
exponential (M5)	0	N/A	134.032	2.871E+01	1.226E+01	
Hill [b]	**1**	**0.939**	**132.032**	**2.214E+01**	**1.117E+01**	**n upper bound hit (n = 18)**
linear	2	0.091	134.825	1.349E+02	8.118E+01	
polynomial, 3-degree	2	0.091	134.825	1.349E+02	8.118E+01	
power	2	0.091	134.825	1.349E+02	8.118E+01	power bound hit (power = 1)
power, unrestricted [c]	1	0.133	134.281	3.721E+01	1.439E-07	unrestricted (power = 0.336)

[a] Constant variance model selected ($p = 0.2262$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.27.2. *Output for Selected Model: Hill*

Markowski et al., 2001: FR5 Run Opportunities

```
 ================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\35_Mark_2001_FR5opp_HillCV_1.(d)
          Gnuplot Plotting File:  C:\1\35_Mark_2001_FR5opp_HillCV_1.plt
                                                Tue Feb 16 18:16:39 2010
 ================================================================

 Table 3
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit
```

```
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =      77.4849
                            rho =            0   Specified
                      intercept =        26.14
                              v =       -13.34
                              n =      2.36002
                              k =      35.0654


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -n
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                    alpha    intercept            v            k

      alpha             1    -3.6e-009     9.8e-009     3.6e-008

  intercept    -3.6e-009            1        -0.81        -0.51

          v     9.8e-009        -0.81            1         0.36

          k     3.6e-008        -0.51         0.36            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha          64.5863          18.6445             28.0438             101.129
       intercept            26.14          3.03753             20.1865             32.0935
               v         -13.1569           3.7676            -20.5413            -5.77257
               n               18               NA
               k          21.5963          2.68136             16.3409             26.8517

 NA - Indicates that this parameter has hit a bound
      implied by some inequality constraint and thus
      has no standard error.


      Table of Data and Estimated Values of Interest

  Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------      ---   --------     --------   -----------  -----------   ----------

     0      7       26.1         26.1         12.3         8.04      1.02e-008
    20      4       23.5         23.5         7.04         8.04     -1.39e-007
    60      6       12.8           13         6.17         8.04        -0.0558
   180      7       13.1           13         7.14         8.04         0.0517


  Model Descriptions for likelihoods calculated


  Model A1:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
```

```
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1          -62.013133            5     134.026266
              A2          -59.839035            8     135.678070
              A3          -62.013133            5     134.026266
           fitted         -62.016024            4     132.032049
               R          -67.530040            2     139.060081


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1               15.382          6         0.01748
   Test 2               4.3482          3          0.2262
   Test 3               4.3482          3          0.2262
   Test 4            0.0057833          1          0.9394

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =            1

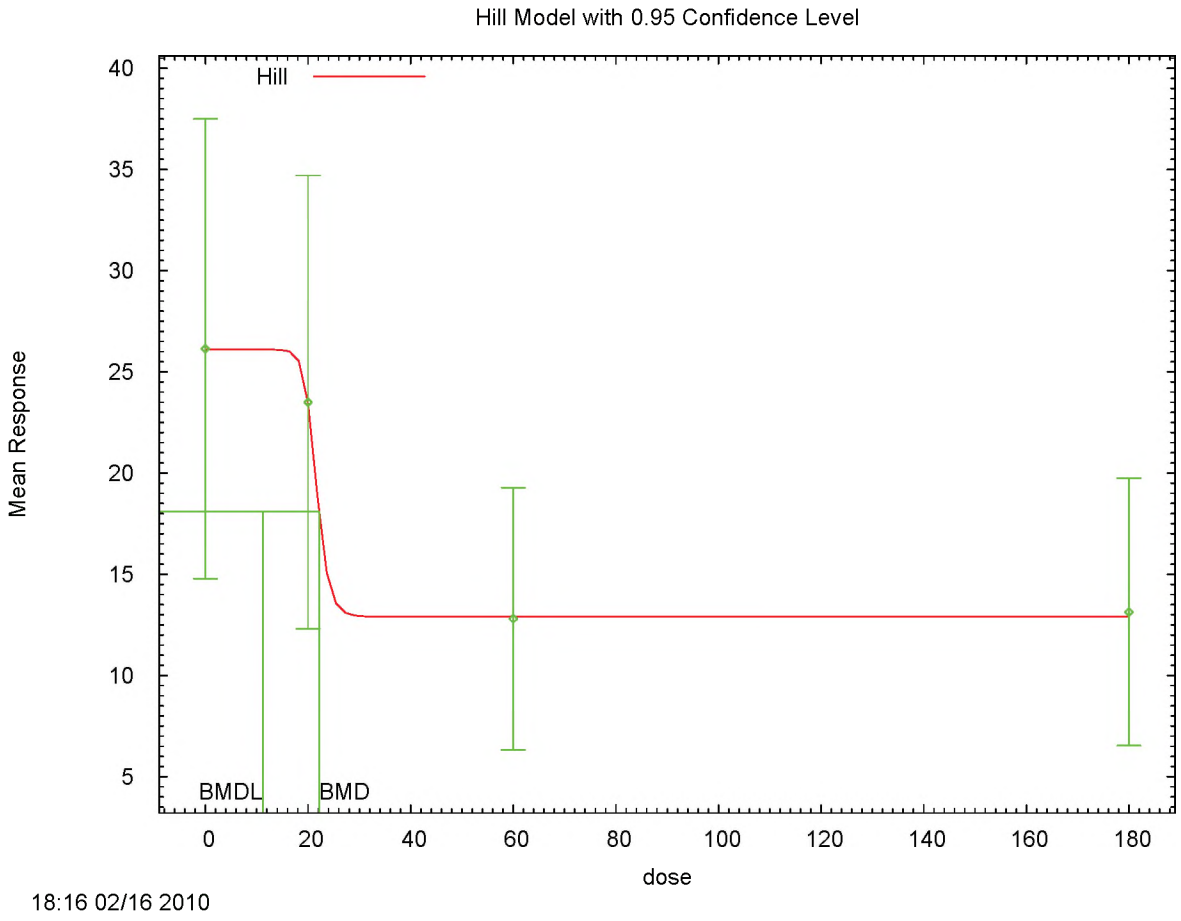
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        22.144

            BMDL =       11.165
```

E.3.27.3. *Figure for Selected Model: Hill*



E.3.27.4. *Output for Additional Model Presented: Power, Unrestricted*

Markowski et al., 2001: FR5 Run Opportunities

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\1\35_Mark_2001_FR5opp_PwrCV_U_1.(d)
         Gnuplot Plotting File:  C:\1\35_Mark_2001_FR5opp_PwrCV_U_1.plt
                                                Tue Feb 16 18:16:40 2010
 ================================================================

 Table 3
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The power is not restricted
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial Parameter Values
                        alpha =      77.4849
                          rho =            0   Specified
                      control =        26.14
                        slope =     -0.39517
                        power =     0.725538


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                 alpha      control        slope        power

    alpha            1     7.4e-009     4.3e-008     4.8e-008

  control     7.4e-009            1        -0.51        -0.34

    slope     4.3e-008        -0.51            1         0.97

    power     4.8e-008        -0.34         0.97            1


                                Parameter Estimates

                                                    95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         alpha          70.9323          20.4764             30.7993             111.065
       control          26.3567          3.13032             20.2213              32.492
         slope         -2.49841          3.16984            -8.71118             3.71437
         power         0.336003         0.242031           -0.138368            0.810375
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	7	26.1	26.4	12.3	8.42	-0.0681
20	4	23.5	19.5	7.04	8.42	0.945
60	6	12.8	16.5	6.17	8.42	-1.07
180	7	13.1	12.1	7.14	8.42	0.341

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-62.013133	5	134.026266
A2	-59.839035	8	135.678070
A3	-62.013133	5	134.026266
fitted	-63.140714	4	134.281428
R	-67.530040	2	139.060081

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	15.382	6	0.01748
Test 2	4.3482	3	0.2262
Test 3	4.3482	3	0.2262
Test 4	2.25516	1	0.1332

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

```
The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


                Benchmark Dose Computation

Specified effect =             1

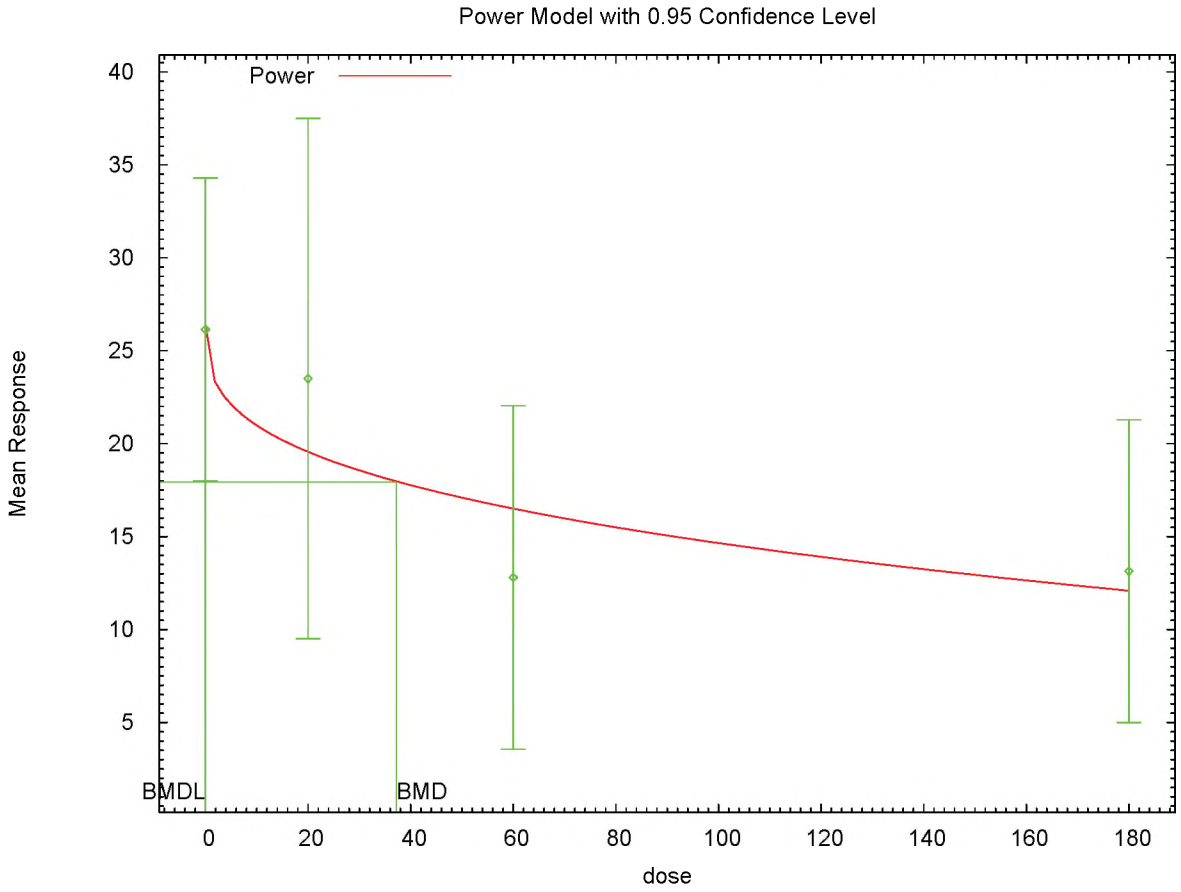
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD = 37.2131


             BMDL = 1.43926e-007
```

E.3.27.5. *Figure for Additional Model Presented: Power, Unrestricted*



18:16 02/16 2010

E.3.28. Miettinen et al., 2006: Cariogenic Lesions, Pups

E.3.28.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	3	0.345	162.699	7.505E+01	4.086E+01	power bound hit (power = 1)
logistic	3	0.315	162.909	8.991E+01	5.250E+01	
log-logistic [a]	**3**	**0.506**	**161.767**	**3.130E+01**	**1.054E+01**	**slope bound hit (slope = 1)**
log-probit	3	0.257	163.393	1.390E+02	6.729E+01	slope bound hit (slope = 1)
multistage, 4-degree	3	0.345	162.699	7.505E+01	4.086E+01	final ß = 0
probit	3	0.299	163.031	9.941E+01	6.208E+01	
Weibull	3	0.345	162.699	7.505E+01	4.086E+01	power bound hit (power = 1)
gamma, unrestricted	2	0.797	161.805	1.591E-02	1.335E-240	unrestricted (power = 0.184)
log-logistic, unrestricted [b]	2	0.723	161.998	3.713E-01	error	unrestricted (slope = 0.403)
log-probit, unrestricted	2	0.726	161.987	5.098E-01	error	unrestricted (slope = 0.25)
Weibull, unrestricted	2	0.761	161.897	1.174E-01	error	unrestricted (power = 0.281)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.3.28.2. *Output for Selected Model: Log-Logistic*

Miettinen et al., 2006: Cariogenic Lesions, Pups

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\36_Miet_2006_Cariogenic_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\36_Miet_2006_Cariogenic_LogLogistic_1.plt
                                          Tue Feb 16 18:17:16 2010
 ================================================================

 Table 2 converting the percentage into the number of animals, and control is Control II from the
study. Dose is in ng per kg and is from Table 1
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
```

```
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                  Default Initial Parameter Values
                     background =     0.595238
                      intercept =     -5.52519
                          slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             background    intercept

background            1        -0.64

 intercept        -0.64            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background         0.658158            *               *                   *
      intercept         -5.64068            *               *                   *
          slope                1            *               *                   *

* - Indicates that this value is not calculated.



                         Analysis of Deviance Table

        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -77.6769         5
   Fitted model         -78.8837         2       2.41374      3          0.4911
  Reduced model         -83.2067         1       11.0597      4          0.0259

           AIC:          161.767


                                Goodness  of  Fit
                                                                    Scaled
      Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
    0.0000     0.6582        27.643    25.000          42       -0.860
   30.0000     0.6911        20.041    23.000          29        1.189
  100.0000     0.7477        18.693    19.000          25        0.141
  300.0000     0.8345        20.027    20.000          24       -0.015
 1000.0000     0.9249        29.596    29.000          32       -0.400

 Chi^2 = 2.33      d.f. = 3        P-value = 0.5062


   Benchmark Dose Computation
```

```
Specified effect =           0.1

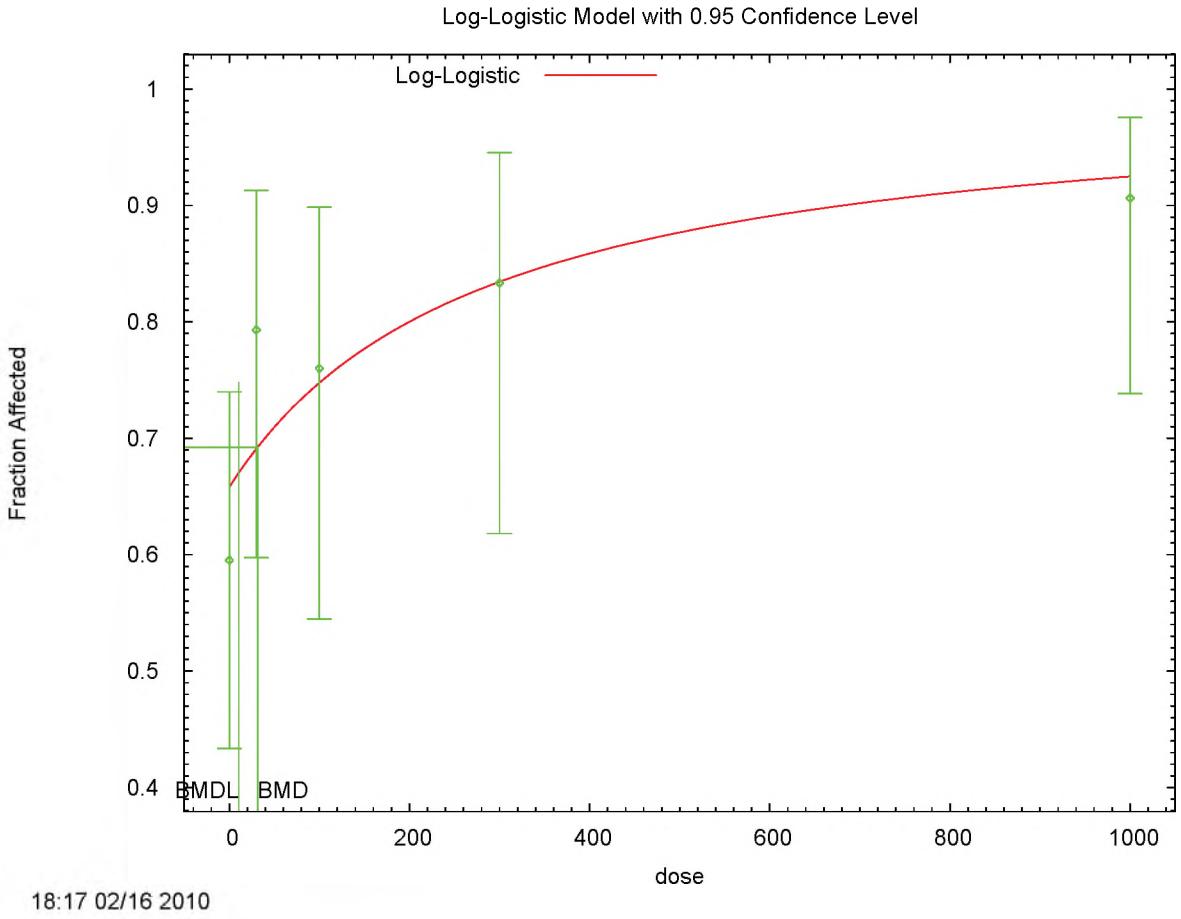
Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       31.2951

            BMDL =       10.5354
```

E.3.28.3. *Figure for Selected Model: Log-Logistic*



E.3.28.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

Miettinen et al., 2006: Cariogenic Lesions, Pups

```
================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\1\36_Miet_2006_Cariogenic_LogLogistic_U_1.(d)
        Gnuplot Plotting File:  C:\1\36_Miet_2006_Cariogenic_LogLogistic_U_1.plt
                                          Tue Feb 16 18:17:18 2010
================================================================
```

```
 Table 2 converting the percentage into the number of animals, and control is Control II from the
study. Dose is in ng per kg and is from Table 1
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =     0.595238
                     intercept =     -1.68849
                         slope =     0.382632


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.41         0.24

 intercept        -0.41            1        -0.96

     slope         0.24        -0.96            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background         0.597778            *               *                   *
      intercept         -1.79836            *               *                   *
          slope         0.402606            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -77.6769         5
   Fitted model         -77.9988         3      0.643944      2          0.7247
  Reduced model         -83.2067         1       11.0597      4          0.0259

           AIC:         161.998


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ----------------------------------------------------------------------
    0.0000     0.5978        25.107    25.000          42        -0.034
   30.0000     0.7564        21.936    23.000          29         0.460
```

```
  100.0000     0.8045        20.112    19.000          25       -0.561
  300.0000     0.8480        20.351    20.000          24       -0.200
 1000.0000     0.8905        28.495    29.000          32        0.286

 Chi^2 = 0.65      d.f. = 2        P-value = 0.7227


   Benchmark Dose Computation

Specified effect =            0.1

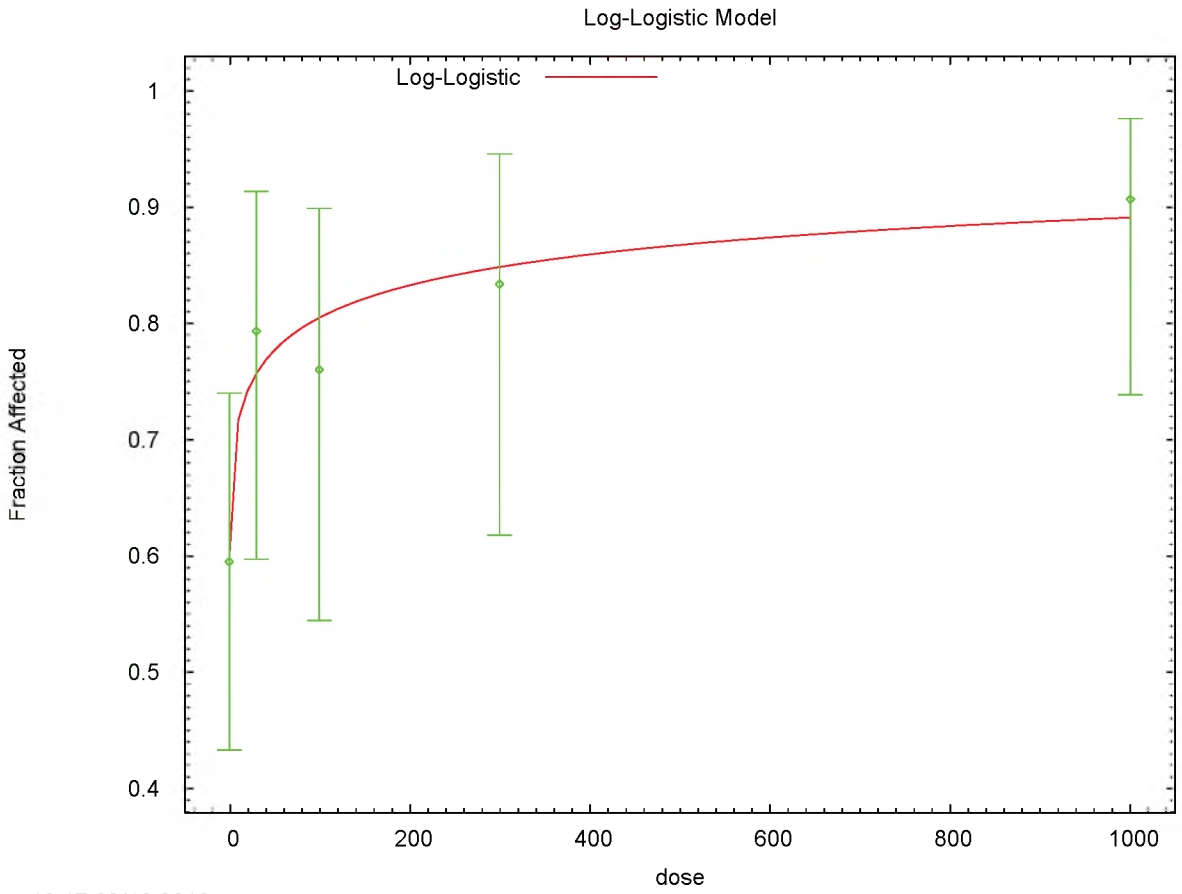
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.371315

           Benchmark dose computation failed.  Lower limit includes zero.
```

E.3.28.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.3.29. Murray et al., 1979: Fertility in F2 Generation

E.3.29.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	0	N/A	61.729	7.016E+00	1.698E+00	
logistic	1	0.072	60.497	4.007E+00	2.836E+00	negative intercept (intercept = -2.53)
log-logistic	0	N/A	61.729	7.902E+00	1.584E+00	
multistage, 1-degree	1	0.053	61.644	2.380E+00	1.320E+00	
multistage, 2-degree [a]	**1**	**0.094**	**59.935**	**4.548E+00**	**1.635E+00**	
probit	1	0.070	60.613	3.707E+00	2.615E+00	negative intercept (intercept = -1.446)
Weibull	0	N/A	61.729	8.115E+00	1.698E+00	
log-probit, unrestricted	0	N/A	61.729	6.373E+00	1.503E+00	unrestricted (slope = 2.306)

[a] Best-fitting model, BMDS output presented in this appendix

E.3.29.2. *Output for Selected Model: Multistage, 2-Degree*

Murray et al., 1979: Fertility in F2 Generation

```
 ================================================================
         Multistage Model. (Version: 3.0;  Date: 05/16/2008)
         Input Data File: C:\1\Murray_1979_fert_index_f2_Multi2_1.(d)
         Gnuplot Plotting File:  C:\1\Murray_1979_fert_index_f2_Multi2_1.plt
                                                Tue Feb 16 20:08:06 2010
 ================================================================

 Table 1 but expressed as number of dams who do not produce offspring
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2
```

```
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =    0.0624181
                        Beta(1) =            0
                        Beta(2) =   0.00532688


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(1)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(2)

Background            1        -0.44

   Beta(2)        -0.44            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0772201            *               *                   *
        Beta(1)                0            *               *                   *
        Beta(2)       0.00509404            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
      Full model        -25.8194         3
    Fitted model        -27.9673         2       4.29584      1         0.03821
   Reduced model        -34.0009         1        16.363      2       0.0002798

           AIC:         59.9347


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ---------------------------------------------------------------------
    0.0000     0.0772         2.471     4.000          32         1.013
    1.0000     0.0819         1.638     0.000          20        -1.336
   10.0000     0.4455         8.911     9.000          20         0.040

 Chi^2 = 2.81      d.f. = 1        P-value = 0.0936


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

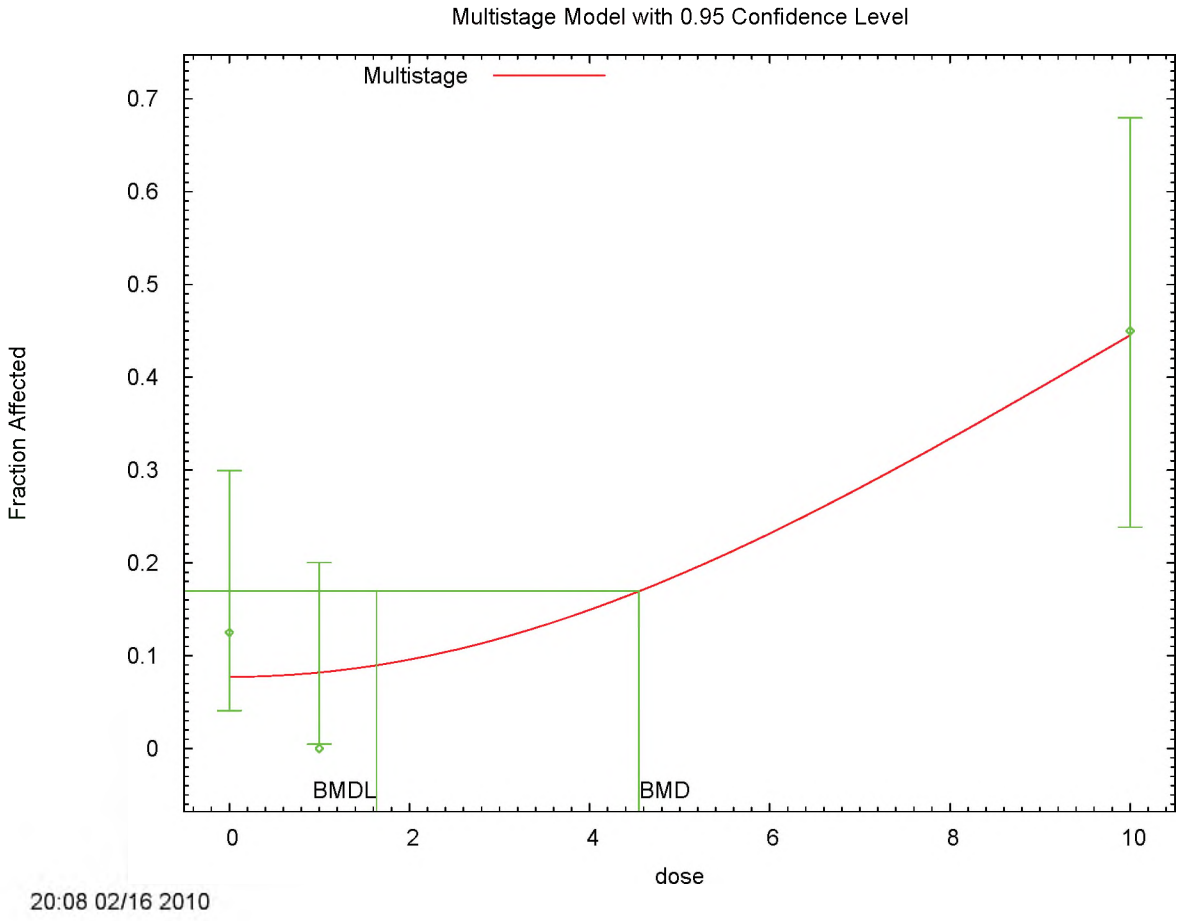
             BMD =        4.54787
```

```
                BMDL =        1.63487

                BMDU =        6.79105

Taken together, (1.63487, 6.79105) is a 90      % two-sided confidence
interval for the BMD
```

E.3.29.3. *Figure for Selected Model: Multistage, 2-Degree*



E.3.30. National Toxicology Program, 1982: Toxic Hepatitis, Male Mice

E.3.30.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	1	0.026	113.097	1.552E+01	5.155E+00	
logistic	2	0.093	110.712	1.769E+01	1.383E+01	negative intercept (intercept = -3.087)
log-logistic	1	0.027	113.093	1.499E+01	6.628E+00	
log-probit	1	0.027	113.111	1.360E+01	7.237E+00	
multistage, 3-degree [a]	**1**	**0.028**	**112.555**	**1.488E+01**	**4.676E+00**	
probit	2	0.088	110.696	1.564E+01	1.261E+01	negative intercept (intercept = -1.731)
Weibull	1	0.026	113.056	1.619E+01	4.903E+00	

[a] Best-fitting model, BMDS output presented in this appendix

E.3.30.2. *Output for Selected Model: Multistage, 3-Degree*

National Toxicology Program, 1982: Toxic Hepatitis, Male Mice

```
 ================================================================
         Multistage Model. (Version: 3.0;  Date: 05/16/2008)
         Input Data File: C:\1\37_NTP_1982_ToxHep_Multi3_1.(d)
         Gnuplot Plotting File:  C:\1\37_NTP_1982_ToxHep_Multi3_1.plt
                                          Tue Feb 16 18:17:51 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0525767
Beta(1) = 0.00243254
Beta(2) = 0
Beta(3) = 5.29052e-006

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(1)	Beta(3)
Background	1	-0.69	0.66
Beta(1)	-0.69	1	-0.98
Beta(3)	0.66	-0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0383474	*	*	*
Beta(1)	0.00605732	*	*	*
Beta(2)	0	*	*	*
Beta(3)	4.60855e-006	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-51.0633	4			
Fitted model	-53.2776	3	4.42854	1	0.03534
Reduced model	-121.743	1	141.358	3	<.0001

AIC: 112.555

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0383	2.799	1.000	73	-1.097
1.4000	0.0465	2.278	5.000	49	1.847
7.1000	0.0803	3.937	3.000	49	-0.492
71.0000	0.8798	43.990	44.000	50	0.004

Chi^2 = 4.86 d.f. = 1 P-value = 0.0275

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

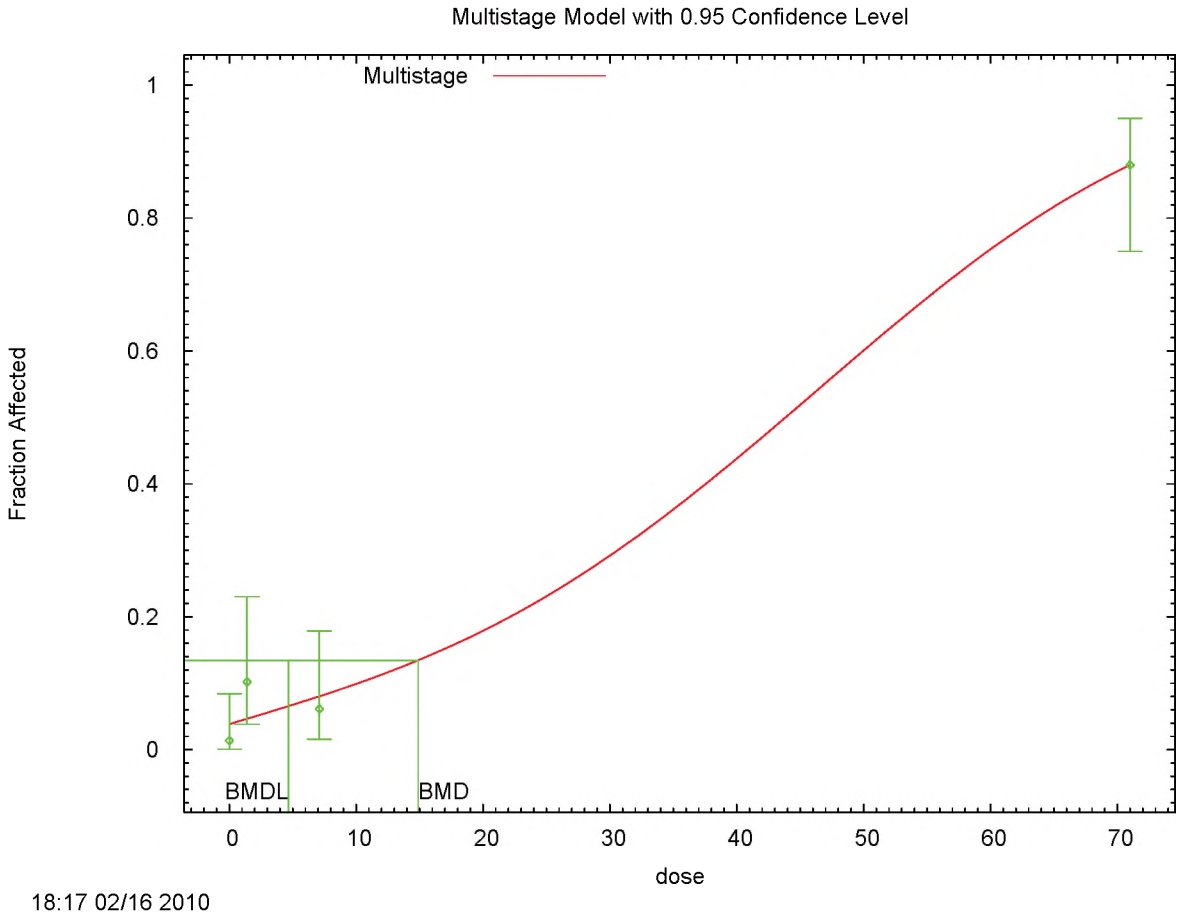
```
                BMD =        14.8848

               BMDL =        4.67636

               BMDU =        28.8293

Taken together, (4.67636, 28.8293) is a 90     % two-sided confidence
interval for the BMD
```

E.3.30.3. *Figure for Selected Model: Multistage, 3-Degree*



E.3.31. National Toxicology Program, 2006: Alveolar Metaplasia

E.3.31.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	4	<0.001	340.127	2.240E+00	1.791E+00	power bound hit (power = 1)
logistic	4	<0.001	358.346	4.997E+00	4.149E+00	negative intercept (intercept = -0.687)
log-logistic [a]	**4**	**0.409**	**312.970**	**6.644E-01**	**5.041E-01**	**slope bound hit (slope = 1)**
log-probit	4	<0.001	340.296	3.291E+00	2.517E+00	slope bound hit (slope = 1)
multistage, 5-degree	4	<0.001	340.127	2.240E+00	1.791E+00	final ß = 0
probit	4	<0.001	362.181	5.656E+00	4.810E+00	negative intercept (intercept = -0.381)
Weibull	4	<0.001	340.127	2.240E+00	1.791E+00	power bound hit (power = 1)
gamma, unrestricted	3	0.407	314.135	2.211E-02	8.081E-04	unrestricted (power = 0.297)
log-logistic, unrestricted [b]	3	0.739	312.487	3.062E-01	7.972E-02	unrestricted (slope = 0.785)
log-probit, unrestricted	3	0.727	312.543	3.316E-01	8.968E-02	unrestricted (slope = 0.471)
Weibull, unrestricted	3	0.586	313.176	9.000E-02	1.341E-02	unrestricted (power = 0.465)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.3.31.2. *Output for Selected Model: Log-Logistic*

National Toxicology Program, 2006: Alveolar Metaplasia

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\40_NTP_2006_AlvMeta_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\40_NTP_2006_AlvMeta_LogLogistic_1.plt
                                              Tue Feb 16 18:19:30 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
```

```
   Slope parameter is restricted as slope >= 1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0377358
                     intercept =     -2.03745
                         slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1         -0.4

 intercept         -0.4            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0448753            *               *                   *
      intercept         -1.78837            *               *                   *
          slope                1            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -152.615         6
   Fitted model        -154.485         2        3.7393      4          0.4424
  Reduced model        -216.802         1       128.374      5         <.0001

           AIC:          312.97


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ---------------------------------------------------------------------
    0.0000     0.0449         2.378     2.000          53       -0.251
    2.1400     0.2966        16.017    19.000          54        0.889
    7.1400     0.5647        29.928    33.000          53        0.851
   15.7000     0.7366        38.301    35.000          52       -1.039
   32.9000     0.8531        45.214    45.000          53       -0.083
   71.4000     0.9262        48.162    46.000          52       -1.147

 Chi^2 = 3.98      d.f. = 4        P-value = 0.4088


   Benchmark Dose Computation
```

```
Specified effect =           0.1

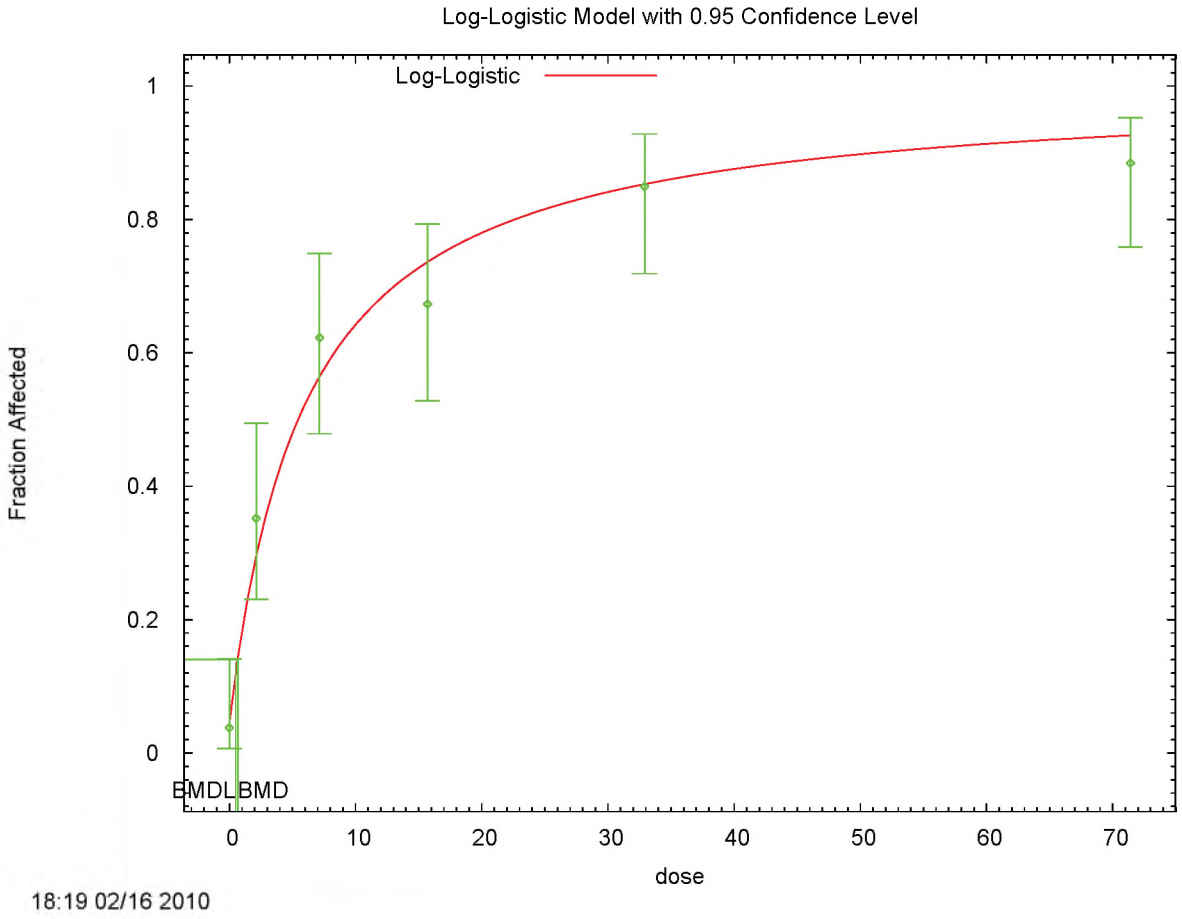
Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =      0.664411

            BMDL =      0.504109
```

E.3.31.3. *Figure for Selected Model: Log-Logistic*



E.3.31.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

National Toxicology Program, 2006: Alveolar Metaplasia

```
================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\1\40_NTP_2006_AlvMeta_LogLogistic_U_1.(d)
        Gnuplot Plotting File:  C:\1\40_NTP_2006_AlvMeta_LogLogistic_U_1.plt
                                          Tue Feb 16 18:19:31 2010
================================================================

0
```

```
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0377358
                     intercept =     -1.26694
                         slope =     0.784484


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.24         0.11

 intercept        -0.24            1         -0.9

     slope         0.11         -0.9            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0375286            *               *                   *
      intercept         -1.26811            *               *                   *
          slope         0.785033            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -152.615         6
   Fitted model        -153.244         3        1.2566      3          0.7395
  Reduced model        -216.802         1       128.374      5         <.0001

           AIC:         312.487


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
    0.0000     0.0375         1.989     2.000          53        0.008
    2.1400     0.3631        19.609    19.000          54       -0.172
    7.1400     0.5845        30.980    33.000          53        0.563
   15.7000     0.7205        37.468    35.000          52       -0.763
```

```
     32.9000      0.8207         43.498     45.000          53         0.538
     71.4000      0.8934         46.455     46.000          52        -0.204

  Chi^2 = 1.26       d.f. = 3         P-value = 0.7388


    Benchmark Dose Computation

Specified effect =            0.1

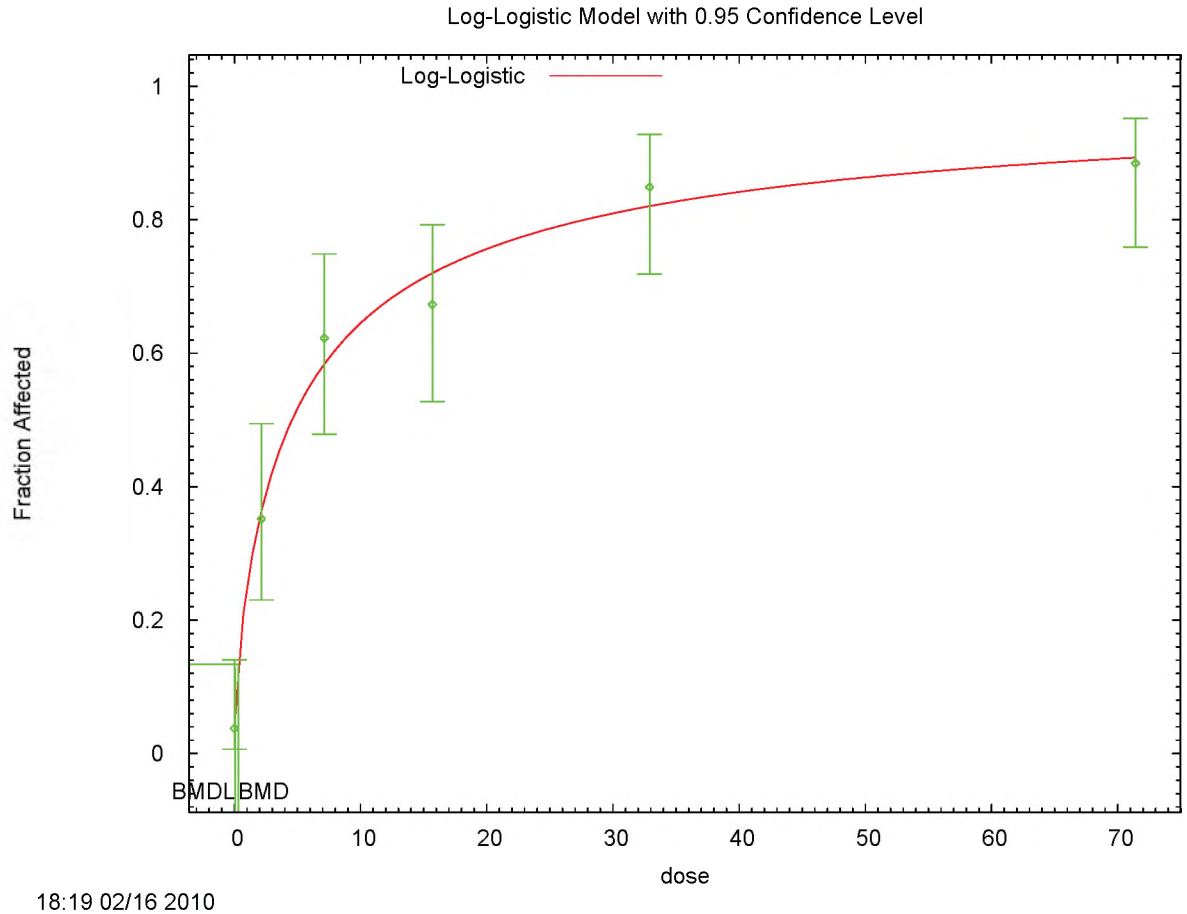
Risk Type        =       Extra risk

Confidence level =           0.95

             BMD =       0.306194

            BMDL =      0.0797223
```

E.3.31.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.3.32. National Toxicology Program, 2006: Eosinophilic Focus, Liver

E.3.32.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	4	0.367	330.457	5.676E+00	4.532E+00	power bound hit (power = 1)
logistic	4	0.167	333.343	1.258E+01	1.071E+01	negative intercept (intercept = -1.747)
log-logistic	3	0.117	334.148	4.727E+00	2.867E+00	
log-probit	4	0.084	334.683	1.078E+01	8.514E+00	
multistage, 5-degree	3	0.313	331.771	6.568E+00	4.666E+00	
probit [a]	**4**	**0.187**	**332.962**	**1.196E+01**	**1.031E+01**	**negative intercept (intercept = -1.061)**
Weibull	4	0.367	330.457	5.675E+00	4.532E+00	power bound hit (power = 1)
log-probit, unrestricted	3	0.087	334.849	4.750E+00	1.757E+00	unrestricted (slope = 0.643)

[a] Best-fitting model, BMDS output presented in this appendix

E.3.32.2. *Output for Selected Model: Probit*

National Toxicology Program, 2006: Eosinophilic Focus, Liver

```
 ================================================================
         Probit Model. (Version: 3.1;  Date: 05/16/2008)
         Input Data File: C:\1\45_NTP_2006_LivEosFoc_Probit_1.(d)
         Gnuplot Plotting File:  C:\1\45_NTP_2006_LivEosFoc_Probit_1.plt
                                              Tue Feb 16 18:25:56 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial (and Specified) Parameter Values
background = 0 Specified
intercept = -1.11935
slope = 0.0279665

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.69
slope	-0.69	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-1.06148	0.109177	-1.27546	-0.847497
slope	0.0269279	0.00327788	0.0205034	0.0333525

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-161.07	6			
Fitted model	-164.481	2	6.8221	4	0.1456
Reduced model	-202.816	1	83.4925	5	<.0001

AIC: 332.962

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1442	7.645	3.000	53	-1.816
2.1400	0.1577	8.517	8.000	54	-0.193
7.1400	0.1924	10.195	14.000	53	1.326
15.7000	0.2615	13.860	17.000	53	0.982
32.9000	0.4303	22.807	22.000	53	-0.224
71.4000	0.8054	42.688	42.000	53	-0.239

Chi^2 = 6.16 d.f. = 4 P-value = 0.1873

Benchmark Dose Computation

Specified effect = 0.1

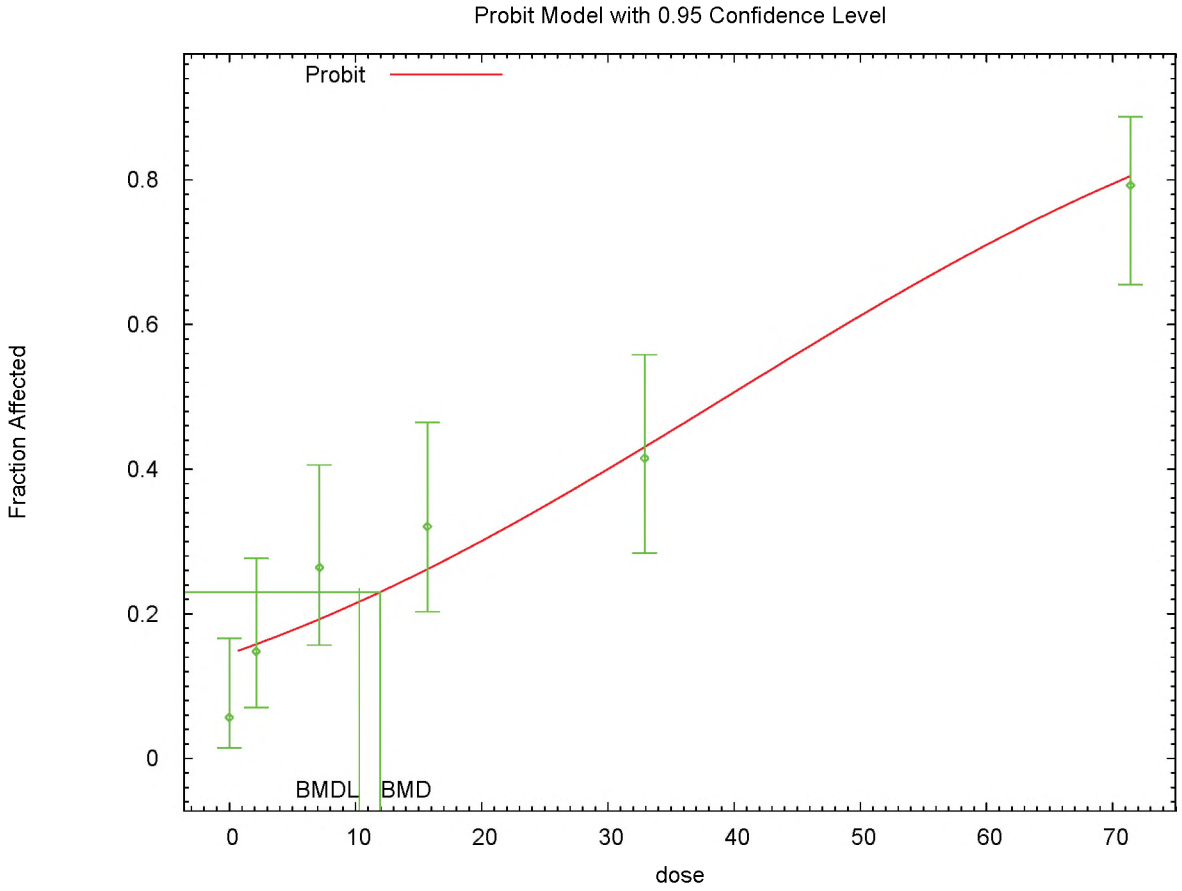
Risk Type = Extra risk

Confidence level = 0.95

BMD = 11.9584

BMDL = 10.3075

E.3.32.3. *Figure for Selected Model: Probit*



E.3.33. National Toxicology Program, 2006: Fatty Change Diffuse, Liver

E.3.33.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	4	0.668	252.294	4.224E+00	3.166E+00	
logistic	4	0.005	269.825	1.092E+01	9.292E+00	negative intercept (intercept = -2.298)
log-logistic	4	0.292	255.082	4.697E+00	3.153E+00	
log-probit	4	0.118	257.548	6.236E+00	5.204E+00	slope bound hit (slope = 1)
multistage, 5-degree	4	0.808	251.545	4.021E+00	3.250E+00	
probit	4	0.005	269.430	1.052E+01	9.068E+00	negative intercept (intercept = -1.36)
Weibull [a]	**4**	**0.679**	**252.218**	**4.252E+00**	**3.174E+00**	
log-probit, unrestricted	4	0.282	255.258	4.581E+00	3.193E+00	unrestricted (slope = 0.824)

[a] Best-fitting model, BMDS output presented in this appendix

E.3.33.2. *Output for Selected Model: Weibull*

National Toxicology Program, 2006: Fatty Change Diffuse, Liver

```
 ======================================================================
         Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
         Input Data File: C:\1\47_NTP_2006_LivFatDiff_Weibull_1.(d)
         Gnuplot Plotting File:  C:\1\47_NTP_2006_LivFatDiff_Weibull_1.plt
                                                Tue Feb 16 18:26:57 2010
 ======================================================================

 NTP_liver_fatty_change_diffuse
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = DichEff
   Independent variable = Dose
   Power parameter is restricted as power >=1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial (and Specified) Parameter Values
Background = 0.00925926
Slope = 0.00962604
Power = 1.28042

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Slope	Power
Slope	1	-0.97
Power	-0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	0.0223474	0.00951041	0.0037073	0.0409874
Power	1.07133	0.122134	0.831952	1.31071

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-122.992	6			
Fitted model	-124.109	2	2.23388	4	0.6928
Reduced model	-204.846	1	163.708	5	<.0001

AIC: 252.218

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	53	0.000
2.1400	0.0492	2.659	2.000	54	-0.414
7.1400	0.1677	8.889	12.000	53	1.144
15.7000	0.3475	18.420	17.000	53	-0.409
32.9000	0.6107	32.365	30.000	53	-0.666
71.4000	0.8851	46.909	48.000	53	0.470

Chi^2 = 2.31 d.f. = 4 P-value = 0.6785

Benchmark Dose Computation

Specified effect = 0.1

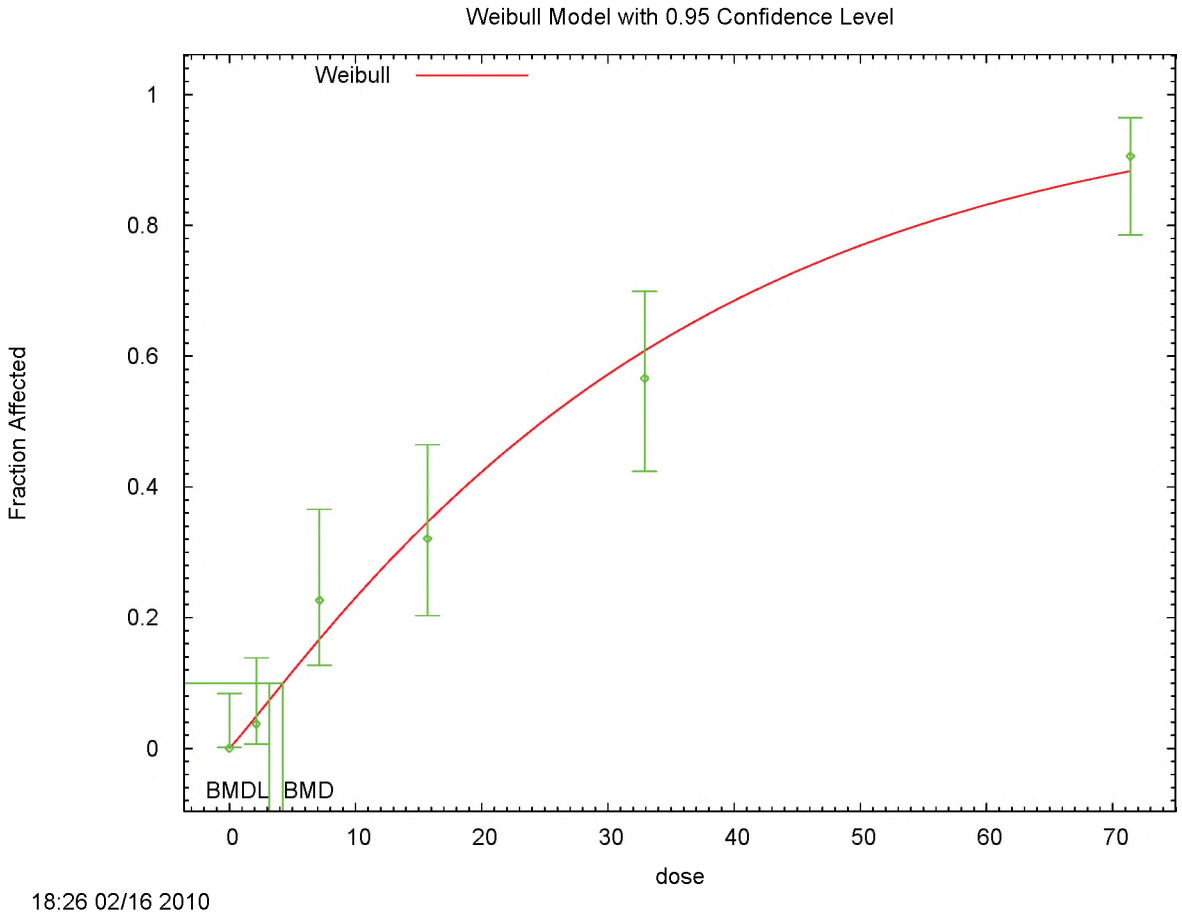
Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.25219

BMDL = 3.17375

E.3.33.3. *Figure for Selected Model: Weibull*



18:26 02/16 2010

E.3.34. National Toxicology Program, 2006: Gingival Hyperplasia, Squamous, 2 Years

E.3.34.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	4	0.012	318.867	2.295E+01	1.417E+01	power bound hit (power = 1)
logistic	4	0.008	320.908	3.594E+01	2.564E+01	negative intercept (intercept = -1.711)
log-logistic [a]	**4**	**0.015**	**317.969**	**1.838E+01**	**1.044E+01**	**slope bound hit (slope = 1)**
log-probit	4	0.003	323.633	4.313E+01	2.794E+01	slope bound hit (slope = 1)
multistage, 5-degree	4	0.012	318.867	2.295E+01	1.417E+01	final ß = 0
probit	4	0.008	320.687	3.436E+01	2.425E+01	negative intercept (intercept = -1.034)
Weibull	4	0.012	318.867	2.295E+01	1.417E+01	power bound hit (power = 1)
gamma, unrestricted	3	0.651	307.529	2.480E-01	5.096E-09	unrestricted (power = 0.199)
log-logistic, unrestricted [b]	3	0.675	307.416	3.710E-01	1.505E-07	unrestricted (slope = 0.265)
log-probit, unrestricted	3	0.688	307.354	4.688E-01	8.851E-07	unrestricted (slope = 0.156)
Weibull, unrestricted	3	0.663	307.471	3.076E-01	3.210E-08	unrestricted (power = 0.23)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.3.34.2. *Output for Selected Model: Log-Logistic*

National Toxicology Program, 2006: Gingival Hyperplasia, Squamous, 2 Years

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\42_NTP_2006_GingHypSq_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\42_NTP_2006_GingHypSq_LogLogistic_1.plt
                                              Tue Feb 16 18:20:29 2010
 ================================================================

 [insert study notes]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
```

```
   Slope parameter is restricted as slope >= 1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0188679
                     intercept =      -4.5509
                         slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.71

 intercept        -0.71            1



                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background         0.117717            *               *                   *
      intercept         -5.10866            *               *                   *
          slope                1            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -149.95         6
   Fitted model        -156.985         2       14.0696      4        0.007076
  Reduced model        -162.631         1       25.3627      5       0.0001186

           AIC:         317.969


                                 Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ---------------------------------------------------------------------
    0.0000     0.1177         6.239     1.000          53       -2.233
    2.1400     0.1290         6.965     7.000          54        0.014
    7.1400     0.1542         8.174    14.000          53        2.216
   15.7000     0.1942        10.292    13.000          53        0.940
   32.9000     0.2641        13.995    15.000          53        0.313
   71.4000     0.3837        20.335    16.000          53       -1.225

 Chi^2 = 12.38     d.f. = 4        P-value = 0.0147


   Benchmark Dose Computation
```

```
Specified effect =          0.1

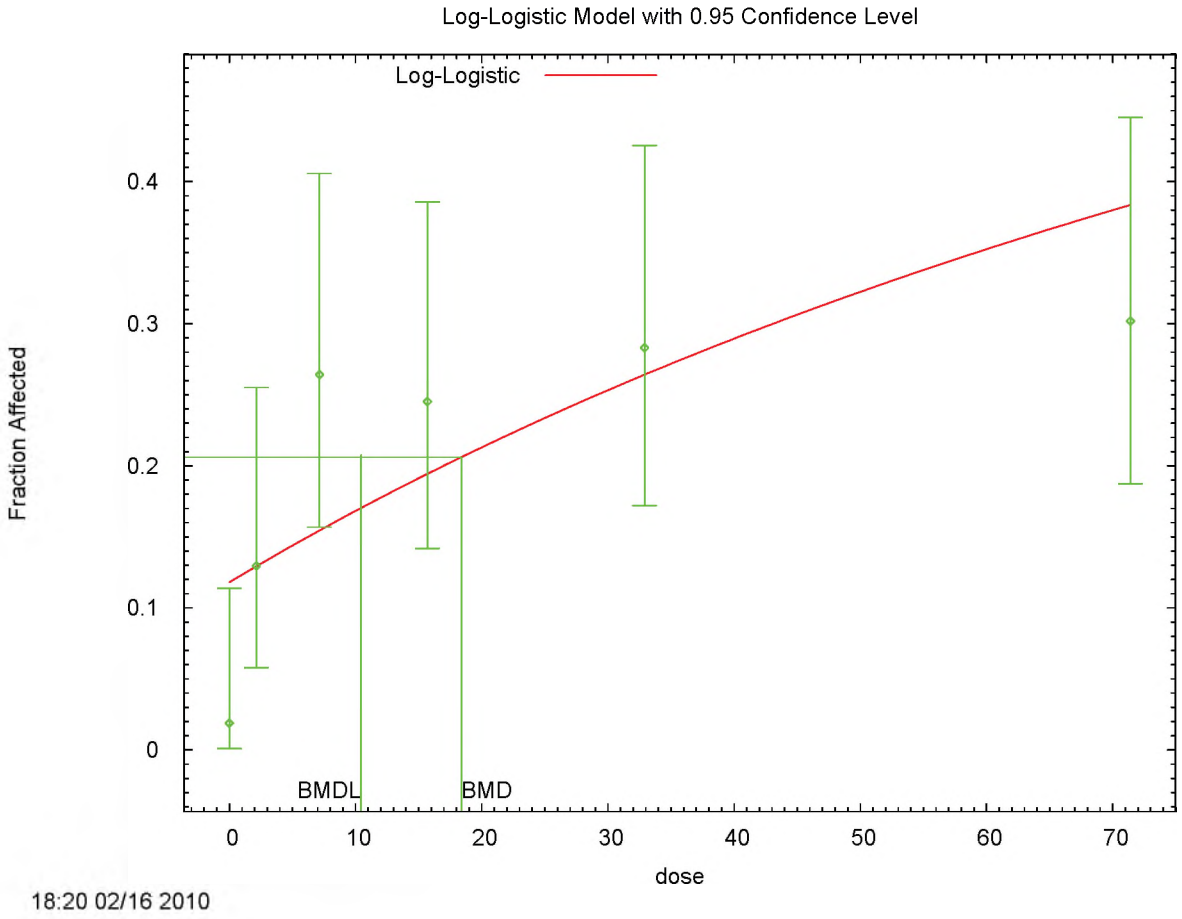
Risk Type        =     Extra risk

Confidence level =         0.95

             BMD =      18.3832

            BMDL =      10.4359
```

E.3.34.3. *Figure for Selected Model: Log-Logistic*



E.3.34.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

National Toxicology Program, 2006: Gingival Hyperplasia, Squamous, 2 Years

```
================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\1\42_NTP_2006_GingHypSq_LogLogistic_U_1.(d)
        Gnuplot Plotting File:  C:\1\42_NTP_2006_GingHypSq_LogLogistic_U_1.plt
                                          Tue Feb 16 18:20:29 2010
================================================================

[insert study notes]
```

```
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0188679
                     intercept =     -2.04571
                         slope =     0.299277


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1         -0.3         0.12

 intercept         -0.3            1        -0.91

     slope         0.12        -0.91            1


                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0185126            *               *                   *
      intercept         -1.93464            *               *                   *
          slope         0.264795            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -149.95         6
   Fitted model        -150.708         3        1.5163      3          0.6785
  Reduced model        -162.631         1       25.3627      5       0.0001186

           AIC:         307.416


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
    0.0000     0.0185         0.981     1.000          53         0.019
    2.1400     0.1659         8.959     7.000          54        -0.717
    7.1400     0.2105        11.155    14.000          53         0.959
   15.7000     0.2447        12.972    13.000          53         0.009
```

```
     32.9000      0.2806        14.873    15.000          53        0.039
     71.4000      0.3219        17.059    16.000          53       -0.311

  Chi^2 = 1.53      d.f. = 3        P-value = 0.6750


    Benchmark Dose Computation

Specified effect =            0.1

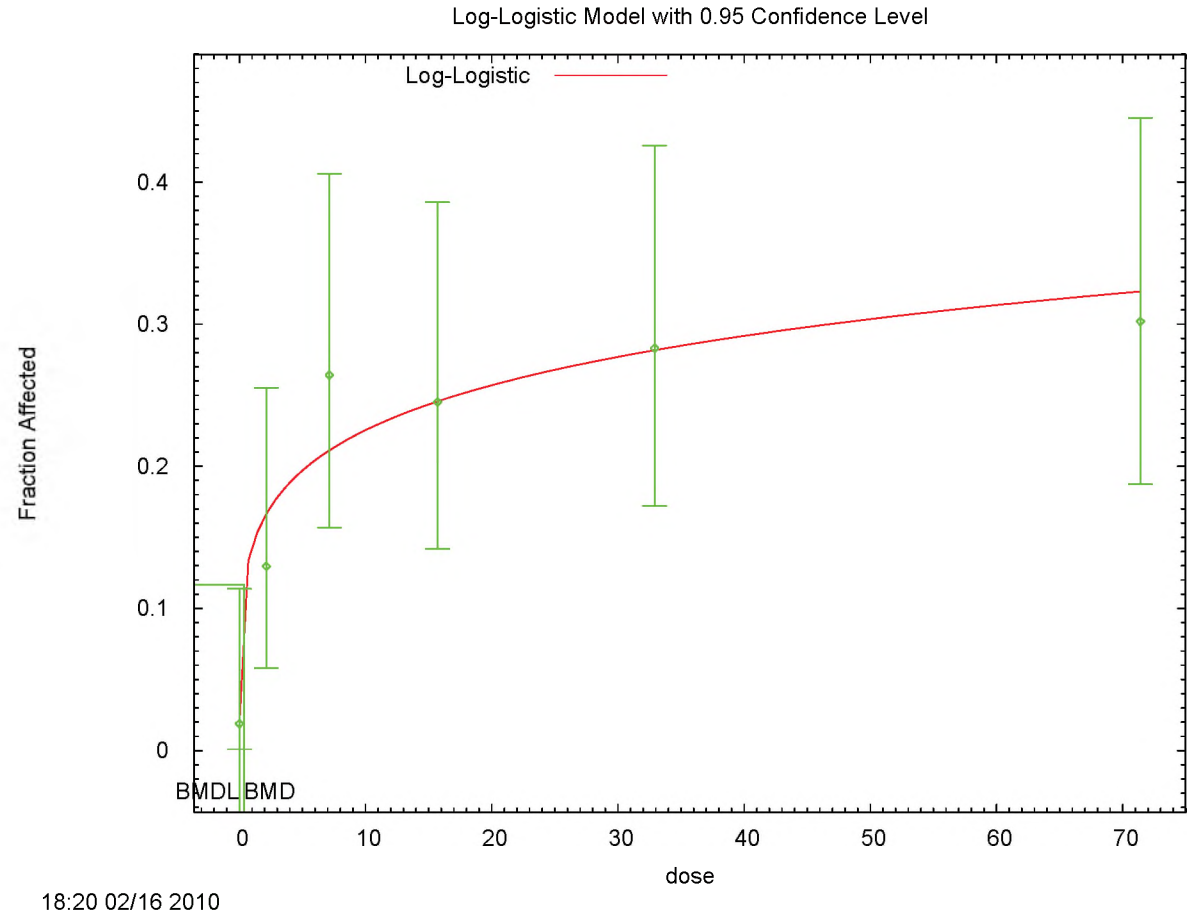
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.370958

            BMDL =   1.50494e-007
```

E.3.34.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.3.35. National Toxicology Program, 2006: Hepatocyte Hypertrophy, 2 Years

E.3.35.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	4	<0.001	290.365	1.647E+00	1.340E+00	power bound hit (power = 1)
logistic	4	<0.001	310.492	4.315E+00	3.650E+00	negative intercept (intercept = -1.237)
log-logistic	5	0.010	278.082	6.978E-01	5.454E-01	slope bound hit (slope = 1)
log-probit	4	<0.001	297.168	2.930E+00	2.267E+00	slope bound hit (slope = 1)
multistage, 5-degree [a]	**4**	**<0.001**	**290.365**	**1.647E+00**	**1.340E+00**	**final ß = 0**
probit	4	<0.001	313.841	4.564E+00	3.923E+00	negative intercept (intercept = -0.714)
Weibull	4	<0.001	290.365	1.647E+00	1.340E+00	power bound hit (power = 1)
gamma, unrestricted	4	0.029	275.042	error	error	unrestricted (power = 0.478)
log-logistic, unrestricted	4	0.005	280.068	6.672E-01	2.939E-01	unrestricted (slope = 0.984)
log-probit, unrestricted	4	0.006	279.204	7.167E-01	3.322E-01	unrestricted (slope = 0.594)
Weibull, unrestricted	4	0.019	275.967	3.709E-01	1.315E-01	unrestricted (power = 0.64)

[a] Best-fitting model, BMDS output presented in this appendix

E.3.35.2. *Output for Selected Model: Multistage, 5-Degree*

National Toxicology Program, 2006: Hepatocyte Hypertrophy, 2 Years

```
 ================================================================
         Multistage Model. (Version: 3.0;  Date: 05/16/2008)
         Input Data File: C:\1\43_NTP_2006_HepHyper_Multi5_1.(d)
         Gnuplot Plotting File:  C:\1\43_NTP_2006_HepHyper_Multi5_1.plt
                                              Tue Feb 16 18:21:00 2010
 ================================================================

 [insert study notes]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4-beta5*dose^5)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
```

```
  Independent variable = Dose

Total number of observations = 6
Total number of records with missing values = 0
Total number of parameters in model = 6
Total number of specified parameters = 0
Degree of polynomial = 5


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.232262
                        Beta(1) =     0.045074
                        Beta(2) =            0
                        Beta(3) =            0
                        Beta(4) =            0
                        Beta(5) = 2.59945e-010


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(2)    -Beta(3)    -Beta(4)    -Beta(5)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(1)

Background            1        -0.64

   Beta(1)        -0.64            1



                               Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0541647            *               *                   *
        Beta(1)        0.0639585            *               *                   *
        Beta(2)                0            *               *                   *
        Beta(3)                0            *               *                   *
        Beta(4)                0            *               *                   *
        Beta(5)                0            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -129.986         6
   Fitted model        -143.183         2       26.3932      4      2.6361629e-005
  Reduced model         -219.97         1       179.968      5         <.0001

           AIC:         290.365


                                 Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ----------------------------------------------------------------------
    0.0000     0.0542         2.871     0.000          53       -1.742
    2.1400     0.1752         9.458    19.000          54        3.416
    7.1400     0.4009        21.248    19.000          53       -0.630
```

```
    15.7000      0.6535        34.635     42.000          53         2.126
    32.9000      0.8847        46.887     41.000          53        -2.532
    71.4000      0.9902        52.479     52.000          53        -0.667

 Chi^2 = 26.48      d.f. = 4        P-value = 0.0000


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

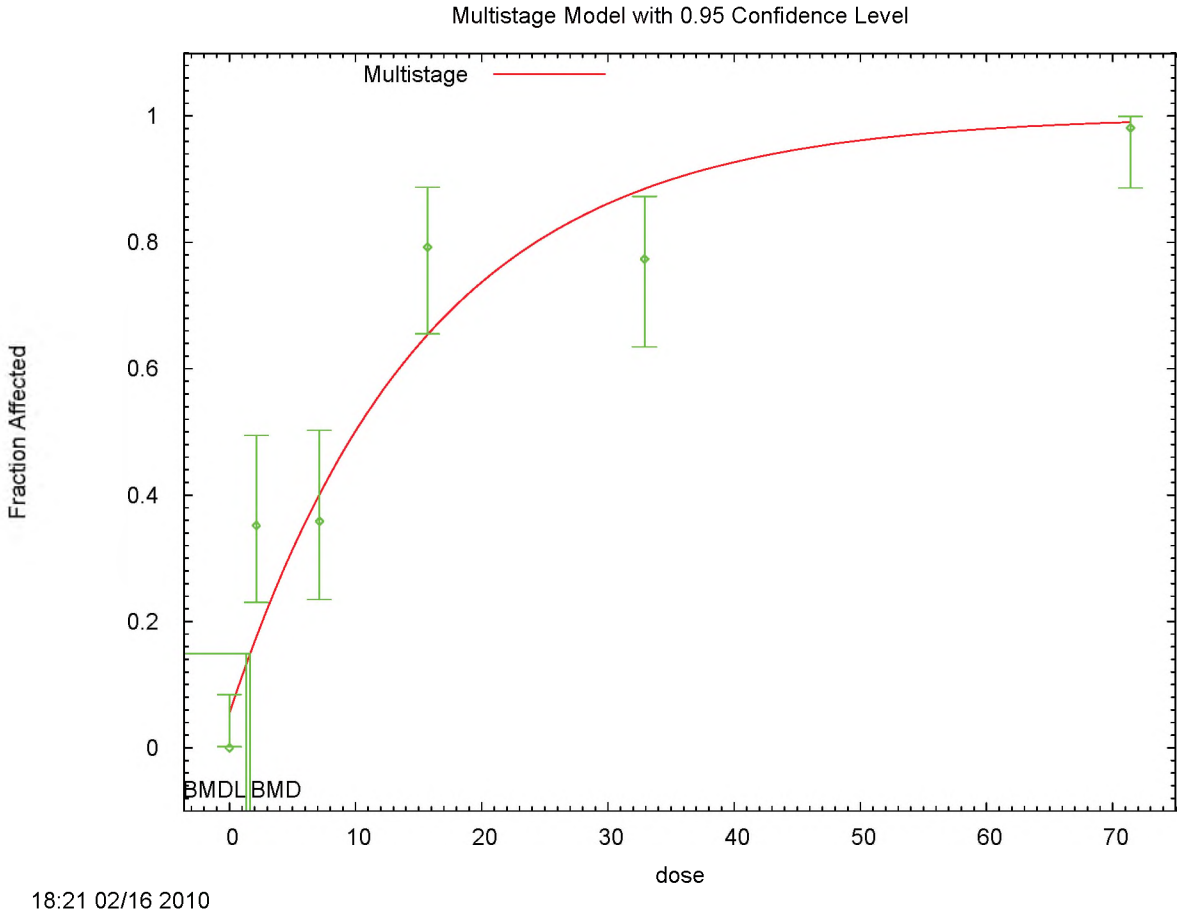
             BMD =        1.64733

            BMDL =        1.34007

            BMDU =         2.0581

Taken together, (1.34007, 2.0581 ) is a 90      % two-sided confidence
interval for the BMD
```

E.3.35.3. *Figure for Selected Model: Multistage, 5-Degree*



E.3.36. National Toxicology Program, 2006: Necrosis, Liver

E.3.36.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
logistic	4	0.397	238.314	3.484E+01	2.842E+01	negative intercept (intercept = -2.601)
log-logistic	4	0.810	235.265	1.791E+01	1.194E+01	slope bound hit (slope = 1)
log-probit	4	0.290	239.107	3.205E+01	2.382E+01	slope bound hit (slope = 1)
multistage, 5-degree	4	0.763	235.581	2.019E+01	1.419E+01	final ß = 0
probit	4	0.445	237.888	3.266E+01	2.637E+01	negative intercept (intercept = -1.508)
Weibull	4	0.763	235.581	2.019E+01	1.419E+01	power bound hit (power = 1)
gamma, unrestricted	3	0.869	236.344	1.114E+01	3.487E+00	unrestricted (power = 0.599)
log-logistic, unrestricted	3	0.833	236.483	1.112E+01	3.581E+00	unrestricted (slope = 0.695)
log-probit, unrestricted [a]	**3**	**0.768**	**236.742**	**1.061E+01**	**3.498E+00**	**unrestricted (slope = 0.367)**
Weibull, unrestricted	3	0.856	236.393	1.117E+01	3.554E+00	unrestricted (power = 0.64)

[a] Best-fitting model, BMDS output presented in this appendix

E.3.36.2. *Output for Selected Model: Log-Probit, Unrestricted*

National Toxicology Program, 2006: Necrosis, Liver

```
 ================================================================
         Probit Model. (Version: 3.1;  Date: 05/16/2008)
         Input Data File: C:\1\50_NTP_2006_LivNec_LogProbit_U_1.(d)
         Gnuplot Plotting File:  C:\1\50_NTP_2006_LivNec_LogProbit_U_1.plt
                                              Tue Feb 16 18:34:31 2010
 ================================================================

 NTP_liver_necrosis
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted
```

```
   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model

                 Default Initial (and Specified) Parameter Values
                    background =    0.0188679
                     intercept =     -1.98094
                         slope =     0.316942


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.69         0.59

 intercept        -0.69            1        -0.97

     slope         0.59        -0.97            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0228339        0.0230818         -0.0224057           0.0680734
      intercept         -2.14844         0.527256           -3.18184            -1.11503
          slope         0.367034         0.139055          0.0944904            0.639577


                          Analysis of Deviance Table

        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
      Full model        -114.813         6
    Fitted model        -115.371         3        1.1157      3          0.7733
   Reduced model         -127.98         1       26.3331      5         <.0001

           AIC:         236.742


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
   ----------------------------------------------------------------------
    0.0000     0.0228         1.210     1.000          53        -0.193
    2.1400     0.0529         2.858     4.000          54         0.694
    7.1400     0.0979         5.187     4.000          53        -0.549
   15.7000     0.1475         7.819     8.000          53         0.070
   32.9000     0.2116        11.215    10.000          53        -0.409
   71.4000     0.2968        15.729    17.000          53         0.382

 Chi^2 = 1.14      d.f. = 3        P-value = 0.7678


   Benchmark Dose Computation

Specified effect =           0.1

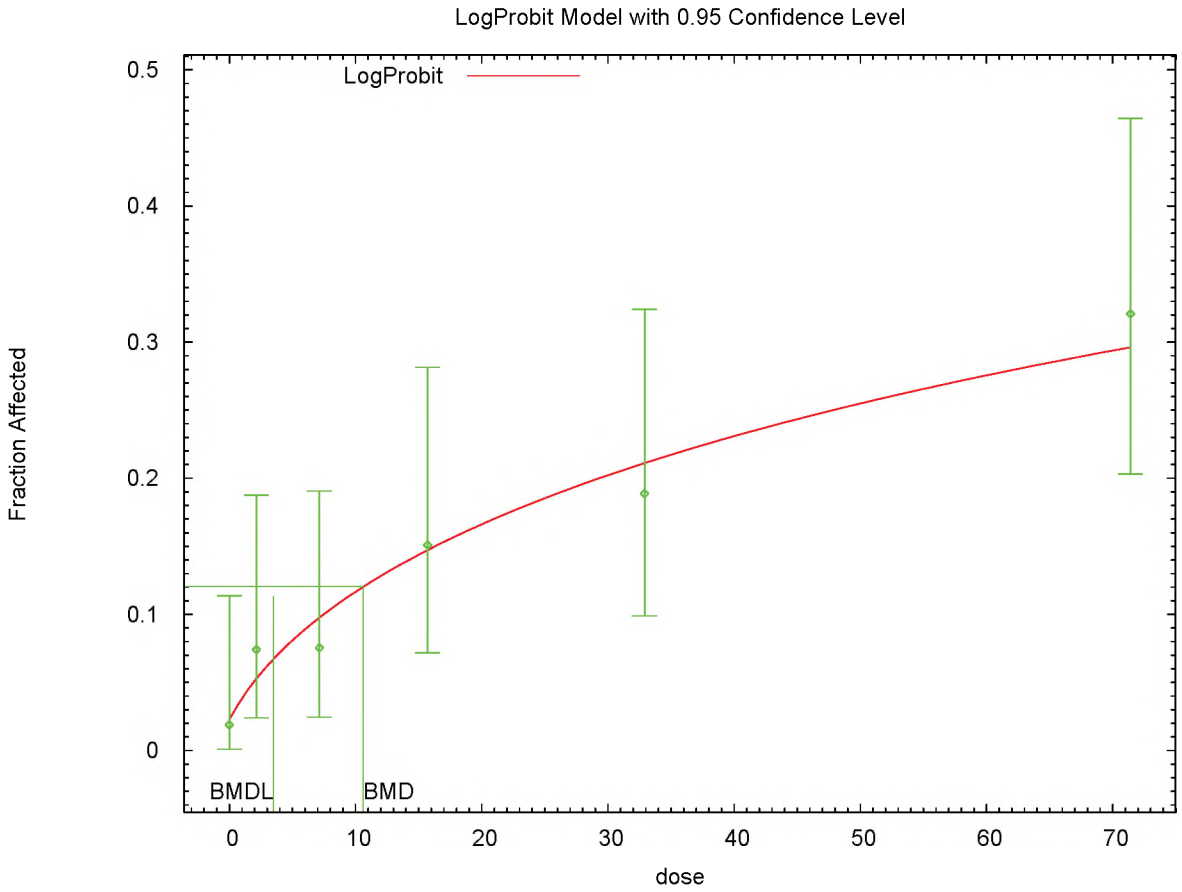
Risk Type        =      Extra risk

Confidence level =          0.95
```

```
BMD =         10.6107

BMDL =        3.49791
```

E.3.36.3. *Figure for Selected Model: Log-Probit, Unrestricted*



E.3.37. National Toxicology Program, 2006: Oval Cell Hyperplasia

E.3.37.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	3	0.072	199.446	8.970E+00	5.499E+00	
logistic	4	0.069	199.875	9.792E+00	8.245E+00	negative intercept (intercept = -3.116)
log-logistic	3	0.039	202.012	9.708E+00	7.247E+00	
log-probit	3	0.068	200.421	9.968E+00	7.758E+00	
multistage, 5-degree	2	0.066	198.641	5.424E+00	3.514E+00	
probit [a]	**4**	**0.112**	**198.166**	**9.103E+00**	**7.701E+00**	**negative intercept (intercept = -1.821)**
Weibull [b]	3	0.075	198.690	7.712E+00	4.692E+00	

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.3.37.2. *Output for Selected Model: Probit*

National Toxicology Program, 2006: Oval Cell Hyperplasia

```
 ================================================================
          Probit Model. (Version: 3.1;  Date: 05/16/2008)
          Input Data File: C:\1\53_NTP_2006_OvalHyper_Probit_1.(d)
          Gnuplot Plotting File:  C:\1\53_NTP_2006_OvalHyper_Probit_1.plt
                                                Tue Feb 16 19:51:52 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial (and Specified) Parameter Values
background = 0 Specified
intercept = -1.92612
slope = 0.0670004

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.8
slope	-0.8	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-1.82129	0.16954	-2.15359	-1.489
slope	0.0767832	0.00835175	0.060414	0.0931523

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-92.4898	6			
Fitted model	-97.0832	2	9.18683	4	0.0566
Reduced model	-210.191	1	235.402	5	<.0001

AIC: 198.166

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0343	1.817	0.000	53	-1.372
2.1400	0.0488	2.633	4.000	54	0.864
7.1400	0.1015	5.379	3.000	53	-1.082
15.7000	0.2690	14.258	20.000	53	1.779
32.9000	0.7596	40.256	38.000	53	-0.725
71.4000	0.9999	52.993	53.000	53	0.082

Chi^2 = 7.50 d.f. = 4 P-value = 0.1119

Benchmark Dose Computation

Specified effect = 0.1

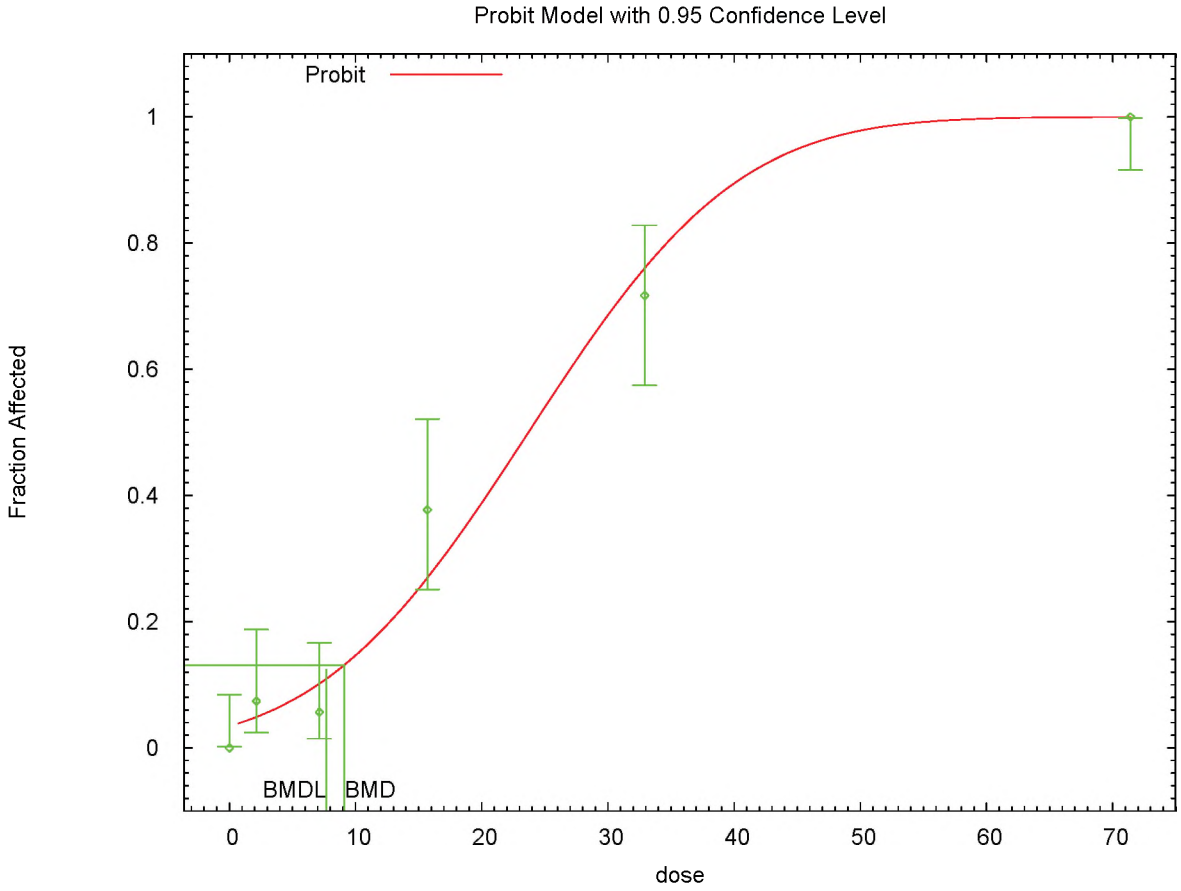
Risk Type = Extra risk

Confidence level = 0.95

BMD = 9.1026

BMDL = 7.7011

E.3.37.3. *Figure for Selected Model: Probit*



E.3.37.4. *Output for Additional Model Presented: Weibull*

National Toxicology Program, 2006: Oval Cell Hyperplasia

```
 ================================================================
         Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
         Input Data File: C:\1\53_NTP_2006_OvalHyper_Weibull_1.(d)
         Gnuplot Plotting File:  C:\1\53_NTP_2006_OvalHyper_Weibull_1.plt
                                          Tue Feb 16 19:51:53 2010
 ================================================================

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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = DichEff
   Independent variable = Dose
   Power parameter is restricted as power >=1

   Total number of observations = 6
```

Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.00925926
Slope = 0.0044452
Power = 1.63009

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope	Power
Background	1	-0.63	0.61
Slope	-0.63	1	-0.99
Power	0.61	-0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.021258	0.0198428	-0.0176332	0.0601492
Slope	0.0028715	0.00303327	-0.0030736	0.0088166
Power	1.76359	0.309457	1.15706	2.37011

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-92.4898	6			
Fitted model	-96.3448	3	7.70998	3	0.0524
Reduced model	-210.191	1	235.402	5	<.0001

AIC: 198.69

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0213	1.127	0.000	53	-1.073
2.1400	0.0320	1.725	4.000	54	1.760
7.1400	0.1073	5.685	3.000	53	-1.192
15.7000	0.3234	17.138	20.000	53	0.840
32.9000	0.7490	39.698	38.000	53	-0.538
71.4000	0.9953	52.750	53.000	53	0.501

Chi^2 = 6.92 d.f. = 3 P-value = 0.0746

Benchmark Dose Computation

Specified effect = 0.1

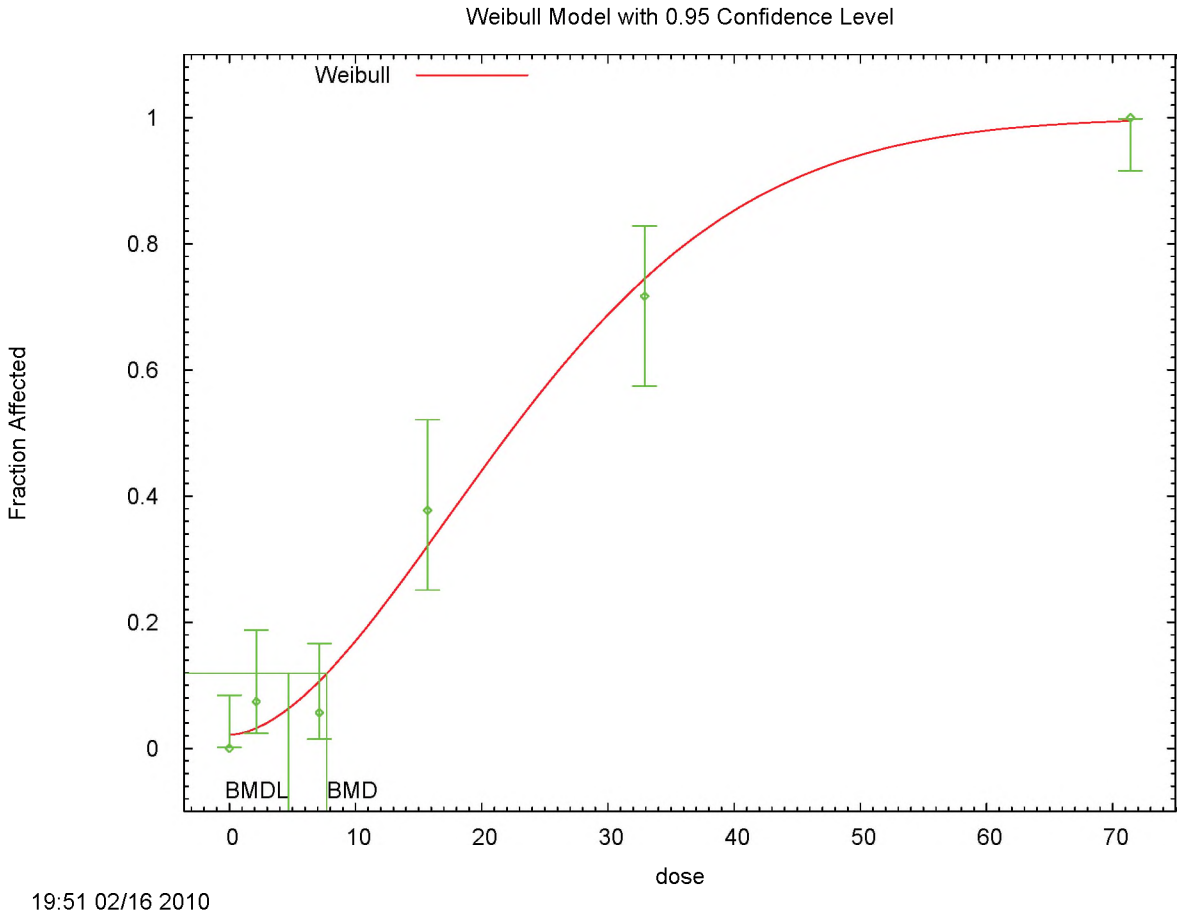
Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.71171

BMDL = 4.69152

E.3.37.5. *Figure for Additional Model Presented: Weibull*



E.3.38. National Toxicology Program, 2006: Pigmentation, Liver

E.3.38.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	3	0.385	197.655	1.547E+00	8.055E-01	
logistic	4	<0.001	203.517	2.259E+00	1.872E+00	negative intercept (intercept = -1.925)
log-logistic	3	0.978	195.600	2.212E+00	1.452E+00	
log-probit [a]	**3**	**0.980**	**195.450**	**2.072E+00**	**1.399E+00**	
multistage, 5-degree	3	0.210	199.850	9.396E-01	7.079E-01	final ß = 0
probit	4	<0.001	210.309	2.259E+00	1.916E+00	negative intercept (intercept = -1.057)
Weibull	3	0.290	198.489	1.280E+00	7.518E-01	

[a] Best-fitting model, BMDS output presented in this appendix

E.3.38.2. *Output for Selected Model: Log-Probit*

National Toxicology Program, 2006: Pigmentation, Liver

```
 ================================================================
         Probit Model. (Version: 3.1;  Date: 05/16/2008)
         Input Data File: C:\1\54_NTP_2006_Pigment_LogProbit_1.(d)
         Gnuplot Plotting File:  C:\1\54_NTP_2006_Pigment_LogProbit_1.plt
                                          Tue Feb 16 19:52:19 2010
 ================================================================

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   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = DichEff
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values
background = 0.0754717
intercept = -1.91144
slope = 1.07385

Asymptotic Correlation Matrix of Parameter Estimates

	background	intercept	slope
background	1	-0.45	0.35
intercept	-0.45	1	-0.94
slope	0.35	-0.94	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0735956	0.0343284	0.00631316	0.140878
intercept	-2.19294	0.400053	-2.97703	-1.40885
slope	1.25068	0.169731	0.918012	1.58335

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-94.6177	6			
Fitted model	-94.7248	3	0.214232	3	0.9753
Reduced model	-210.717	1	232.198	5	<.0001

AIC: 195.45

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0736	3.901	4.000	53	0.052
2.1400	0.1729	9.338	9.000	54	-0.122
7.1400	0.6338	33.591	34.000	53	0.117
15.7000	0.9023	47.822	48.000	53	0.082
32.9000	0.9863	52.275	52.000	53	-0.325
71.4000	0.9992	52.959	53.000	53	0.202

Chi^2 = 0.18 d.f. = 3 P-value = 0.9801

Benchmark Dose Computation

Specified effect = 0.1

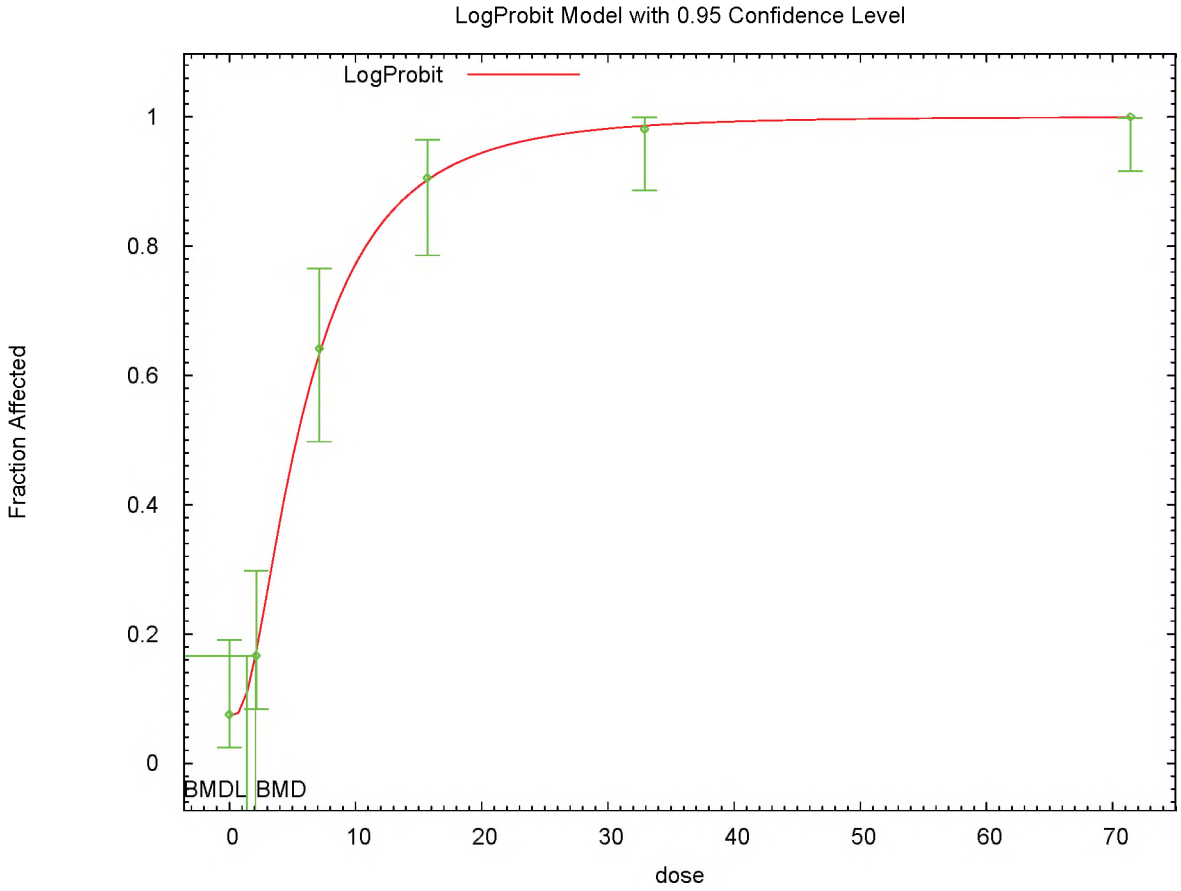
Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.07241

BMDL = 1.39932

E.3.38.3. *Figure for Selected Model: Log-Probit*



E.3.39. National Toxicology Program, 2006: Toxic Hepatopathy

E.3.39.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	4	0.772	185.634	4.668E+00	3.317E+00	
logistic	4	0.012	198.445	7.070E+00	5.925E+00	negative intercept (intercept = -2.925)
log-logistic	3	0.362	190.061	5.676E+00	4.040E+00	
log-probit	3	0.378	189.858	6.061E+00	4.079E+00	
multistage, 5-degree [a]	**4**	**0.577**	**186.521**	**4.163E+00**	**2.701E+00**	**final ß = 0**
probit	4	0.019	197.159	6.784E+00	5.712E+00	negative intercept (intercept = -1.724)
Weibull	4	0.745	185.657	4.454E+00	3.159E+00	

[a] Best-fitting model, BMDS output presented in this appendix

E.3.39.2. *Output for Selected Model: Multistage, 5-Degree*

National Toxicology Program, 2006: Toxic Hepatopathy

```
 ======================================================================
          Multistage Model. (Version: 3.0;  Date: 05/16/2008)
          Input Data File: C:\1\55_NTP_2006_ToxHepa_Multi5_1.(d)
          Gnuplot Plotting File:  C:\1\55_NTP_2006_ToxHepa_Multi5_1.plt
                                              Tue Feb 16 19:52:49 2010
 ======================================================================

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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4-beta5*dose^5)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 0
 Degree of polynomial = 5


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

Background =	0
Beta(1) =	0
Beta(2) =	0
Beta(3) =	0
Beta(4) =	0
Beta(5) =	5.40983e+010

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(3) -Beta(4) -Beta(5)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)	Beta(2)
Beta(1)	1	-0.91
Beta(2)	-0.91	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.019656	*	*	*
Beta(2)	0.00135796	*	*	*
Beta(3)	0	*	*	*
Beta(4)	0	*	*	*
Beta(5)	0	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-89.8076	6			
Fitted model	-91.2606	2	2.90597	4	0.5737
Reduced model	-218.207	1	256.799	5	<.0001

AIC: 186.521

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	53	0.000
2.1400	0.0471	2.545	2.000	54	-0.350
7.1400	0.1891	10.021	8.000	53	-0.709
15.7000	0.4745	25.146	30.000	53	1.335
32.9000	0.8796	46.616	45.000	53	-0.682
71.4000	0.9998	52.987	53.000	53	0.113

Chi^2 = 2.89 d.f. = 4 P-value = 0.5771

Benchmark Dose Computation

Specified effect = 0.1

```
Risk Type          =      Extra risk

Confidence level =            0.95

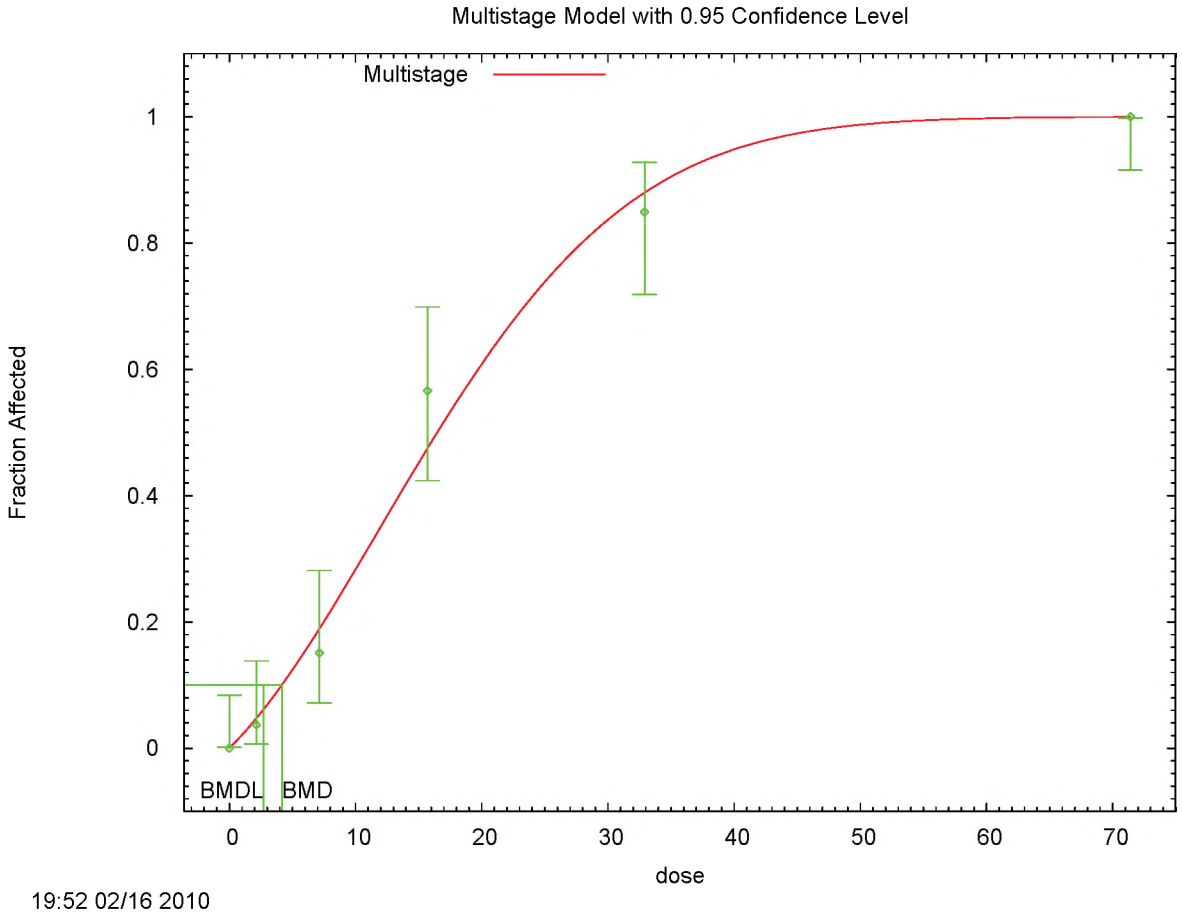
              BMD =         4.16294

             BMDL =         2.70063

             BMDU =         6.00186

Taken together, (2.70063, 6.00186) is a 90      % two-sided confidence
interval for the BMD
```

E.3.39.3. *Figure for Selected Model: Multistage, 5-Degree*



E.3.40. Ohsako et al., 2001: Ano-Genital Length, PND 120

E.3.40.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	3	0.019	171.804	5.650E+02	3.785E+02	
exponential (M3)	3	0.019	171.804	5.650E+02	3.785E+02	power hit bound (d = 1)
exponential (M4)	2	0.117	168.204	2.854E+01	1.054E+01	
exponential (M5)	1	0.049	169.789	2.948E+01	1.135E+01	
Hill [b]	**2**	**0.148**	**167.727**	**3.722E+01**	**9.752E+00**	**n lower bound hit (n = 1)**
linear	3	0.018	171.954	5.852E+02	4.047E+02	
polynomial, 4-degree	3	0.018	171.954	5.852E+02	4.047E+02	
power	3	0.018	171.954	5.852E+02	4.047E+02	power bound hit (power = 1)
Hill, unrestricted [c]	1	0.055	169.600	5.101E+01	3.066E+00	unrestricted (n = 0.502)
power, unrestricted	2	0.151	167.689	6.200E+01	2.291E+00	unrestricted (power = 0.252)

[a] Constant variance model selected ($p = 0.165$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.40.2. *Output for Selected Model: Hill*

Ohsako et al., 2001: Ano-Genital Length, PND 120

```
 ======================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\56_Ohsako_2001_Anogen_HillCV_1.(d)
         Gnuplot Plotting File:  C:\1\56_Ohsako_2001_Anogen_HillCV_1.plt
                                                Tue Feb 16 19:53:25 2010
 ======================================================================

 Figure 7
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
```

Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	7.27386	
rho =	0	Specified
intercept =	28.905	
v =	-5.1065	
n =	1.40226	
k =	33.9669	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	k
alpha	1	-2.2e-009	-2.4e-008	-7.2e-009
intercept	-2.2e-009	1	-0.66	-0.5
v	-2.4e-008	-0.66	1	-0.11
k	-7.2e-009	-0.5	-0.11	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	7.08444	1.3634	4.41223	9.75666
intercept	28.9809	0.745637	27.5195	30.4423
v	-4.79692	0.983318	-6.72418	-2.86965
n	1	NA		
k	29.8628	24.4463	-18.0511	77.7767

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	12	28.9	29	3.13	2.66	-0.0988
12.5	10	27.9	27.6	2.5	2.66	0.442
50	10	25.2	26	3.21	2.66	-0.963
200	10	26	24.8	2.85	2.66	1.42
800	12	23.8	24.4	1.56	2.66	-0.726

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          -77.952340           6     167.904680
              A2          -74.703868          10     169.407736
              A3          -77.952340           6     167.904680
          fitted          -79.863340           4     167.726680
               R          -89.824703           2     183.649405


                    Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              30.2417          8       0.0001916
  Test 2              6.49694          4           0.165
  Test 3              6.49694          4           0.165
  Test 4                3.822          2          0.1479

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =             1

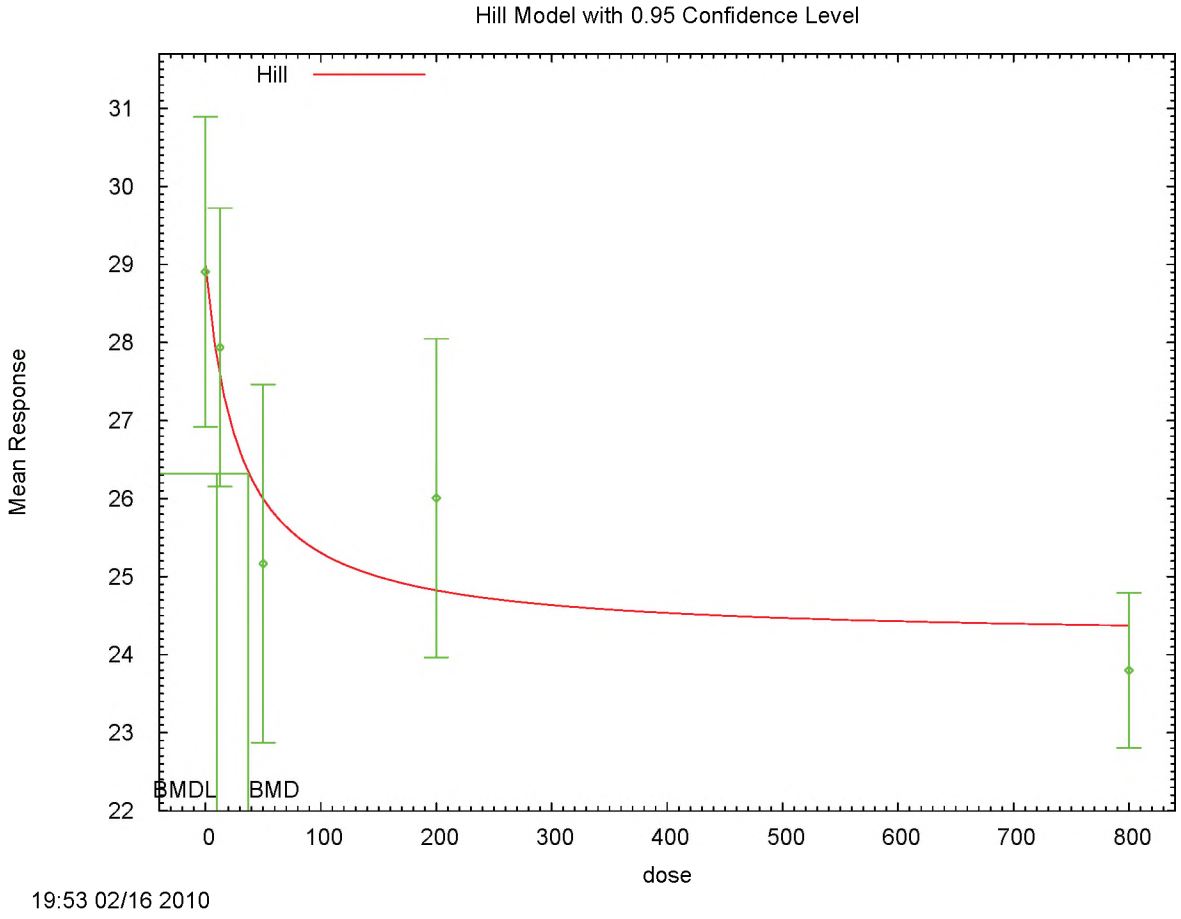
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        37.2249

            BMDL =       9.75249
```

E.3.40.3. *Figure for Selected Model: Hill*



E.3.40.4. *Output for Additional Model Presented: Hill, Unrestricted*

Ohsako et al., 2001: Ano-Genital Length, PND 120

```
 ================================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\56_Ohsako_2001_Anogen_HillCV_U_1.(d)
          Gnuplot Plotting File:  C:\1\56_Ohsako_2001_Anogen_HillCV_U_1.plt
                                                 Tue Feb 16 19:53:26 2010
 ================================================================================

 Figure 7
 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the response function is:

    Y[dose] = intercept + v*dose^n/(k^n + dose^n)


    Dependent variable = Mean
    Independent variable = Dose
    rho is set to 0
    Power parameter is not restricted
    A constant variance model is fit
```

```
   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =      7.27386
                            rho =            0   Specified
                      intercept =       28.905
                              v =      -5.1065
                              n =      1.40226
                              k =      33.9669


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                    alpha    intercept            v            n            k

       alpha            1     2.1e-009    -1.8e-008    -1.7e-008     1.6e-008

   intercept     2.1e-009            1        0.012       0.0075        -0.13

           v    -1.8e-008        0.012            1         0.98        -0.99

           n    -1.7e-008       0.0075         0.98            1        -0.97

           k     1.6e-008        -0.13        -0.99        -0.97            1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha          7.06785          1.36021             4.40189             9.73381
       intercept          28.9608         0.755363             27.4803             30.4413
               v         -6.94236          12.2514            -30.9547               17.07
               n         0.501942         0.915162            -1.29174             2.29563
               k          131.957           1071.9            -1968.92             2232.84


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    12       28.9           29         3.13         2.66        -0.0727
 12.5    10       27.9         27.3          2.5         2.66           0.72
   50    10       25.2         26.3         3.21         2.66          -1.37
  200    10         26         25.1         2.85         2.66           1.04
  800    12       23.8           24         1.56         2.66         -0.287


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
```

```
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -77.952340           6     167.904680
             A2          -74.703868          10     169.407736
             A3          -77.952340           6     167.904680
          fitted         -79.800035           5     169.600070
              R          -89.824703           2     183.649405


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              30.2417          8       0.0001916
   Test 2              6.49694          4           0.165
   Test 3              6.49694          4           0.165
   Test 4              3.69539          1         0.05456

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =            1

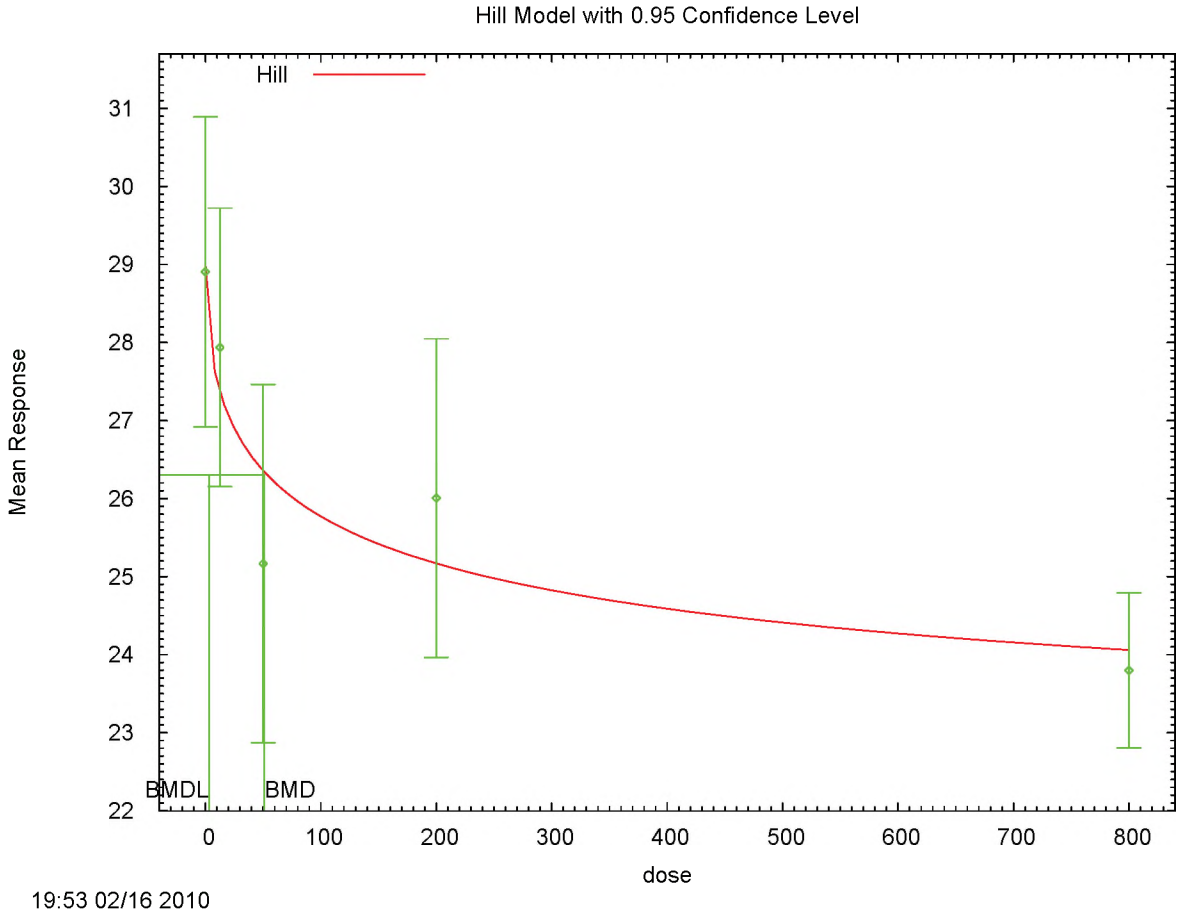
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        51.0107

            BMDL =       3.06631
```

E.3.40.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.3.41. Sewall et al., 1995: T4 In Serum

E.3.41.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	3	0.424	205.966	5.762E+01	3.783E+01	
exponential (M3)	3	0.424	205.966	5.762E+01	3.783E+01	power hit bound (d = 1)
exponential (M5)	2	0.611	206.152	2.523E+01	8.442E+00	power hit bound (d = 1)
Hill [b]	**2**	**0.702**	**205.875**	**2.071E+01**	**5.164E+00**	**n lower bound hit (n = 1)**
linear	3	0.332	206.584	6.788E+01	4.858E+01	
polynomial, 4-degree	3	0.332	206.584	6.788E+01	4.858E+01	
power	3	0.332	206.584	6.788E+01	4.858E+01	power bound hit (power = 1)
Hill, unrestricted [c]	1	0.844	207.205	1.657E+01	1.903E+00	unrestricted (n = 0.427)
power, unrestricted	2	0.983	205.200	1.658E+01	1.820E+00	unrestricted (power = 0.403)

[a] Constant variance model selected ($p = 0.4078$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.41.2. *Output for Selected Model: Hill*

Sewall et al., 1995: T4 In Serum

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\58_Sewall_1995_T4_HillCV_1.(d)
         Gnuplot Plotting File:  C:\1\58_Sewall_1995_T4_HillCV_1.plt
                                                Tue Feb 16 19:54:30 2010
 ================================================================

 Figure 1, Saline noninitiated
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/{k^n + dose^n}


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 5
   Total number of records with missing values = 0
```

```
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                          alpha =      33.0913
                            rho =            0   Specified
                      intercept =      30.6979
                              v =     -12.2937
                              n =     0.695384
                              k =      24.6674


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -n
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                    alpha    intercept            v            k

      alpha             1     1.2e-008     4.1e-008    -2.4e-008

  intercept     1.2e-008            1         0.14        -0.66

          v     4.1e-008         0.14            1        -0.76

          k    -2.4e-008        -0.66        -0.76            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha          29.8807          6.29941            17.5341             42.2274
       intercept          29.9609          1.64749            26.7319             33.1899
               v         -14.2338          4.35645           -22.7723            -5.69537
               n                1               NA
               k          33.2198          37.0852           -39.4658             105.905

 NA - Indicates that this parameter has hit a bound
      implied by some inequality constraint and thus
      has no standard error.


      Table of Data and Estimated Values of Interest

  Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------      ---   --------     --------   -----------  -----------   ----------

     0      9       30.7           30         4.66         5.47          0.404
   3.5      9       27.9         28.6         7.17         5.47         -0.399
  10.7      9       25.9         26.5         6.81         5.47         -0.328
    35      9       23.6         22.7         5.38         5.47          0.493
   125      9       18.4         18.7         4.12         5.47         -0.171


  Model Descriptions for likelihoods calculated


  Model A1:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma(i)^2
```

```
Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          -98.583448           6     209.166896
              A2          -96.590204          10     213.180407
              A3          -98.583448           6     209.166896
          fitted          -98.937315           4     205.874631
               R         -109.013252           2     222.026503


                    Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              24.8461          8        0.001651
  Test 2              3.98649          4          0.4078
  Test 3              3.98649          4          0.4078
  Test 4             0.707735          2           0.702

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =             1

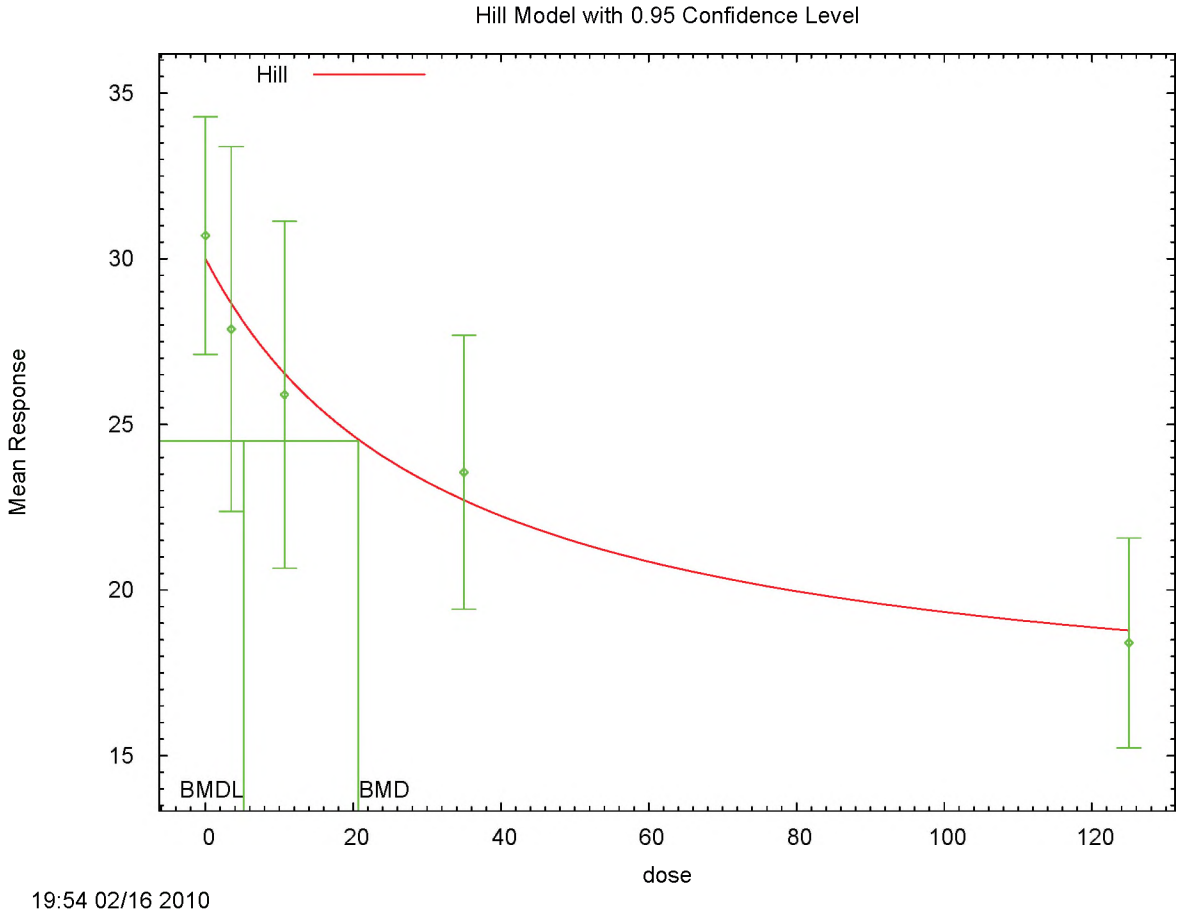
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        20.7117

            BMDL =       5.16405
```

E.3.41.3. *Figure for Selected Model: Hill*



E.3.41.4. *Output for Additional Model Presented: Hill, Unrestricted*

Sewall et al., 1995: T4 In Serum

```
 ================================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\1\58_Sewall_1995_T4_HillCV_U_1.(d)
          Gnuplot Plotting File:  C:\1\58_Sewall_1995_T4_HillCV_U_1.plt
                                                 Tue Feb 16 19:54:31 2010
 ================================================================================

 Figure 1, Saline noninitiated
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the response function is:

    Y[dose] = intercept + v*dose^n/(k^n + dose^n)


    Dependent variable = Mean
    Independent variable = Dose
    rho is set to 0
    Power parameter is not restricted
    A constant variance model is fit
```

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	33.0913	
rho =	0	Specified
intercept =	30.6979	
v =	-12.2937	
n =	0.695384	
k =	24.6674	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	n	k
alpha	1	-0.0004	0.0059	0.0048	-0.0059
intercept	-0.0004	1	-0.026	-0.44	0.07
v	0.0059	-0.026	1	0.77	-1
n	0.0048	-0.44	0.77	1	-0.82
k	-0.0059	0.07	-1	-0.82	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	29.4396	6.20653	17.2751	41.6042
intercept	30.6757	1.77521	27.1963	34.155
v	-141.324	1202.4	-2497.98	2215.33
n	0.426599	0.262207	-0.0873175	0.940515
k	31487	770429	-1.47853e+006	1.5415e+006

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	9	30.7	30.7	4.66	5.43	0.0123
3.5	9	27.9	27.8	7.17	5.43	0.0279
10.7	9	25.9	26.1	6.81	5.43	-0.137
35	9	23.6	23.3	5.38	5.43	0.132
125	9	18.4	18.5	4.12	5.43	-0.0354

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)

```
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -98.583448           6     209.166896
             A2          -96.590204          10     213.180407
             A3          -98.583448           6     209.166896
          fitted         -98.602701           5     207.205403
              R         -109.013252           2     222.026503


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              24.8461          8        0.001651
   Test 2              3.98649          4          0.4078
   Test 3              3.98649          4          0.4078
   Test 4            0.0385071          1          0.8444

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =            1

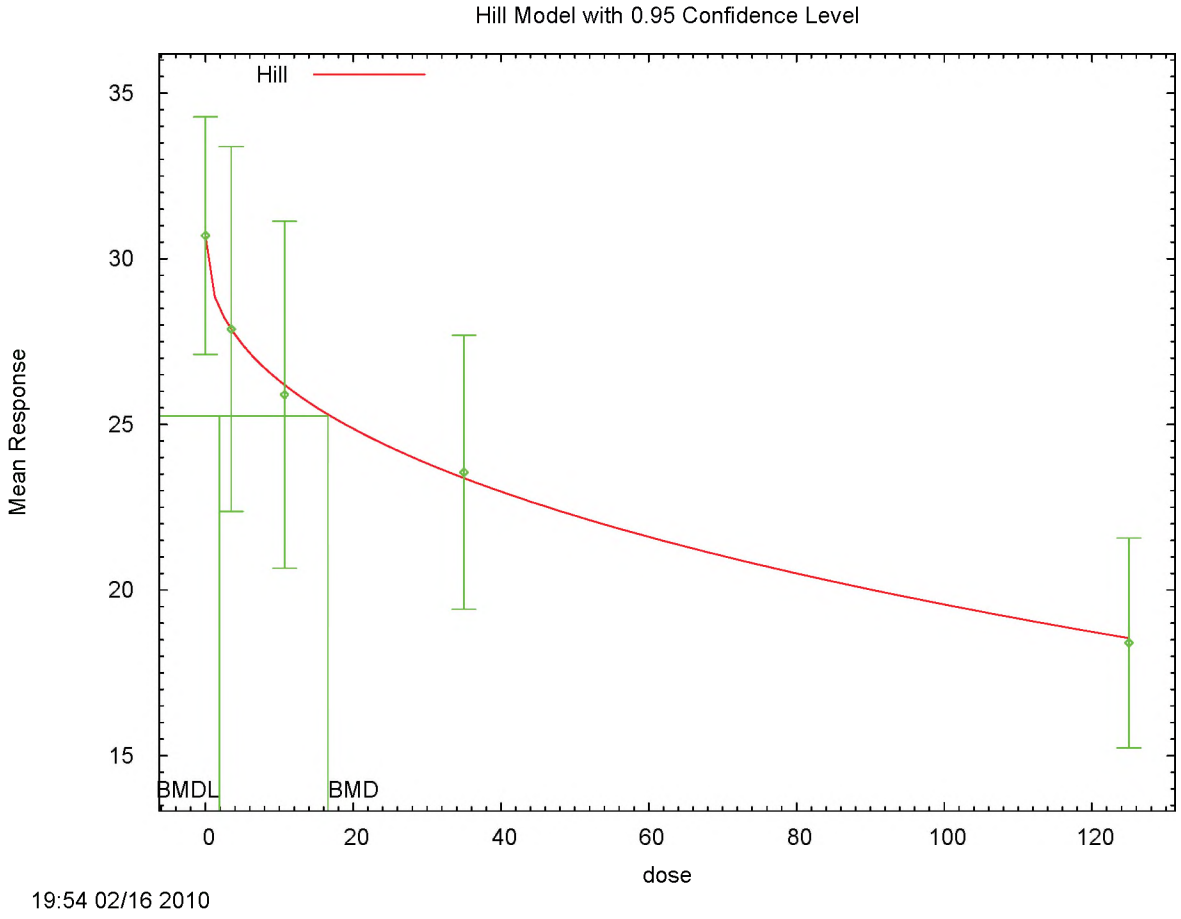
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        16.5689

            BMDL =       1.90347
```

E.3.41.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.3.42. Shi et al., 2007: Estradiol 17B, PE9

E.3.42.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	3	0.001	395.701	1.729E+01	8.956E+00	
exponential (M3)	3	0.001	395.701	1.729E+01	8.956E+00	power hit bound (d = 1)
exponential (M4) [b]	**2**	**0.494**	**383.635**	**5.559E-01**	**2.236E-01**	
exponential (M5)	2	0.494	383.635	5.559E-01	2.236E-01	power hit bound (d = 1)
Hill	2	0.773	382.743	4.434E-01	error	n lower bound hit (n = 1)
linear	3	0.001	397.484	2.243E+01	1.523E+01	
polynomial, 4-degree	3	0.001	397.484	2.243E+01	1.523E+01	
power	3	0.001	397.484	2.243E+01	1.523E+01	power bound hit (power = 1)
Hill, unrestricted	1	0.874	384.251	3.998E-01	error	unrestricted (n = 0.616)
power, unrestricted	2	0.506	383.589	3.409E-01	5.002E-03	unrestricted (power = 0.155)

[a] Non-constant variance model selected ($p = 0.0521$)

[b] Best-fitting model, BMDS output presented in this appendix

E.3.42.2. *Output for Selected Model: Exponential (M4)*

Shi et al., 2007: Estradiol 17B, PE9

```
 ========================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\59_Shi_2007_Estradiol_Exp_1.(d)
         Gnuplot Plotting File:
                                                 Tue Feb 16 19:55:06 2010
 ========================================================================

 Figure 4 PE9 only
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
```

```
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


                Initial Parameter Values

                Variable          Model 4
                --------          --------
                  lnalpha             2.65881
                      rho            0.913414
                        a                 108
                        b            0.136287
                        c            0.340136
                        d                   1



                  Parameter Estimates

                 Variable          Model 4
                 --------          -------
                  lnalpha            1.81331
                      rho            1.12126
                        a            100.526
                        b            1.53823
                        c           0.431796
                        d                  1


         Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0     10        102.9         41.41
  0.143     10        86.19         19.58
  0.714     10        63.33         29.36
   7.14     10         48.1         18.82
   28.6     10        38.57         22.59


              Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0         100.5        32.83           0.2245
  0.143         89.25        30.71          -0.3147
  0.714         62.45        25.14           0.1108
   7.14         43.41         20.5            0.723
   28.6         43.41         20.5          -0.7458


Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
```

```
            Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
            Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                   Model      Log(likelihood)      DF         AIC
                  -------    -----------------    ----   ------------
                      A1       -188.3615           6      388.7231
                      A2        -183.667          10      387.3339
                      A3       -186.1132           7      386.2263
                       R       -203.3606           2      410.7211
                       4       -186.8176           5      383.6352


Additive constant for all log-likelihoods =     -45.95.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                        39.39           8            < 0.0001
  Test 2                        9.389           4             0.05208
  Test 3                        4.892           3              0.1798
 Test 6a                        1.409           2              0.4944


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

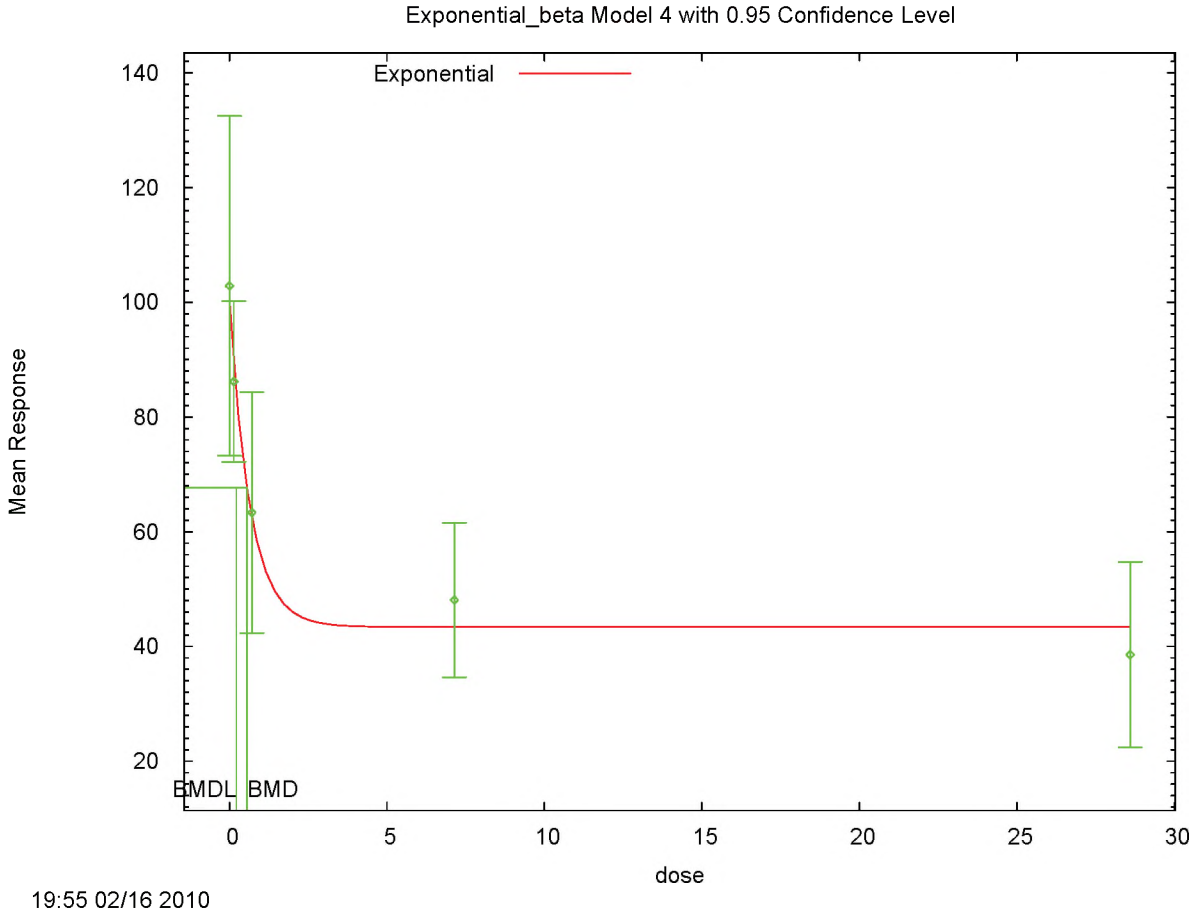
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

              BMD =     0.555948
```

BMDL = 0.223612

E.3.42.3. *Figure for Selected Model: Exponential (M4)*



E.3.43. Smialowicz et al., 2008: PFC per 10^6 Cells

E.3.43.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	3	0.048	903.586	8.234E+01	4.833E+01	
exponential (M3)	3	0.048	903.586	8.234E+01	4.833E+01	power hit bound (d = 1)
exponential (M4)	2	0.019	905.578	8.032E+01	6.220E+00	
exponential (M5)	2	0.019	905.578	8.032E+01	6.220E+00	power hit bound (d = 1)
Hill	2	0.026	904.975	1.617E+01	2.214E+00	n lower bound hit (n = 1)
linear	3	0.016	905.992	1.450E+02	1.102E+02	
polynomial, 4-degree	2	<0.0001	1198.471	1.375E+03	3.331E+01	
power [c]	3	0.016	905.992	1.450E+02	1.102E+02	power bound hit (power = 1)
Hill, unrestricted	1	0.183	901.442	8.297E+00	4.172E-01	unrestricted (n = 0.266)
power, unrestricted [b]	**2**	**0.446**	**899.282**	**7.676E+00**	**4.087E-01**	**unrestricted (power = 0.249)**

[a] Constant variance model selected (*p* = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.43.2. *Output for Selected Model: Power, Unrestricted*

Smialowicz et al., 2008: PFC per 10^6 Cells

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\1\60_Smial_2008_PFCcells_PwrCV_U_1.(d)
         Gnuplot Plotting File:  C:\1\60_Smial_2008_PFCcells_PwrCV_U_1.plt
                                              Tue Feb 16 19:55:53 2010
 ================================================================

 Anti Response to SRBCs, PFC per 10to6 cells, Table 4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
```

```
   The power is not restricted
   A constant variance model is fit

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                          alpha =       232385
                            rho =            0   Specified
                        control =         1491
                          slope =     -384.362
                          power =     0.215085


            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -rho
                  have been estimated at a boundary point, or have been specified by the user,
                  and do not appear in the correlation matrix )

                   alpha      control        slope        power

      alpha            1    -1.5e-009    -8.2e-009    -1.1e-008

    control    -1.5e-009            1        -0.79        -0.65

      slope    -8.2e-009        -0.79            1         0.96

      power    -1.1e-008        -0.65         0.96            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha           220294          38061.1             145696              294893
         control          1470.38           124.07            1227.21             1713.55
           slope         -282.777          145.113           -567.193             1.64025
           power         0.248621        0.0856348          0.0807799            0.416462


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    15  1.49e+003    1.47e+003          716          469           0.17
 1.07    14  1.13e+003    1.18e+003          171          469         -0.429
 10.7    15        945          961          516          469         -0.129
  107    15        677          567          465          469           0.91
  321     8        161          283          117          469         -0.735


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2
```

```
Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -444.832859            6     901.665718
             A2         -425.402825           10     870.805651
             A3         -444.832859            6     901.665718
         fitted         -445.641102            4     899.282205
              R         -463.753685            2     931.507371


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              76.7017          8          <.0001
  Test 2              38.8601          4          <.0001
  Test 3              38.8601          4          <.0001
  Test 4              1.61649          2          0.4456

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  Consider running a
non-homogeneous variance model

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


               Benchmark Dose Computation

Specified effect =             1

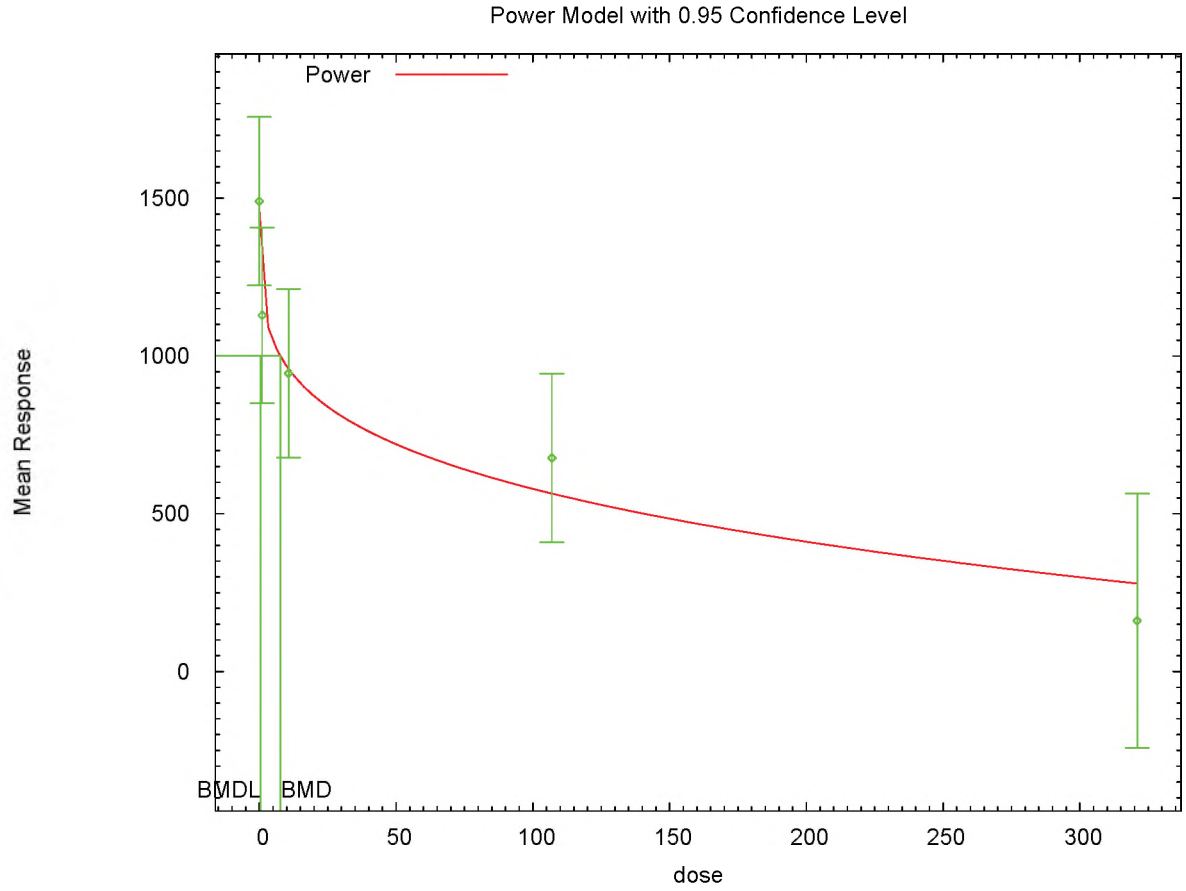
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 7.67564


             BMDL = 0.408661
```

E.3.43.3. *Figure for Selected Model: Power, Unrestricted*



E.3.43.4. *Output for Additional Model Presented: Power*

Smialowicz et al., 2008: PFC per 10^6 Cells

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\1\60_Smial_2008_PFCcells_PwrCV_1.(d)
         Gnuplot Plotting File:  C:\1\60_Smial_2008_PFCcells_PwrCV_1.plt
                                          Tue Feb 16 19:55:53 2010
 ================================================================

 Anti Response to SRBCs, PFC per 10to6 cells, Table 4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The power is restricted to be greater than or equal to 1
   A constant variance model is fit
```

```
   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =       232385
                            rho =            0   Specified
                        control =         1491
                          slope =     -2925.99
                          power =    -0.136613


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -power
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                    alpha      control        slope

     alpha              1     3.6e-009    -1.2e-008

   control     3.6e-009            1        -0.53

     slope    -1.2e-008        -0.53            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha           250878          43345.1             165923              335833
        control          1176.24          72.2586            1034.61             1317.86
          slope         -3.45384         0.592114           -4.61436            -2.29332
          power                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    15  1.49e+003    1.18e+003          716          501           2.43
 1.07    14  1.13e+003    1.17e+003          171          501         -0.325
 10.7    15        945    1.14e+003          516          501           -1.5
  107    15        677          807          465          501             -1
  321     8        161         67.6          117          501          0.528


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2
```

```
Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -444.832859            6     901.665718
              A2         -425.402825           10     870.805651
              A3         -444.832859            6     901.665718
          fitted         -449.996183            3     905.992366
               R         -463.753685            2     931.507371


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              76.7017          8          <.0001
  Test 2              38.8601          4          <.0001
  Test 3              38.8601          4          <.0001
  Test 4              10.3266          3         0.01598

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  Consider running a
non-homogeneous variance model

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is less than .1.  You may want to try a different
model


               Benchmark Dose Computation

Specified effect =             1

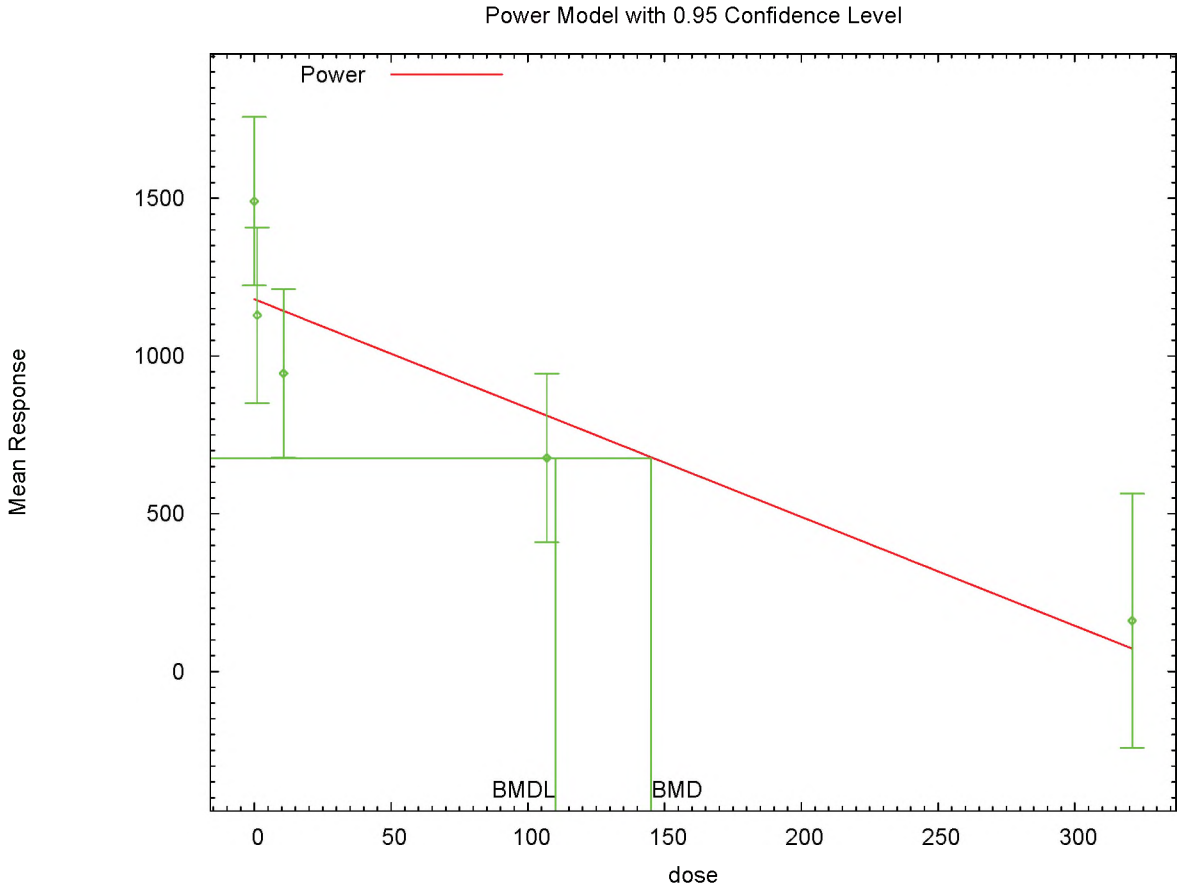
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 145.02


            BMDL = 110.161
```

E.3.43.5. *Figure for Additional Model Presented: Power*



E.3.44. Smialowicz et al., 2008: PFC per Spleen

E.3.44.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	3	0.133	377.395	1.320E+02	8.431E+01	
exponential (M3)	3	0.133	377.395	1.320E+02	8.431E+01	power hit bound (d = 1)
exponential (M4)	3	0.133	377.395	1.320E+02	8.184E+01	
exponential (M5)	2	0.061	379.395	1.320E+02	8.184E+01	power hit bound (d = 1)
Hill	2	0.069	379.150	1.401E+02	error	n lower bound hit (n = 1)
linear	3	0.044	379.895	2.151E+02	1.704E+02	
polynomial, 4-degree	3	0.044	379.895	2.151E+02	1.704E+02	
power[c]	3	0.044	379.895	2.151E+02	1.704E+02	power bound hit (power = 1)
Hill, unrestricted	2	<0.0001	441.885	7.545E-23	error	unrestricted (n = 0.038)
power, unrestricted[b]	**2**	**0.230**	**376.738**	**9.374E+01**	**2.088E+01**	**unrestricted (power = 0.418)**

[a] Non-constant variance model selected ($p = 0.0011$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.44.2. *Output for Selected Model: Power, Unrestricted*

Smialowicz et al., 2008: PFC per Spleen

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\1\61_Smial_2008_PFCspleen_Pwr_U_1.(d)
         Gnuplot Plotting File:  C:\1\61_Smial_2008_PFCspleen_Pwr_U_1.plt
                                              Tue Feb 16 19:56:26 2010
 ================================================================

 Anti Response to SRBCs - PFC x 10 to the 4 per spleen, Table 4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   The power is not restricted
```

The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	4.76607
rho =	0
control =	27.8
slope =	-7.21601
power =	0.213905

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.98	0.25	-0.27	-0.23
rho	-0.98	1	-0.31	0.28	0.23
control	0.25	-0.31	1	-0.81	-0.74
slope	-0.27	0.28	-0.81	1	0.99
power	-0.23	0.23	-0.74	0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	0.747155	1.0244	-1.26063	2.75494
rho	1.36972	0.357098	0.66982	2.06962
control	25.1733	2.93169	19.4273	30.9193
slope	-1.98465	1.82113	-5.554	1.5847
power	0.417867	0.141932	0.139686	0.696048

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	15	27.8	25.2	13.4	13.2	0.769
1.07	14	21	23.1	13.6	12.5	-0.639
10.7	15	17.6	19.8	9.4	11.2	-0.768
107	15	12.6	11.2	8.7	7.59	0.721
321	8	3	3.04	3.1	3.11	-0.0353

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))

```
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -190.565019           6     393.130038
             A2         -181.476284          10     382.952569
             A3         -181.900030           7     377.800059
          fitted        -183.369059           5     376.738118
              R         -204.636496           2     413.272993


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              46.3204          8          <.0001
   Test 2              18.1775          4        0.001139
   Test 3              0.84749          3          0.8381
   Test 4              2.93806          2          0.2301

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


               Benchmark Dose Computation

Specified effect =             1

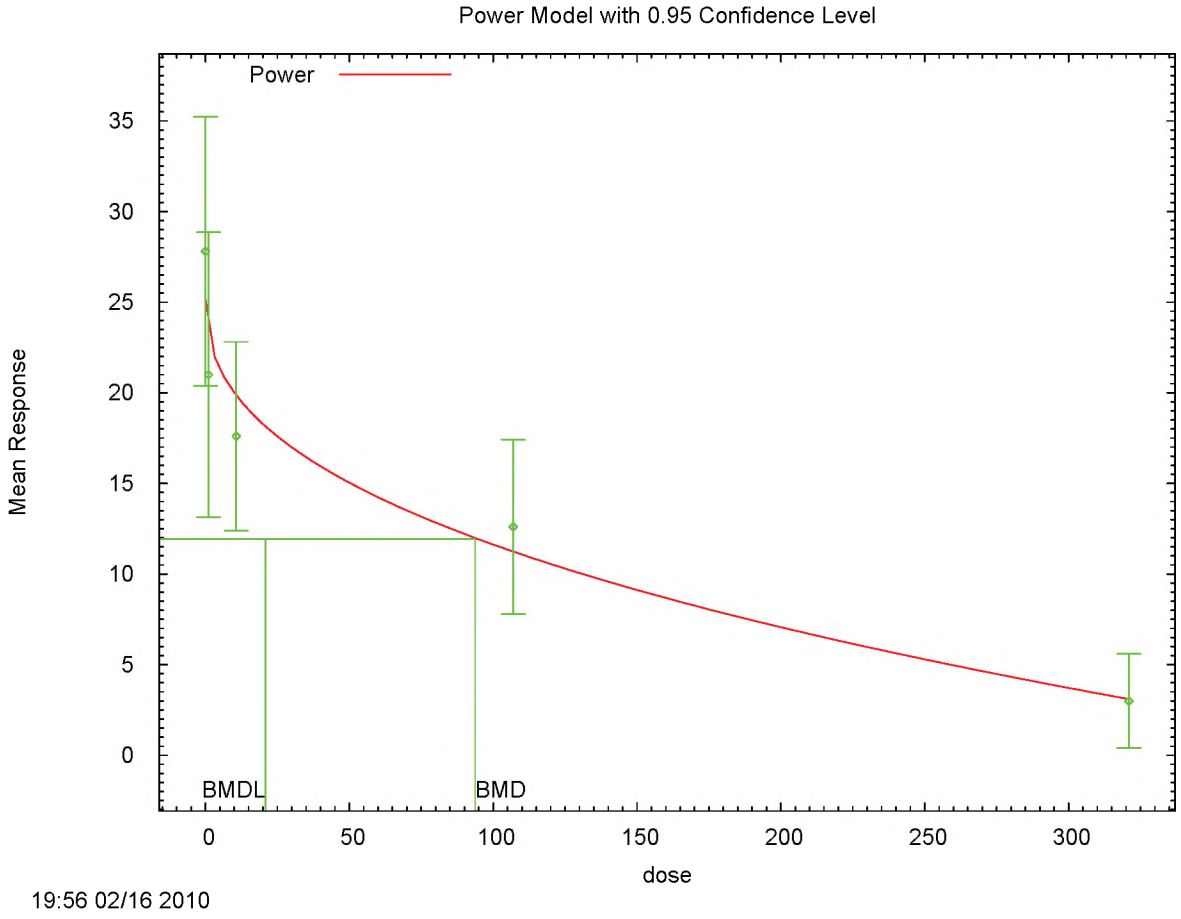
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 93.7416


            BMDL = 20.8758
```

E.3.44.3. *Figure for Selected Model: Power, Unrestricted*



E.3.44.4. *Output for Additional Model Presented: Power*

Smialowicz et al., 2008: PFC per Spleen

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\1\61_Smial_2008_PFCspleen_Pwr_1.(d)
         Gnuplot Plotting File:  C:\1\61_Smial_2008_PFCspleen_Pwr_1.plt
                                          Tue Feb 16 19:56:25 2010
 ================================================================

 Anti Response to SRBCs - PFC x 10 to the 4 per spleen, Table 4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
```

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	4.76607
rho =	0
control =	27.8
slope =	-54.5244
power =	-0.136501

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	control	slope
lalpha	1	-0.98	0.16	-0.48
rho	-0.98	1	-0.25	0.54
control	0.16	-0.25	1	-0.88
slope	-0.48	0.54	-0.88	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	0.474614	1.09569	-1.6729	2.62213
rho	1.48709	0.385029	0.732449	2.24173
control	21.3571	1.69233	18.0402	24.674
slope	-0.0574184	0.00632057	-0.0698064	-0.0450303
power	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	15	27.8	21.4	13.4	12.3	2.02
1.07	14	21	21.3	13.6	12.3	-0.0898
10.7	15	17.6	20.7	9.4	12.1	-1.01
107	15	12.6	15.2	8.7	9.6	-1.05
321	8	3	2.93	3.1	2.82	0.0745

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)

```
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -190.565019           6     393.130038
             A2         -181.476284          10     382.952569
             A3         -181.900030           7     377.800059
          fitted        -185.947278           4     379.894555
              R         -204.636496           2     413.272993


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              46.3204          8          <.0001
   Test 2              18.1775          4        0.001139
   Test 3              0.84749          3          0.8381
   Test 4               8.0945          3          0.0441

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


               Benchmark Dose Computation

Specified effect =            1

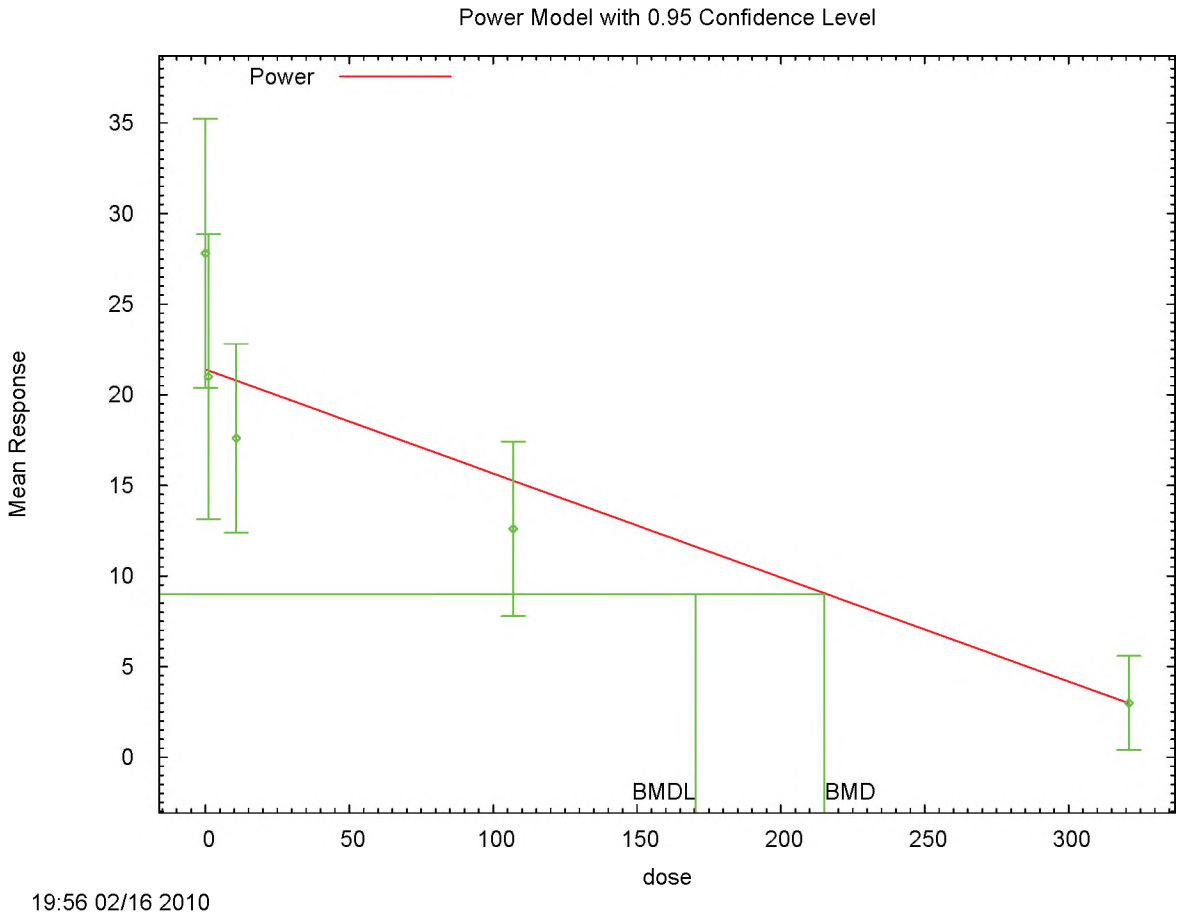
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 215.073


            BMDL = 170.412
```

E.3.44.5. *Figure for Additional Model Presented: Power*



E.3.45. Toth et al., 1979: Amyloidosis

E.3.45.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	2	0.022	150.666	2.296E+02	1.460E+02	power bound hit (power = 1)
logistic	2	0.013	152.187	4.088E+02	3.125E+02	negative intercept (intercept = -2.098)
log-logistic [a]	**2**	**0.028**	**149.984**	**1.759E+02**	**9.729E+01**	**slope bound hit (slope = 1)**
log-probit	2	0.007	153.479	4.402E+02	2.965E+02	slope bound hit (slope = 1)
multistage, 3-degree	2	0.022	150.666	2.296E+02	1.460E+02	final ß = 0
probit	2	0.014	152.040	3.846E+02	2.911E+02	negative intercept (intercept = -1.238)
Weibull	2	0.022	150.666	2.296E+02	1.460E+02	power bound hit (power = 1)
gamma, unrestricted	2	0.917	140.208	7.687E-01	7.637E-04	unrestricted (power = 0.187)
log-logistic, unrestricted [b]	2	0.847	140.370	8.465E-01	1.565E-03	unrestricted (slope = 0.238)
log-probit, unrestricted	2	0.811	140.458	8.545E-01	2.334E-03	unrestricted (slope = 0.135)
Weibull, unrestricted	2	0.882	140.287	8.179E-01	1.140E-03	unrestricted (power = 0.212)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.3.45.2. *Output for Selected Model: Log-Logistic*

Toth et al., 1979: Amyloidosis

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\62_Toth_1979_Amy1yr_LogLogistic_1.(d)
         Gnuplot Plotting File:  C:\1\62_Toth_1979_Amy1yr_LogLogistic_1.plt
                                              Tue Feb 16 19:56:59 2010
 ================================================================

 Table 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = DichEff
   Independent variable = Dose
```

```
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =            0
                     intercept =     -6.90711
                         slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             background    intercept

background            1        -0.47

 intercept        -0.47            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background        0.0848984            *               *                   *
      intercept         -7.36716            *               *                   *
          slope                1            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -68.017         4
   Fitted model        -72.9918         2        9.9496      2         0.00691
  Reduced model        -82.0119         1         27.99      3         <.0001

           AIC:         149.984


                                 Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
    0.0000     0.0849         3.226     0.000          38       -1.878
    1.0000     0.0855         3.761     5.000          44        0.668
  100.0000     0.1393         6.128    10.000          44        1.686
 1000.0000     0.4392        18.884    17.000          43       -0.579

 Chi^2 = 7.15      d.f. = 2        P-value = 0.0280


   Benchmark Dose Computation

Specified effect =           0.1
```

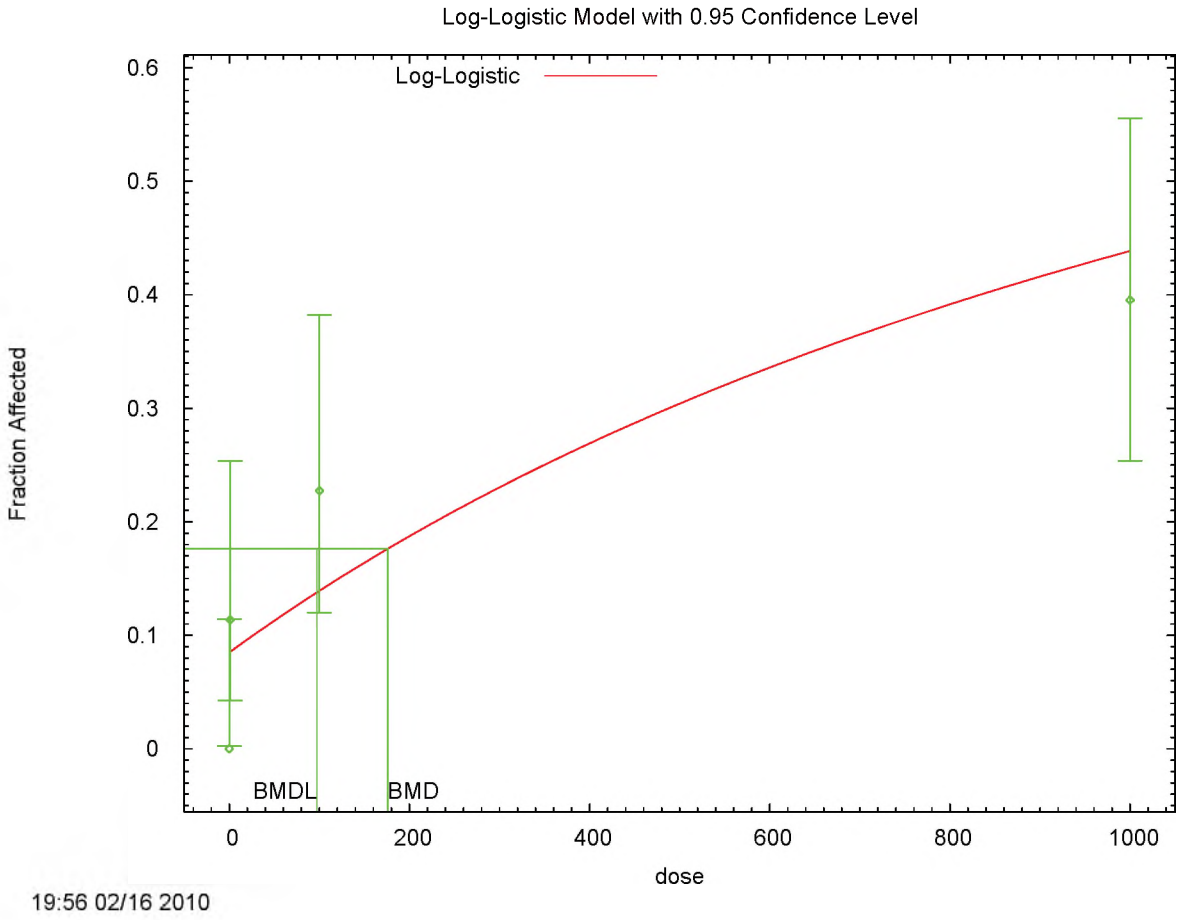
```
Risk Type          =      Extra risk

Confidence level   =           0.95

              BMD  =        175.903

             BMDL  =        97.2899
```

E.3.45.3. *Figure for Selected Model: Log-Logistic*



E.3.45.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

Toth et al., 1979: Amyloidosis

```
 ========================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\62_Toth_1979_Amylyr_LogLogistic_U_1.(d)
         Gnuplot Plotting File:  C:\1\62_Toth_1979_Amylyr_LogLogistic_U_1.plt
                                                 Tue Feb 16 19:57:00 2010
 ========================================================================

 Table 2
 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

```
The form of the probability function is:

P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


Dependent variable = DichEff
Independent variable = Dose
Slope parameter is not restricted

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



User has chosen the log transformed model


                Default Initial Parameter Values
                   background =            0
                    intercept =     -2.10894
                        slope =     0.227921


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -background
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

             intercept        slope

 intercept            1        -0.89

     slope        -0.89            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background                0            *               *                   *
      intercept         -2.15753            *               *                   *
          slope         0.238304            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -68.017         4
   Fitted model        -68.1848         2       0.33571      2          0.8455
  Reduced model        -82.0119         1         27.99      3         <.0001

           AIC:          140.37


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
     0.0000     0.0000         0.000     0.000          38        0.000
     1.0000     0.1036         4.560     5.000          44        0.218
   100.0000     0.2573        11.321    10.000          44       -0.456
  1000.0000     0.3749        16.119    17.000          43        0.277
```

```
 Chi^2 = 0.33      d.f. = 2        P-value = 0.8471


   Benchmark Dose Computation

Specified effect =            0.1

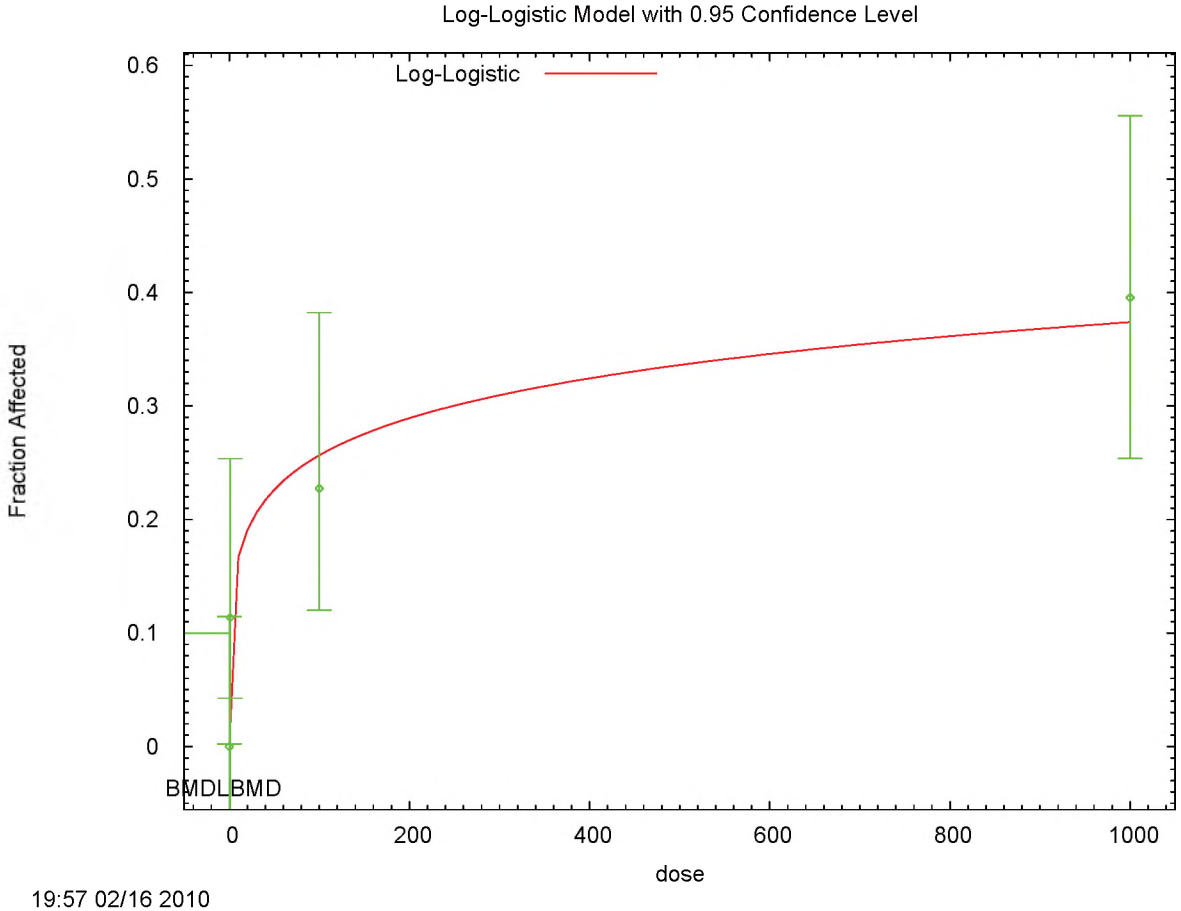
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.846547

            BMDL =     0.00156534
```

E.3.45.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.3.46. Toth et al., 1979: Skin Lesions

E.3.46.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
gamma	2	0.009	159.223	1.181E+02	8.308E+01	power bound hit (power = 1)
logistic [a]	**2**	**0.002**	**162.974**	**2.709E+02**	**2.147E+02**	**negative intercept (intercept = -2.098)**
log-logistic	2	0.029	156.567	6.750E+01	4.057E+01	slope bound hit (slope = 1)
log-probit	2	0.001	164.598	2.446E+02	1.626E+02	slope bound hit (slope = 1)
multistage, 3-degree	2	0.009	159.223	1.181E+02	8.308E+01	final ß = 0
probit	2	0.003	162.684	2.522E+02	2.015E+02	negative intercept (intercept = -1.238)
Weibull	2	0.009	159.223	1.181E+02	8.308E+01	power bound hit (power = 1)
gamma, unrestricted	2	0.882	147.287	error	error	unrestricted (power = 0.251)
log-logistic, unrestricted [b]	2	0.630	147.969	1.137E+00	5.477E-02	unrestricted (slope = 0.351)
log-probit, unrestricted	2	0.558	148.218	1.096E+00	6.847E-02	unrestricted (slope = 0.202)
Weibull, unrestricted	2	0.762	147.581	1.077E+00	4.080E-02	unrestricted (power = 0.3)

[a] Best-fitting model, BMDS output presented in this appendix

[b] Alternate model, BMDS output also presented in this appendix

E.3.46.2. *Output for Selected Model: Logistic*

Toth et al., 1979: Skin Lesions

```
 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File: C:\1\63_Toth_1979_SkinLes_Logistic_1.(d)
         Gnuplot Plotting File:  C:\1\63_Toth_1979_SkinLes_Logistic_1.plt
                                              Tue Feb 16 19:57:29 2010
 ================================================================

 Table 2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = 1/[1+EXP(-intercept-slope*dose)]


   Dependent variable = DichEff
   Independent variable = Dose
```

```
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                    background =            0   Specified
                     intercept =     -2.53484
                         slope =   0.00299511


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -background
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

             intercept        slope

intercept            1        -0.67

    slope        -0.67            1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     intercept         -1.91768          0.26892            -2.44475            -1.39061
         slope       0.00230499      0.000419329          0.00148312          0.00312686



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model         -71.5177         4
  Fitted model          -79.487         2       15.9387      2       0.0003459
 Reduced model         -95.8498         1       48.6642      3         <.0001

          AIC:         162.974


                                 Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
     0.0000     0.1281         4.869     0.000          38       -2.363
     1.0000     0.1284         5.649     5.000          44       -0.292
   100.0000     0.1561         6.870    13.000          44        2.546
  1000.0000     0.5956        25.612    25.000          43       -0.190

 Chi^2 = 12.19     d.f. = 2        P-value = 0.0023


   Benchmark Dose Computation

Specified effect =           0.1

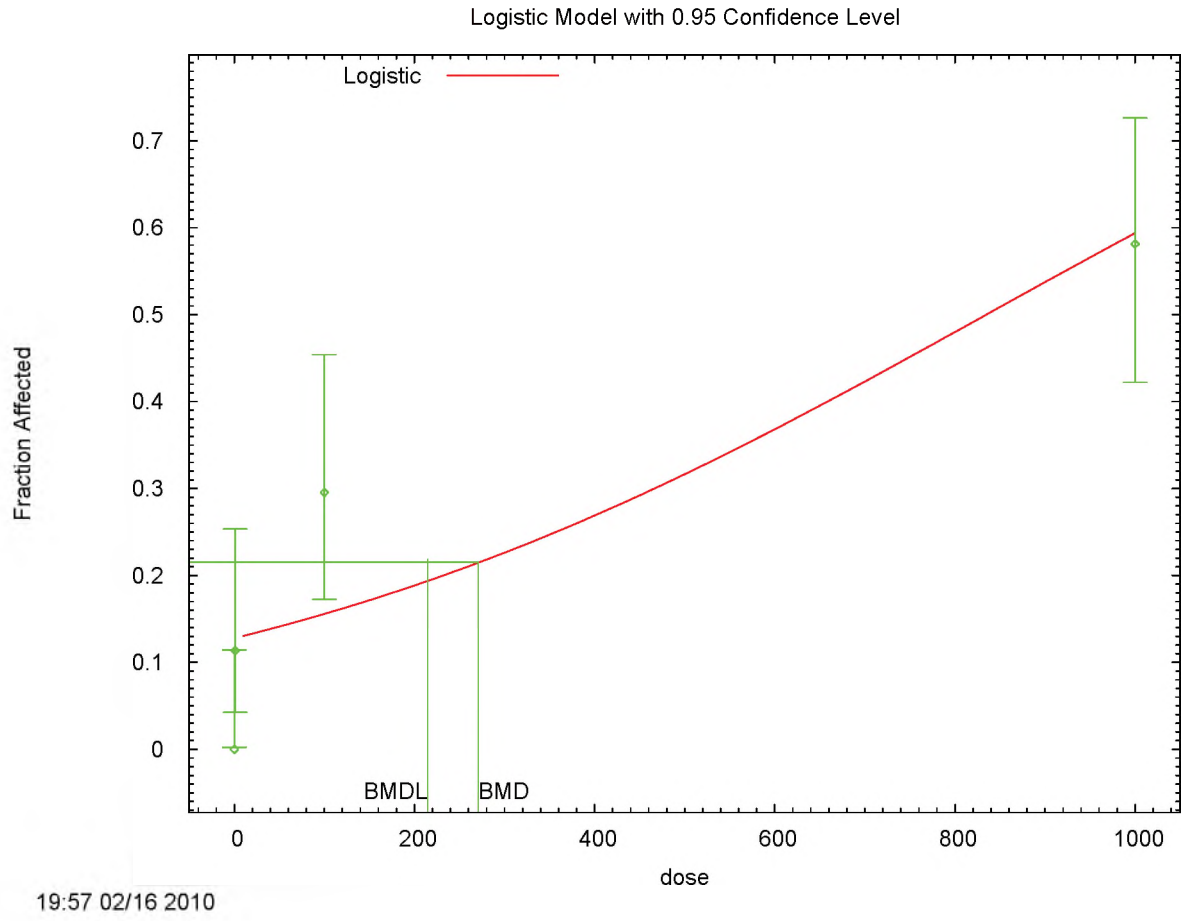
Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =        270.917
```

```
BMDL =          214.66
```

E.3.46.3. *Figure for Selected Model: Logistic*



E.3.46.4. *Output for Additional Model Presented: Log-Logistic, Unrestricted*

Toth et al., 1979: Skin Lesions

```
========================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\1\63_Toth_1979_SkinLes_LogLogistic_U_1.(d)
        Gnuplot Plotting File:  C:\1\63_Toth_1979_SkinLes_LogLogistic_U_1.plt
                                          Tue Feb 16 20:01:56 2010
========================================================================

Table 2
------------------------------------------------------------------------

  The form of the probability function is:

  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


  Dependent variable = DichEff
```

```
  Independent variable = Dose
  Slope parameter is not restricted

  Total number of observations = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008



  User has chosen the log transformed model


                Default Initial Parameter Values
                   background =            0
                    intercept =     -2.14055
                        slope =     0.332409


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -background
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

             intercept        slope

 intercept            1         -0.9

     slope         -0.9            1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background                0               *                 *                   *
      intercept         -2.24241               *                 *                   *
          slope         0.350932               *                 *                   *

* - Indicates that this value is not calculated.


                         Analysis of Deviance Table

        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -71.5177         4
   Fitted model         -71.9844         2       0.93345      2          0.6271
  Reduced model         -95.8498         1       48.6642      3         <.0001

           AIC:          147.969


                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
    0.0000     0.0000          0.000     0.000          38         0.000
    1.0000     0.0960          4.224     5.000          44         0.397
  100.0000     0.3483         15.327    13.000          44        -0.736
 1000.0000     0.5453         23.448    25.000          43         0.475

 Chi^2 = 0.93      d.f. = 2        P-value = 0.6295


   Benchmark Dose Computation
```

```
Specified effect =          0.1

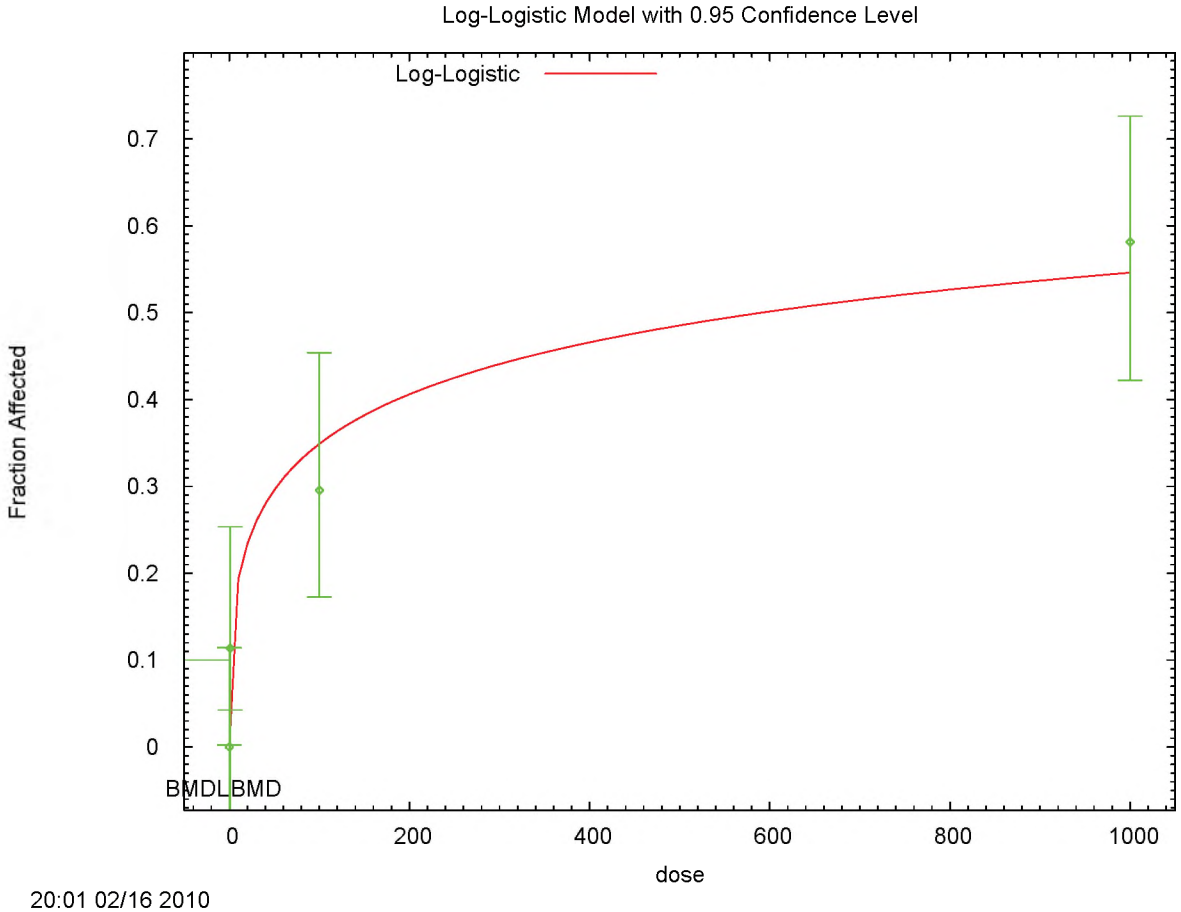
Risk Type        =      Extra risk

Confidence level =         0.95

             BMD =       1.1374

            BMDL =    0.0547689
```

E.3.46.5. *Figure for Additional Model Presented: Log-Logistic, Unrestricted*



E.3.47. Van Birgelen et al., 1995a: Hepatic Retinol

E.3.47.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	4	<0.0001	164.340	2.912E+02	error	
exponential (M3)	4	<0.0001	164.340	2.912E+02	error	power hit bound (d = 1)
exponential (M4) [b]	**3**	**<0.0001**	**148.052**	**1.151E+02**	**7.098E+01**	
exponential (M5)	3	<0.0001	148.052	1.151E+02	7.098E+01	power hit bound (d = 1)
Hill	3	0.044	128.757	1.314E+01	error	n lower bound hit (n = 1)
linear	4	<0.0001	178.734	7.815E+02	5.997E+02	
polynomial, 5-degree	0	N/A	283.606	2.481E+03	error	
power	4	<0.0001	178.734	7.815E+02	5.997E+02	power bound hit (power = 1)
Hill, unrestricted	2	0.269	125.273	5.561E+00	error	unrestricted (n = 0.571)
power, unrestricted [c]	3	0.025	129.990	4.205E-01	8.504E-03	unrestricted (power = 0.118)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.47.2. *Output for Selected Model: Exponential (M4)*

Van Birgelen et al., 1995a: Hepatic Retinol

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\1\65_VanB_1995a_HepRet_Exp_1.(d)
         Gnuplot Plotting File:
                                                Tue Feb 16 20:03:05 2010
 ================================================================

 Tbl3, hepatic retinol
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
```

```
      sign = -1 for decreasing trend.

   Model 2 is nested within Models 3 and 4.
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


              Initial Parameter Values

              Variable          Model 4
              --------          --------
                lnalpha            -1.16065
                    rho             1.53688
                      a              15.645
                      b           0.00625117
                      c            0.0365247
                      d                    1



                Parameter Estimates

               Variable          Model 4
               --------          -------
                lnalpha           -0.882225
                    rho             1.82707
                      a             10.5294
                      b           0.00720346
                      c            0.0688661
                      d                    1


        Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
     0      8         14.9         8.768
    14      8          8.4         3.394
    26      8          8.2         2.263
    47      8          5.1        0.8485
   320      8          2.2        0.8485
  1024      8          0.6        0.5657


              Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
  ------    ----------    ---------    ----------------
      0         10.53        5.526            2.237
     14         9.589        5.073          -0.6628
     26         8.855        4.717          -0.3926
     47         7.714        4.159           -1.778
    320         1.703        1.046            1.343
   1024        0.7313       0.4833          -0.7681
```

Other models for which likelihoods are calculated:

```
  Model A1:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-87.1567	7	188.3134
A2	-47.28742	12	118.5748
A3	-55.32422	8	126.6484
R	-109.967	2	223.934
4	-69.02619	5	148.0524

Additive constant for all log-likelihoods = -44.11. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	125.4	10	< 0.0001
Test 2	79.74	5	< 0.0001
Test 3	16.07	4	0.002922
Test 6a	27.4	3	< 0.0001

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 6a is less than .1. Model 4 may not adequately describe the data; you may want to consider another model.

Benchmark Dose Computations:

Specified Effect = 1.000000

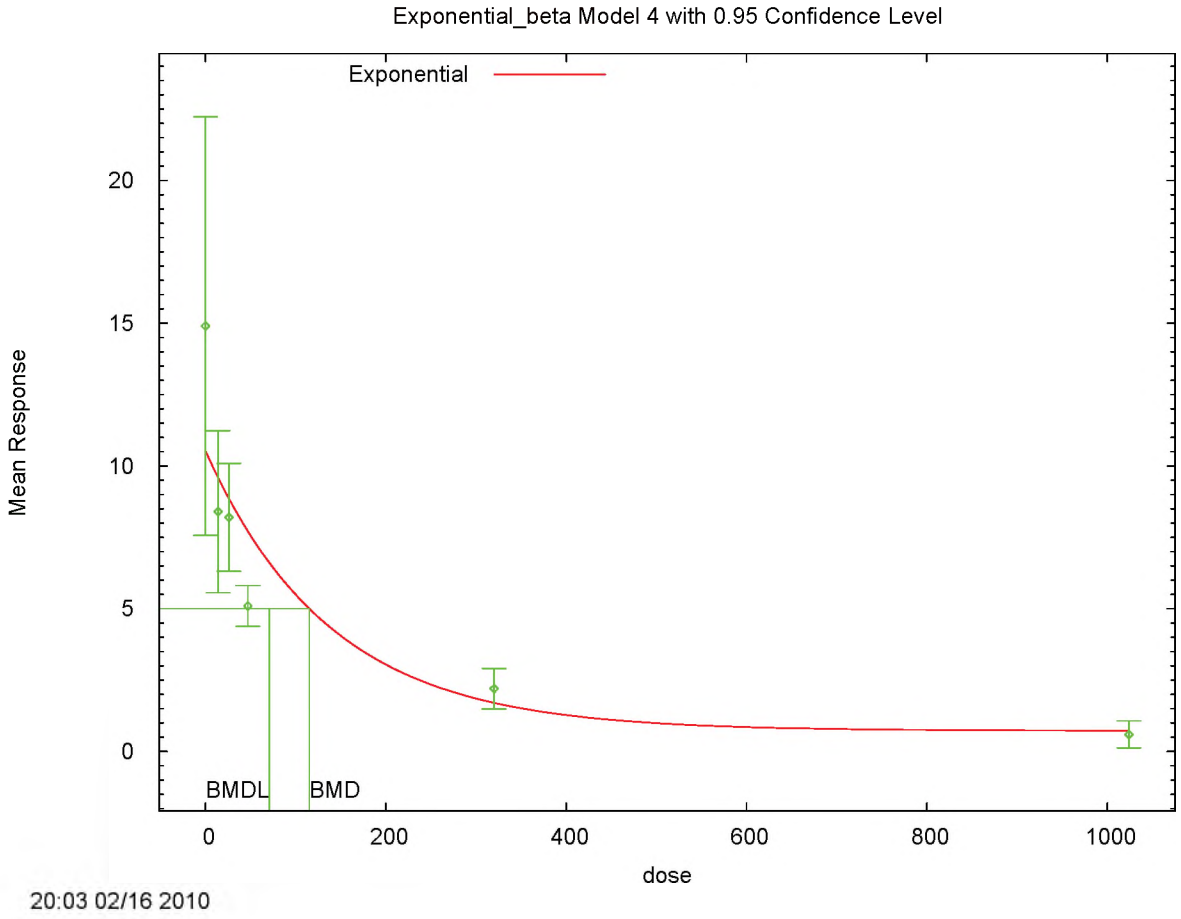
```
            Risk Type = Estimated standard deviations from control

      Confidence Level = 0.950000

                   BMD =      115.128

                  BMDL =       70.981
```

E.3.47.3. *Figure for Selected Model: Exponential (M4)*



E.3.47.4. *Output for Additional Model Presented: Power, Unrestricted*

Van Birgelen et al., 1995a: Hepatic Retinol

```
  ================================================================
          Power Model. (Version: 2.15;  Date: 04/07/2008)
          Input Data File: C:\1\65_VanB_1995a_HepRet_Pwr_U_1.(d)
          Gnuplot Plotting File:  C:\1\65_VanB_1995a_HepRet_Pwr_U_1.plt
                                                Tue Feb 16 20:03:11 2010
  ================================================================

  Tbl3, hepatic retinol
 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the response function is:
```

```
Y[dose] = control + slope * dose^power


Dependent variable = Mean
Independent variable = Dose
The power is not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                Default Initial Parameter Values
                       lalpha =      2.76506
                          rho =            0
                      control =         14.9
                        slope =     -3.78637
                        power =     0.191713


          Asymptotic Correlation Matrix of Parameter Estimates

                lalpha          rho      control        slope        power

    lalpha           1         -0.8       -0.047        0.042        0.065

       rho        -0.8            1       -0.085      -0.0029        -0.11

   control      -0.047       -0.085            1        -0.95        -0.81

     slope       0.042      -0.0029        -0.95            1         0.96

     power       0.065        -0.11        -0.81         0.96            1


                                Parameter Estimates

                                                    95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha         -1.02622         0.389164            -1.78897           -0.263475
            rho          1.68421         0.199212             1.29376             2.07466
        control          16.9577          2.21133             12.6235             21.2918
          slope         -7.19097          1.99708            -11.1052            -3.27676
          power         0.117935        0.0225396           0.0737578            0.162111


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0       8       14.9           17         8.77         6.49         -0.896
   14       8        8.4         7.14         3.39         3.13           1.14
   26       8        8.2          6.4         2.26         2.86           1.78
   47       8        5.1         5.63        0.849         2.57         -0.588
  320       8        2.2         2.76        0.849         1.41          -1.12
 1024       8        0.6        0.672        0.566        0.428         -0.475


 Model Descriptions for likelihoods calculated
```

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          -87.156698           7     188.313395
              A2          -47.287416          12     118.574833
              A3          -55.324218           8     126.648436
          fitted          -59.994980           5     129.989960
               R         -109.967018           2     223.934036


                    Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              125.359         10          <.0001
  Test 2              79.7386          5          <.0001
  Test 3              16.0736          4        0.002922
  Test 4              9.34152          3         0.02508

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is less than .1.  You may want to try a different
model


               Benchmark Dose Computation

Specified effect =             1

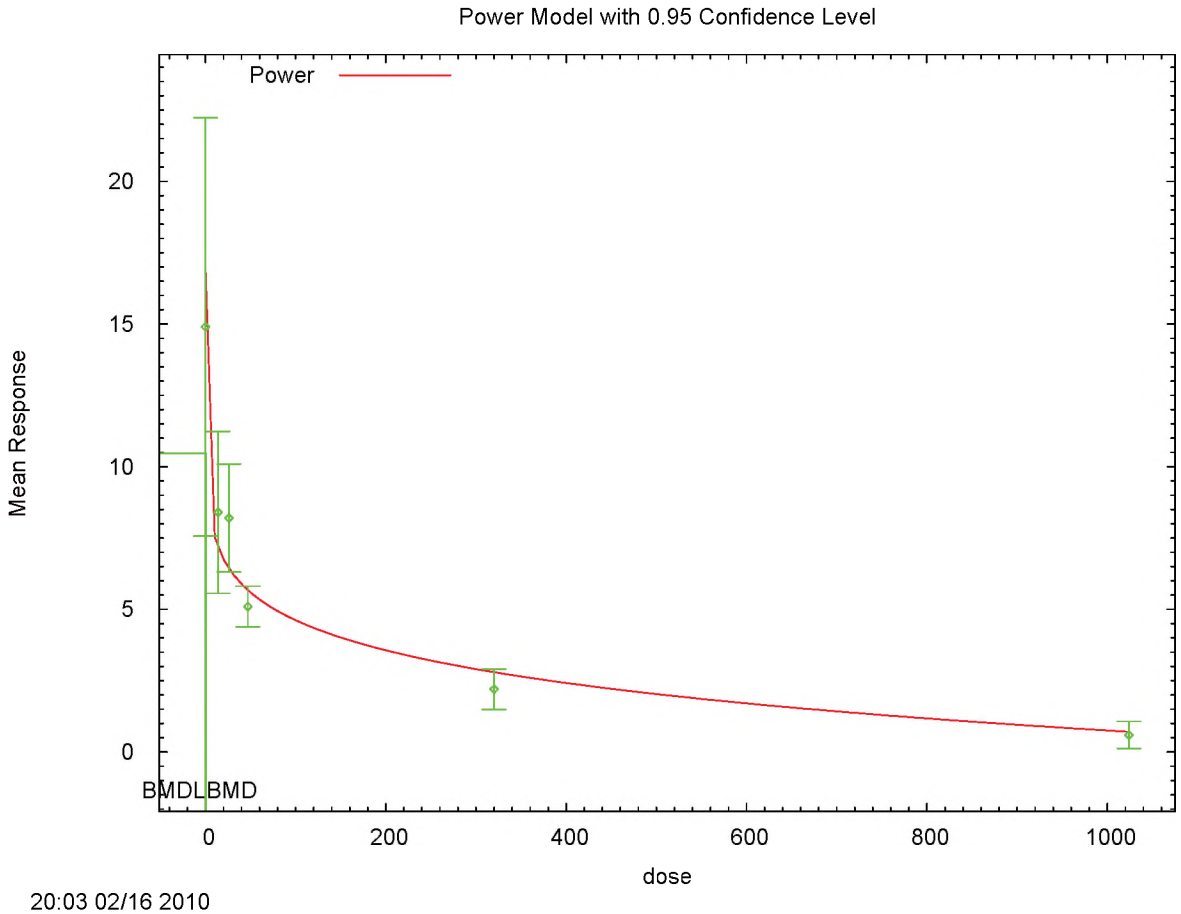
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 0.420475


            BMDL = 0.00850422
```

E.3.47.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.3.48. Van Birgelen et al., 1995a: Hepatic Retinol Palmitate

E.3.48.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	4	<0.0001	467.446	error	error	
exponential (M3)	4	<0.0001	467.446	error	error	power hit bound (d = 1)
exponential (M4)	3	<0.0001	454.087	error	error	
exponential (M5)	3	<0.0001	454.087	error	error	power hit bound (d = 1)
Hill	3	<0.0001	563.579	error	error	
linear [b]	**4**	**<0.0001**	**488.446**	**1.420E+03**	**9.889E+02**	
polynomial, 5-degree	0	N/A	573.977	error	error	
power	4	<0.0001	488.446	1.420E+03	9.889E+02	power bound hit (power = 1)
Hill, unrestricted	3	<0.0001	522.322	2.418E-12	2.418E-12	unrestricted (n = 0.452)
power, unrestricted [c]	3	0.348	408.062	3.765E-02	1.208E-05	unrestricted (power = 0.054)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.48.2. *Output for Selected Model: Linear*

Van Birgelen et al., 1995a: Hepatic Retinol Palmitate

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\1\66_VanB_1995a_HepRetPalm_Linear_1.(d)
         Gnuplot Plotting File:  C:\1\66_VanB_1995a_HepRetPalm_Linear_1.plt
                                              Tue Feb 16 20:03:46 2010
 ================================================================

 Tbl3, hepatic retinol palmitate
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
```

The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	9.57332
rho =	0
beta_0 =	177.506
beta_1 =	-0.204775

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-0.95	-0.017	0.022
rho	-0.95	1	0.00019	-0.0048
beta_0	-0.017	0.00019	1	-1
beta_1	0.022	-0.0048	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-0.723216	0.638291	-1.97424	0.527811
rho	2.26615	0.140196	1.99137	2.54093
beta_0	150.535	31.5457	88.7064	212.363
beta_1	-0.143931	0.0308317	-0.20436	-0.0835018

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	472	151	272	204	4.45
14	8	94	149	67.9	201	-0.766
26	8	107	147	76.4	199	-0.567
47	8	74	144	39.6	194	-1.02
320	8	22	104	22.6	135	-1.73
1024	8	3	3.15	2.83	2.56	-0.166

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

```
Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -250.554817           7     515.109634
             A2         -196.755746          12     417.511491
             A3         -197.383174           8     410.766347
          fitted        -240.223107           4     488.446215
              R         -276.789644           2     557.579287


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              160.068         10          <.0001
   Test 2              107.598          5          <.0001
   Test 3              1.25486          4           0.869
   Test 4              85.6799          4          <.0001

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


              Benchmark Dose Computation

Specified effect =             1

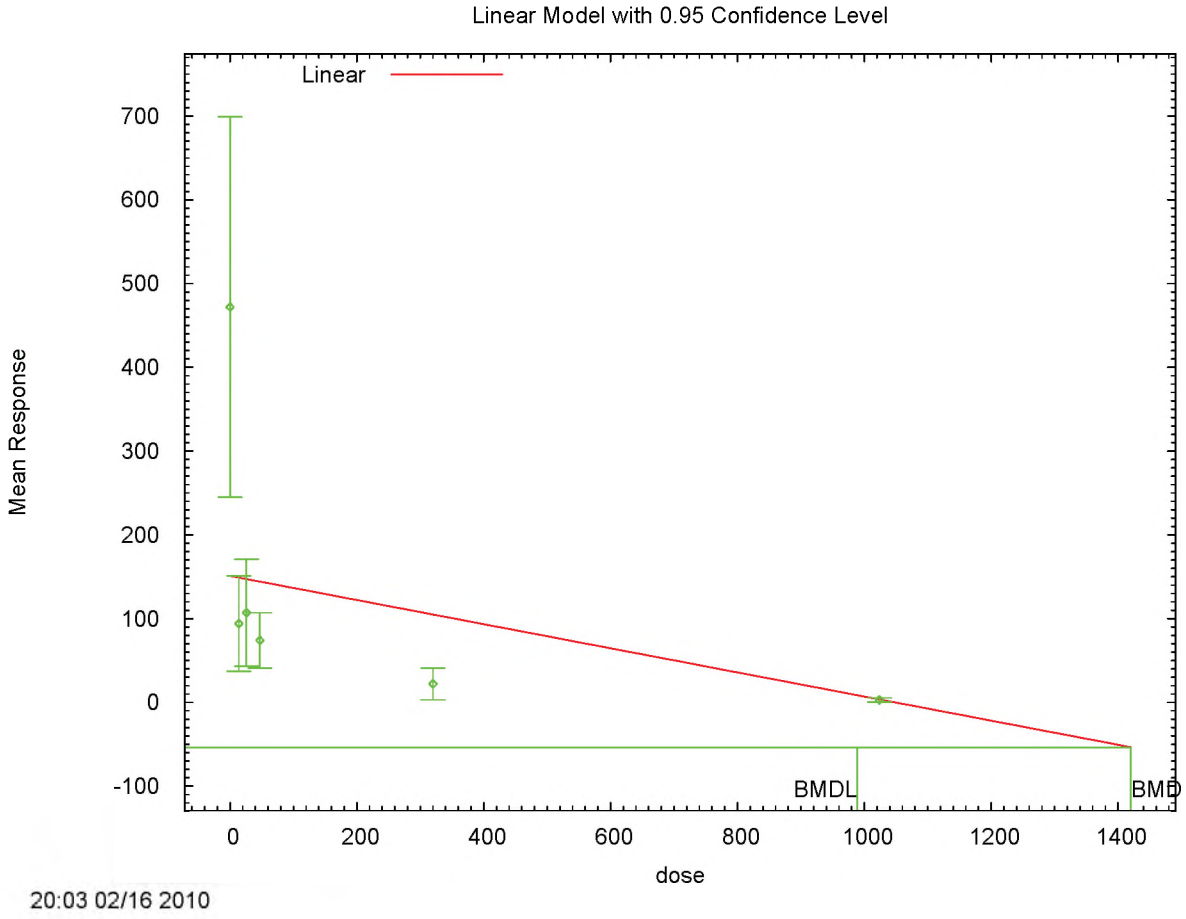
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD =        1419.81


             BMDL =        988.945
```

E.3.48.3. *Figure for Selected Model: Linear*



E.3.48.4. *Output for Additional Model Presented: Power, Unrestricted*

Van Birgelen et al., 1995a: Hepatic Retinol Palmitate

```
 ======================================================================
          Power Model. (Version: 2.15;  Date: 04/07/2008)
          Input Data File: C:\1\66_VanB_1995a_HepRetPalm_Pwr_U_1.(d)
          Gnuplot Plotting File:  C:\1\66_VanB_1995a_HepRetPalm_Pwr_U_1.plt
                                                Tue Feb 16 20:03:50 2010
 ======================================================================

 Tbl3, hepatic retinol palmitate
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the response function is:

    Y[dose] = control + slope * dose^power


    Dependent variable = Mean
    Independent variable = Dose
    The power is not restricted
    The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
```

```
Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =      9.57332
                            rho =            0
                        control =          472
                          slope =     -315.054
                          power =    0.0586881


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho      control        slope        power

  lalpha              1        -0.95         0.29        -0.31         -0.3

     rho          -0.95            1        -0.4         0.39         0.29

 control           0.29        -0.4            1        -0.98        -0.82

   slope          -0.31         0.39        -0.98            1         0.91

   power           -0.3         0.29        -0.82         0.91            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
        lalpha        0.0734958         0.849559           -1.59161              1.7386
           rho          1.80632         0.194602            1.42491             2.18774
       control          465.497           86.914            295.149             635.845
         slope          -318.06          82.4127           -479.586            -156.534
         power        0.0540573        0.0117709          0.0309869           0.0771278


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0       8        472          465          272          266          0.069
   14       8         94         98.7         67.9         65.6         -0.201
   26       8        107         86.2         76.4         58.1           1.01
   47       8         74         73.8         39.6         50.5         0.0086
  320       8         22         31.1         22.6         23.1          -1.11
 1024       8          3         2.86         2.83         2.68          0.145


Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
```

were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-250.554817	7	515.109634
A2	-196.755746	12	417.511491
A3	-197.383174	8	410.766347
fitted	-199.031154	5	408.062307
R	-276.789644	2	557.579287

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	160.068	10	<.0001
Test 2	107.598	5	<.0001
Test 3	1.25486	4	0.869
Test 4	3.29596	3	0.3482

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

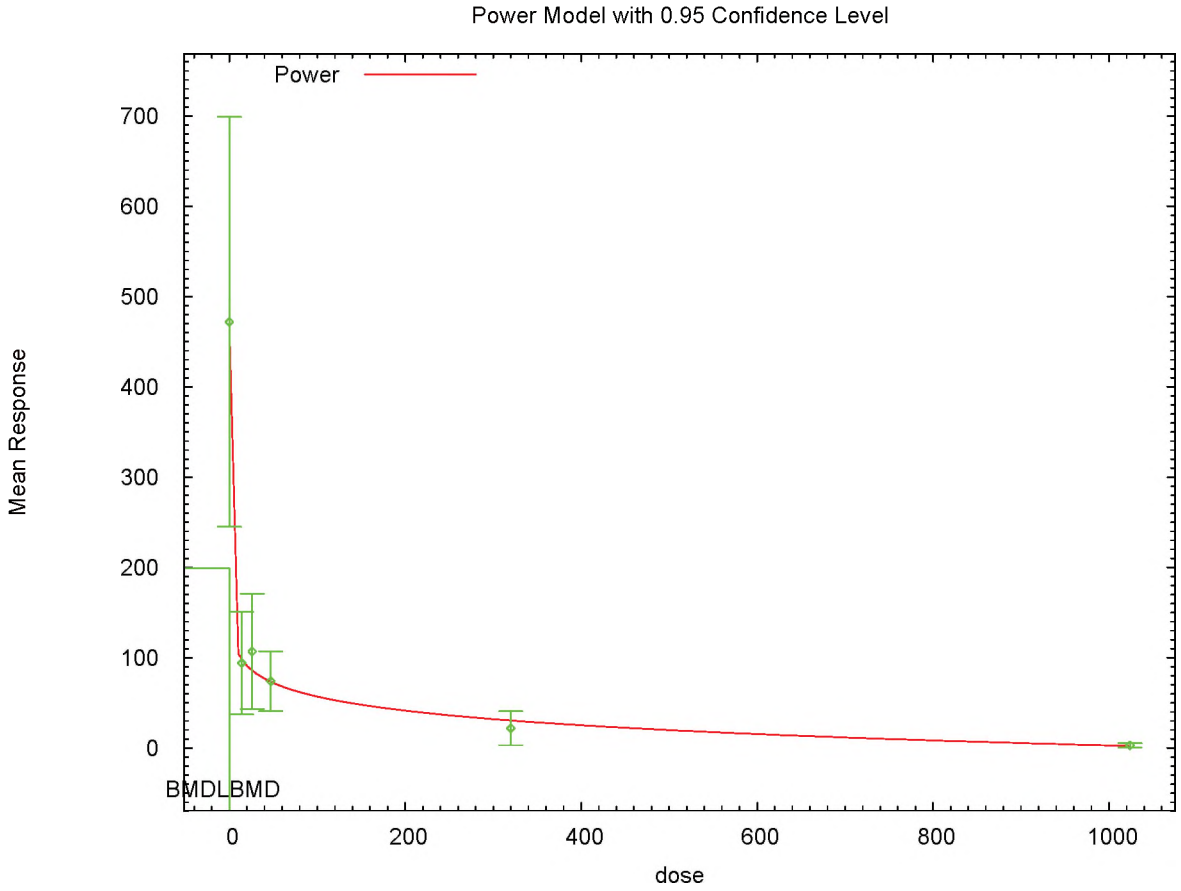
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 0.0376489

BMDL = 1.20769e-005

E.3.48.5. *Figure for Additional Model Presented: Power, Unrestricted*



E.3.49. White et al., 1986: CH50

E.3.49.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	5	0.001	391.472	4.480E+02	2.844E+02	
exponential (M3)	5	0.001	391.472	4.480E+02	2.844E+02	power hit bound (d = 1)
exponential (M4)	4	0.001	392.128	3.126E+02	1.140E+02	
exponential (M5)	4	0.001	392.128	3.126E+02	1.140E+02	power hit bound (d = 1)
Hill [b]	**4**	**0.001**	**391.223**	**2.042E+02**	**3.585E+01**	**n lower bound hit (n = 1)**
linear	5	<0.0001	396.430	8.065E+02	5.899E+02	
polynomial, 6-degree	3	<0.0001	643.059	9.600E+02	error	
power	5	<0.0001	396.430	8.065E+02	5.899E+02	power bound hit (power = 1)
Hill, unrestricted [c]	3	0.058	381.943	9.677E-01	1.900E-01	unrestricted (n = 0.211)
power, unrestricted	4	0.131	379.574	7.186E-01	1.157E-02	unrestricted (power = 0.188)

[a] Non-constant variance model selected ($p = 0.0871$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

E.3.49.2. *Output for Selected Model: Hill*

White et al., 1986: CH50

```
 ================================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\1\71_White_1986_CH50_Hill_1.(d)
         Gnuplot Plotting File:  C:\1\71_White_1986_CH50_Hill_1.plt
                                                 Tue Feb 16 20:06:45 2010
 ================================================================================

 [insert study notes]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
```

The variance is to be modeled as Var(i) = exp(lalpha + rho * ln(mean(i)))

Total number of dose groups = 7
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	5.60999
rho =	0
intercept =	91
v =	-74
n =	0.0969998
k =	10

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	intercept	v	k
lalpha	1	-0.99	0.19	0.13	-0.22
rho	-0.99	1	-0.2	-0.14	0.23
intercept	0.19	-0.2	1	0.33	-0.7
v	0.13	-0.14	0.33	1	-0.86
k	-0.22	0.23	-0.7	-0.86	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	4.34761	1.59601	1.21948	7.47574
rho	0.381496	0.413764	-0.429467	1.19246
intercept	71.6585	5.38454	61.105	82.212
v	-62.7464	14.9646	-92.0765	-33.4163
n	1	NA		
k	441.016	460.151	-460.864	1342.9

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	91	71.7	14.1	19.9	2.75
10	8	54	70.3	8.49	19.8	-2.33
50	8	63	65.3	11.3	19.5	-0.329
100	8	56	60.1	25.5	19.2	-0.598
500	8	41	38.3	17	17.6	0.43
1000	8	32	28.1	17	16.6	0.661
2000	8	17	20.2	17	15.6	-0.589

```
Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -181.340979           8     378.681959
              A2         -175.820265          14     379.640529
              A3         -181.238690           9     380.477380
          fitted         -190.611743           5     391.223485
               R         -212.367055           2     428.734109


                    Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              73.0936         12          <.0001
  Test 2              11.0414          6          0.0871
  Test 3              10.8369          5         0.05471
  Test 4              18.7461          4       0.0008815

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =             1

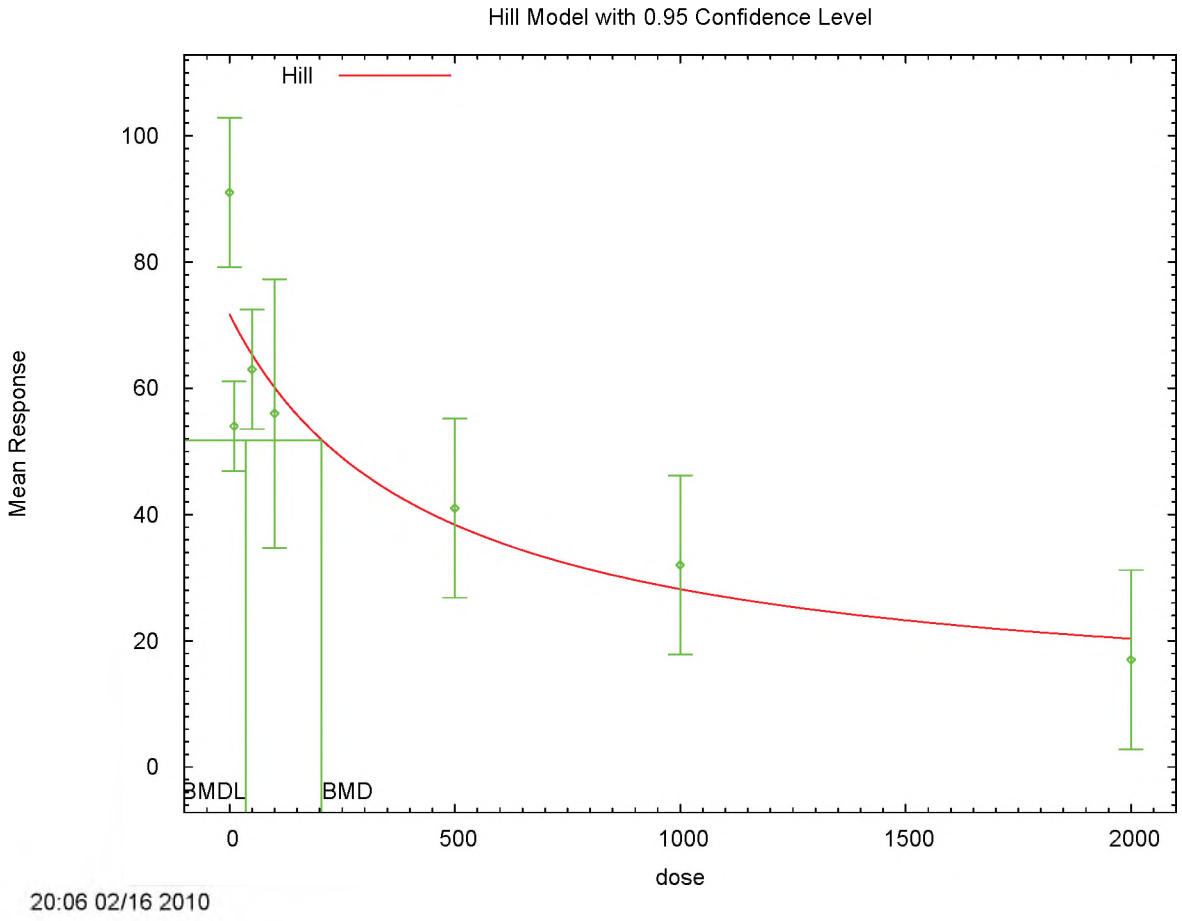
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       204.214
```

```
BMDL =        35.8504
```

E.3.49.3. *Figure for Selected Model: Hill*



E.3.49.4. *Output for Additional Model Presented: Hill, Unrestricted*

White et al., 1986: CH50

```
=============================================================================
        Hill Model. (Version: 2.14;  Date: 06/26/2008)
        Input Data File: C:\1\71_White_1986_CH50_Hill_U_1.(d)
        Gnuplot Plotting File:  C:\1\71_White_1986_CH50_Hill_U_1.plt
                                                Tue Feb 16 20:06:46 2010
=============================================================================

[insert study notes]

  The form of the response function is:

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = Mean
```

```
Independent variable = Dose
Power parameter is not restricted
The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

Total number of dose groups = 7
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                       lalpha =      5.60999
                          rho =            0
                    intercept =           91
                            v =          -74
                            n =    0.0969998
                            k =           10


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho    intercept            v            n            k

    lalpha            1           -1         0.17         0.22        -0.42       -0.022

       rho           -1            1        -0.17        -0.22         0.42        0.019

 intercept         0.17        -0.17            1         0.16        -0.58       0.0069

         v         0.22        -0.22         0.16            1       -0.048        -0.91

         n        -0.42         0.42        -0.58       -0.048            1        -0.35

         k       -0.022        0.019       0.0069        -0.91        -0.35            1


                                 Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha          6.62767          2.14235           2.42875             10.8266
            rho        -0.266376         0.555274          -1.35469            0.821941
      intercept           89.579          5.61106           78.5815             100.576
              v         -458.615          402.837          -1248.16              330.93
              n         0.210614        0.0503369          0.111956            0.309273
              k     9.00638e+006     4.61231e+007      -8.13933e+007       9.94061e+007


    Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0      8         91         89.6         14.1         15.1          0.266
   10      8         54         65.4         8.49         15.8          -2.04
   50      8         63         56.3         11.3         16.1           1.18
  100      8         56         51.5         25.5         16.3          0.777
  500      8         41         37.9           17         16.9          0.516
 1000      8         32         30.8           17         17.4          0.191
 2000      8         17         22.9           17         18.1         -0.927


Model Descriptions for likelihoods calculated
```

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -181.340979           8     378.681959
              A2         -175.820265          14     379.640529
              A3         -181.238690           9     380.477380
          fitted         -184.971691           6     381.943382
               R         -212.367055           2     428.734109


                    Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              73.0936         12          <.0001
  Test 2              11.0414          6          0.0871
  Test 3              10.8369          5         0.05471
  Test 4                7.466          3         0.05844

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =             1

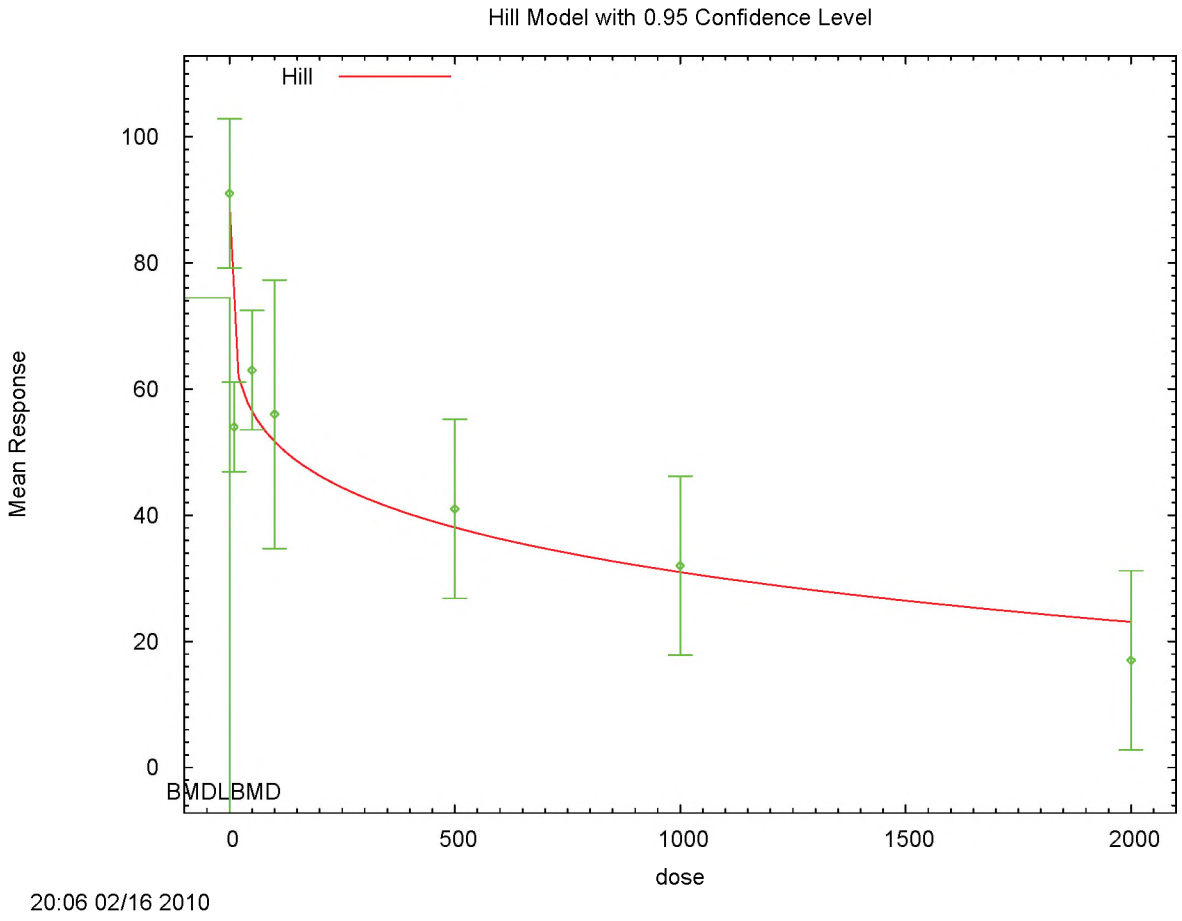
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       0.967689

            BMDL =      0.189992
```

E.3.49.5. *Figure for Additional Model Presented: Hill, Unrestricted*



E.4. REFERENCES

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APPENDIX F

Cancer Benchmark Dose Modeling

NOTICE

THIS DOCUMENT IS AN EXTERNAL REVIEW DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

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APPENDIX F. CANCER BENCHMARK DOSE MODELING

F.1. BLOOD BMDS RESULTS

F.1.1. Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

F.1.1.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**3**	**0.815**	**31.564**	**5.763E+00**	**2.795E+00**	
Multistage Cancer, 2-Degree	3	0.985	30.170	1.369E+01	3.416E+00	
Multistage Cancer, 3-Degree	3	0.999	29.930	1.917E+01	3.578E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.1.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\4\Blood\1_msc1_1Perc_palate_nasal.(d)
         Gnuplot Plotting File:  C:\4\Blood\1_msc1_1Perc_palate_nasal.plt
                                              Thu Apr 01 15:56:03 2010
 ======================================================================

 Source - Table 4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008
```

```
                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =   0.00226154


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                Beta(1)

   Beta(1)            1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background                0            *               *                   *
        Beta(1)        0.0017438            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -13.9385         4
   Fitted model        -14.7819         1       1.68696      3          0.6398
  Reduced model        -20.2589         1       12.6409      3        0.005481

           AIC:         31.5639


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          85        0.000
    1.5617     0.0027         0.136     0.000          50       -0.369
    7.1600     0.0124         0.620     0.000          50       -0.793
   38.7212     0.0653         3.265     4.000          50        0.421

 Chi^2 = 0.94      d.f. = 3        P-value = 0.8153


   Benchmark Dose Computation

Specified effect =           0.01

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        5.76347

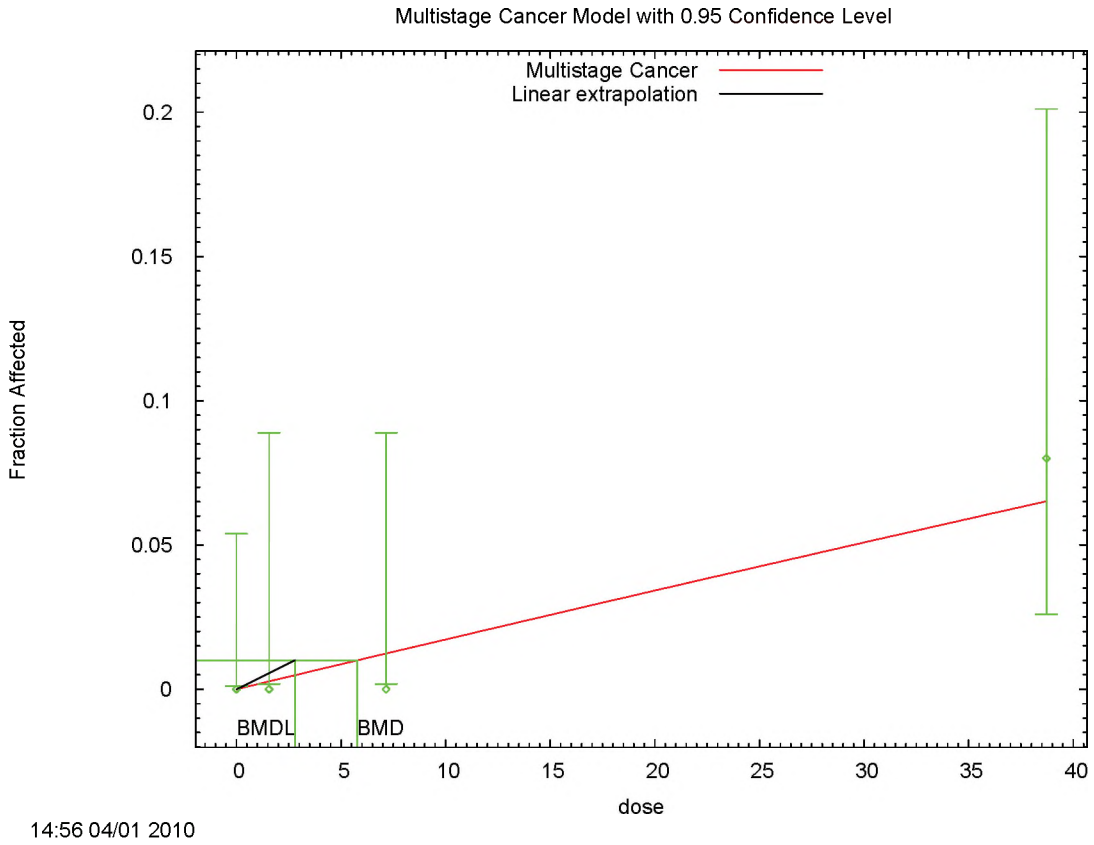
            BMDL =        2.79485

            BMDU =        14.9396

Taken together, (2.79485, 14.9396) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =      0.003578
```

F.1.1.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

F.1.2. Kociba et al., 1978: Stratified squamous cell carcinoma of tongue

F.1.2.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.472**	**47.933**	**6.091E+00**	**2.600E+00**	
Multistage Cancer, 2-Degree	2	0.472	47.933	6.091E+00	2.600E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.472	47.933	6.091E+00	2.600E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.2.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Stratified squamous cell carcinoma of tongue

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\4\Blood\2_msc1_1Perc_tongue.(d)
         Gnuplot Plotting File:  C:\4\Blood\2_msc1_1Perc_tongue.plt
                                                Thu Apr 01 15:56:35 2010
 ======================================================================

 Source - Table 4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0092514
                        Beta(1) =   0.00137224
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.58
Beta(1)	-0.58	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.00510501	*	*	*
Beta(1)	0.00165011	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-21.1523	4			
Fitted model	-21.9667	2	1.62881	2	0.4429
Reduced model	-24.1972	1	6.08976	3	0.1073

AIC: 47.9334

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0051	0.434	0.000	85	-0.660
1.5617	0.0077	0.383	1.000	50	1.000
7.1600	0.0168	0.840	1.000	50	0.177
38.7212	0.0667	3.334	3.000	50	-0.189

Chi^2 = 1.50 d.f. = 2 P-value = 0.4716

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 6.0907

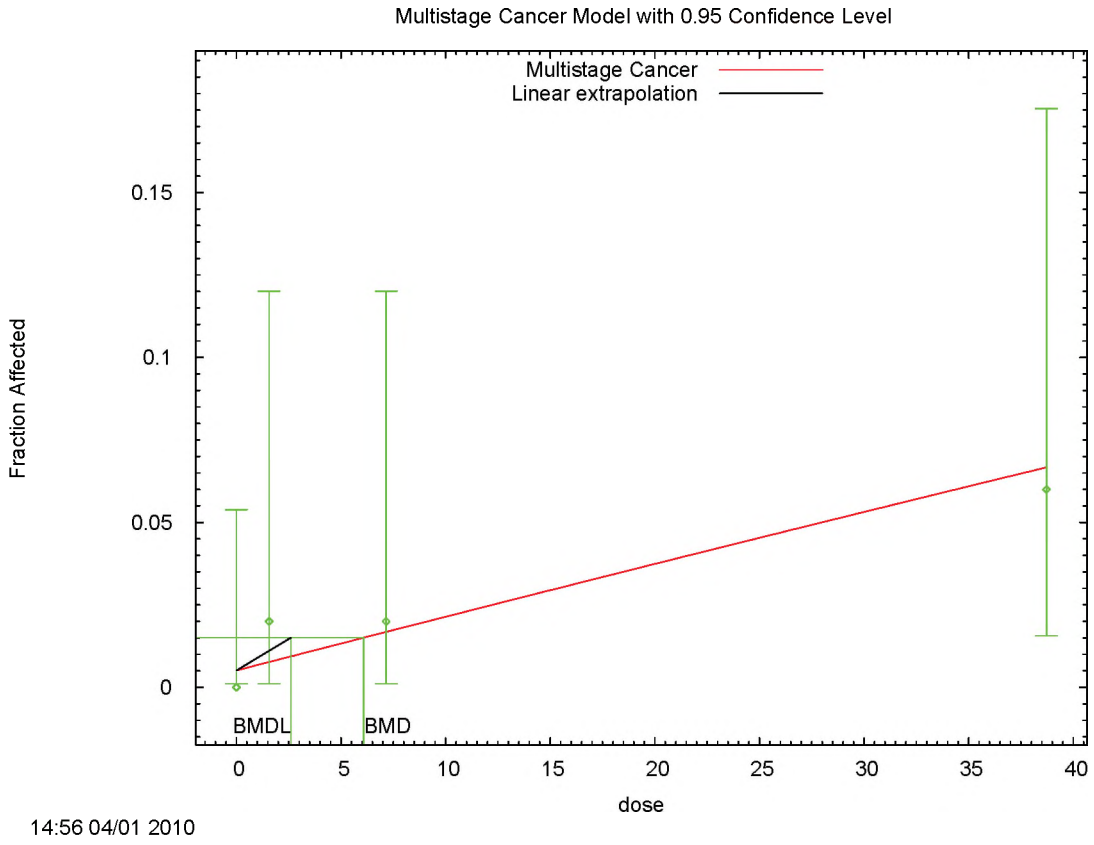
BMDL = 2.60049

BMDU = 519124

Taken together, (2.60049, 519124) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00384542

F.1.2.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Stratified squamous cell carcinoma of tongue

F.1.3. Kociba et al., 1978: Adenoma of adrenal cortex

F.1.3.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**3**	**0.779**	**52.488**	**3.254E+00**	**1.852E+00**	
Multistage Cancer, 2-Degree	3	0.779	52.488	3.254E+00	1.852E+00	final ß=0
Multistage Cancer, 3-Degree	3	0.779	52.488	3.254E+00	1.852E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.3.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Adenoma of adrenal cortex

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\4\Blood\3_msc1_1Perc_adre_adenoma.(d)
         Gnuplot Plotting File:  C:\4\Blood\3_msc1_1Perc_adre_adenoma.plt
                                                Thu Apr 01 15:57:07 2010
 ================================================================

 Source - Table 5
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =   0.00493756
                        Beta(1) =    0.0026639
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00308883	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-24.6514	4			
Fitted model	-25.2438	1	1.18487	3	0.7566
Reduced model	-31.4904	1	13.6781	3	0.003378

AIC: 52.4876

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	85	0.000
1.5617	0.0048	0.241	0.000	50	-0.492
7.1600	0.0219	1.094	2.000	50	0.876
38.7212	0.1127	5.636	5.000	50	-0.285

Chi^2 = 1.09 d.f. = 3 P-value = 0.7793

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.25376

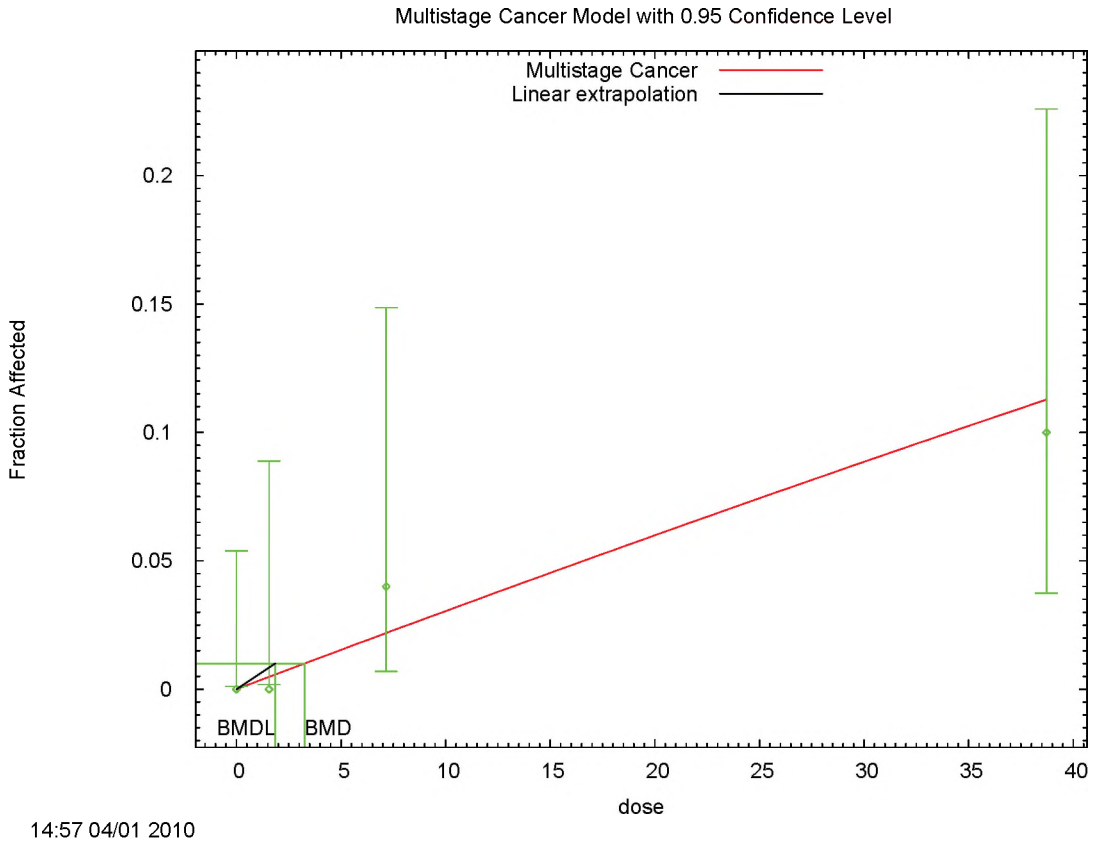
BMDL = 1.85162

BMDU = 6.58595

Taken together, (1.85162, 6.58595) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00540067

F.1.3.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Adenoma of adrenal cortex

F.1.4. Kociba et al., 1978: Hepatocellular adenoma(s) or carcinoma(s)

F.1.4.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.245**	**143.261**	**7.010E-01**	**5.013E-01**	
Multistage Cancer, 2-Degree	2	0.245	143.261	7.010E-01	5.013E-01	final ß=0
Multistage Cancer, 3-Degree	2	0.245	143.261	7.010E-01	5.013E-01	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.4.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Hepatocellular adenoma(s) or carcinoma(s)

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\4\Blood\4_msc1_1Perc_liver_ad_carc.(d)
        Gnuplot Plotting File:  C:\4\Blood\4_msc1_1Perc_liver_ad_carc.plt
                                          Thu Apr 01 15:57:41 2010
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Source - Table 1 in Goodman and Sauer 1992
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
               -beta1*dose^1)]

  The parameter betas are restricted to be positive


  Dependent variable = Mean
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                    Background =    0.0400263
                       Beta(1) =    0.0124752
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.51
Beta(1)	-0.51	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0221468	*	*	*
Beta(1)	0.0143372	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-68.2561	4			
Fitted model	-69.6304	2	2.74857	2	0.253
Reduced model	-89.1983	1	41.8843	3	<.0001

AIC: 143.261

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0221	1.905	2.000	86	0.070
1.5473	0.0436	2.180	1.000	50	-0.817
7.1546	0.1175	5.874	9.000	50	1.373
38.5608	0.4374	19.685	18.000	45	-0.506

Chi^2 = 2.81 d.f. = 2 P-value = 0.2449

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.700996

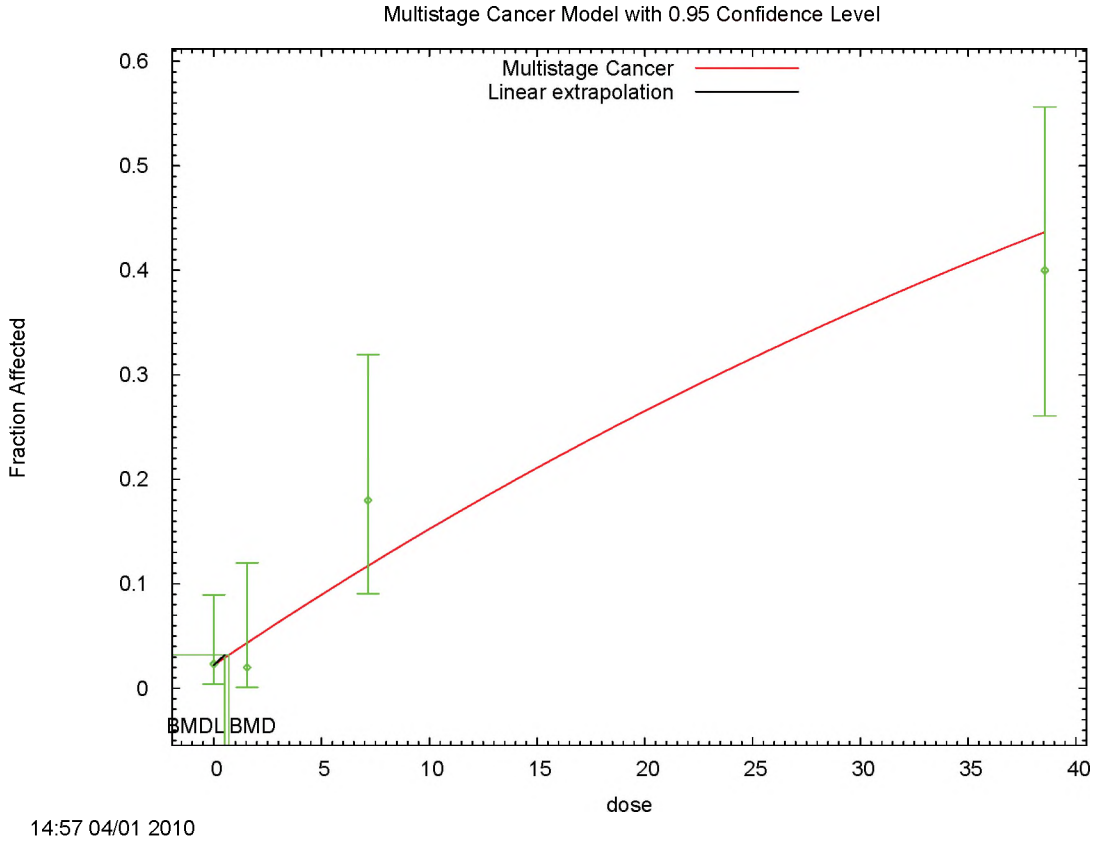
BMDL = 0.501345

BMDU = 1.04839

Taken together, (0.501345, 1.04839) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0199463

F.1.4.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Hepatocellular adenoma(s) or carcinoma(s)

F.1.5. Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

F.1.5.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**3**	**0.815**	**31.564**	**5.763E+00**	**2.795E+00**	
Multistage Cancer, 2-Degree	3	0.985	30.170	1.369E+01	3.416E+00	
Multistage Cancer, 3-Degree	3	0.999	29.930	1.917E+01	3.578E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.5.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\4\Blood\5_msc1_1Perc_nasal.(d)
        Gnuplot Plotting File:  C:\4\Blood\5_msc1_1Perc_nasal.plt
                                          Thu Apr 01 15:58:14 2010
=================================================================

Source - Table 5
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
               -beta1*dose^1)]

  The parameter betas are restricted to be positive


  Dependent variable = Mean
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                Default Initial Parameter Values
                   Background = 7.10818e-005
                      Beta(1) =   0.00222324
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.0022294	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-18.7562	4			
Fitted model	-18.9547	1	0.397012	3	0.9409
Reduced model	-24.1972	1	10.882	3	0.01238

AIC: 39.9093

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	86	0.000
1.5473	0.0034	0.172	0.000	50	-0.416
7.1546	0.0158	0.791	1.000	50	0.237
38.5608	0.0824	4.036	4.000	49	-0.019

Chi^2 = 0.23 d.f. = 3 P-value = 0.9728

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.50809

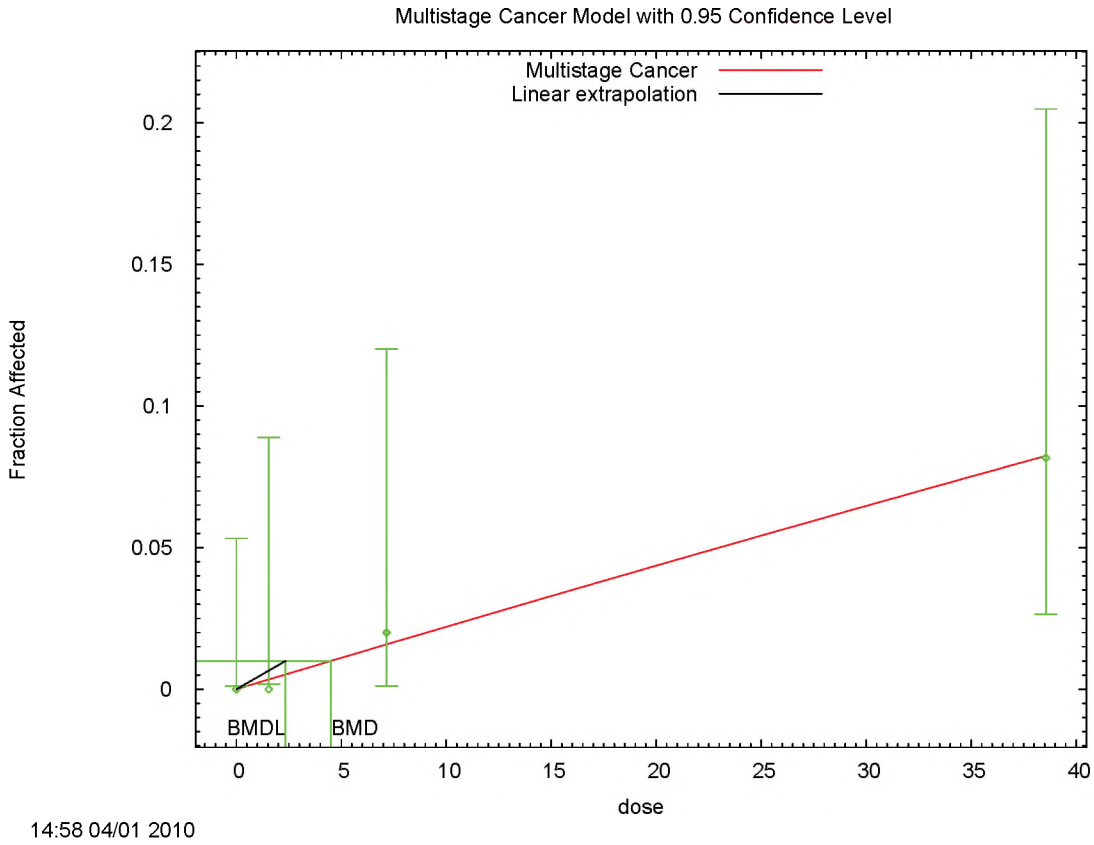
BMDL = 2.34012

BMDU = 10.4588

Taken together, (2.34012, 10.4588) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00427329

F.1.5.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

F.1.6. Kociba et al., 1978: Keratinizing squamous cell carcinoma of lung

F.1.6.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**3**	**0.626**	**45.298**	**3.140E+00**	**1.786E+00**	
Multistage Cancer, 2-Degree	3	0.964	42.736	1.004E+01	2.707E+00	
Multistage Cancer, 3-Degree	3	0.997	42.291	1.556E+01	3.135E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.6.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Keratinizing squamous cell carcinoma of lung

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\4\Blood\6_msc1_1Perc_kera_carc.(d)
         Gnuplot Plotting File:  C:\4\Blood\6_msc1_1Perc_kera_carc.plt
                                                Thu Apr 01 15:58:49 2010
 ================================================================

 Source - Table 5
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =   0.00419802
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00320098	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-20.0957	4			
Fitted model	-21.6489	1	3.10639	3	0.3755
Reduced model	-31.4904	1	22.7894	3	<.0001

AIC: 45.2978

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	86	0.000
1.5473	0.0049	0.247	0.000	50	-0.498
7.1546	0.0226	1.132	0.000	50	-1.076
38.5608	0.1161	5.690	7.000	49	0.584

Chi^2 = 1.75 d.f. = 3 P-value = 0.6263

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.13977

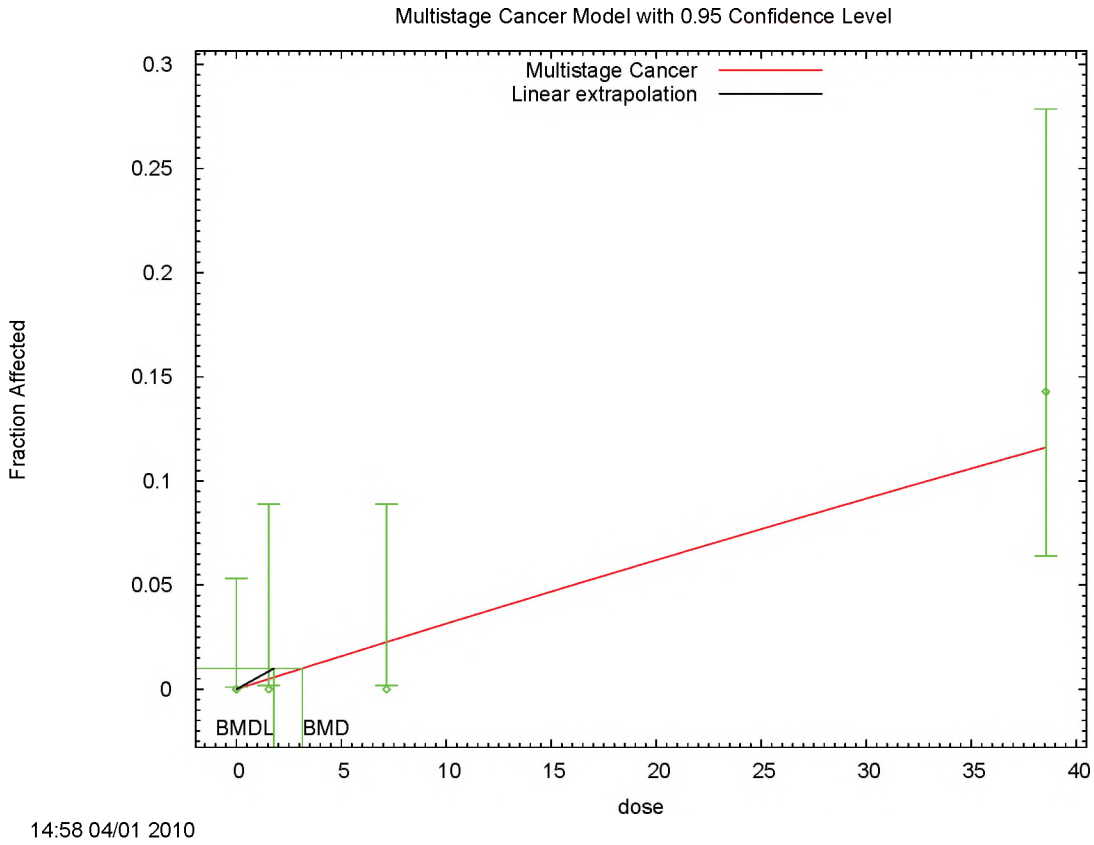
BMDL = 1.78648

BMDU = 6.28288

Taken together, (1.78648, 6.28288) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0055976

F.1.6.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Keratinizing squamous cell carcinoma of lung

F.1.7. National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.1.7.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree[a]	**2**	**0.179**	**75.385**	**3.127E+00**	**1.380E+00**	
Multistage Cancer, 2-Degree	2	0.179	75.385	3.127E+00	1.380E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.179	75.385	3.127E+00	1.380E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.7.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\4\Blood\7_msc1_1Perc_sub_fibro.(d)
        Gnuplot Plotting File:  C:\4\Blood\7_msc1_1Perc_sub_fibro.plt
                                          Thu Apr 01 15:59:25 2010
=================================================================

Source - Table 10
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  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
               -beta1*dose^1)]

  The parameter betas are restricted to be positive


  Dependent variable = Mean
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                    Background =    0.0268183
                       Beta(1) =   0.00211524
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.63
Beta(1)	-0.63	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0149841	*	*	*
Beta(1)	0.00321423	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-33.5998	4			
Fitted model	-35.6923	2	4.18508	2	0.1234
Reduced model	-37.7465	1	8.29346	3	0.04032

AIC: 75.3847

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0150	1.124	0.000	75	-1.068
1.9574	0.0212	1.058	2.000	50	0.926
5.6942	0.0328	1.642	3.000	50	1.077
29.7519	0.1048	5.136	4.000	49	-0.530

Chi^2 = 3.44 d.f. = 2 P-value = 0.1792

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.12683

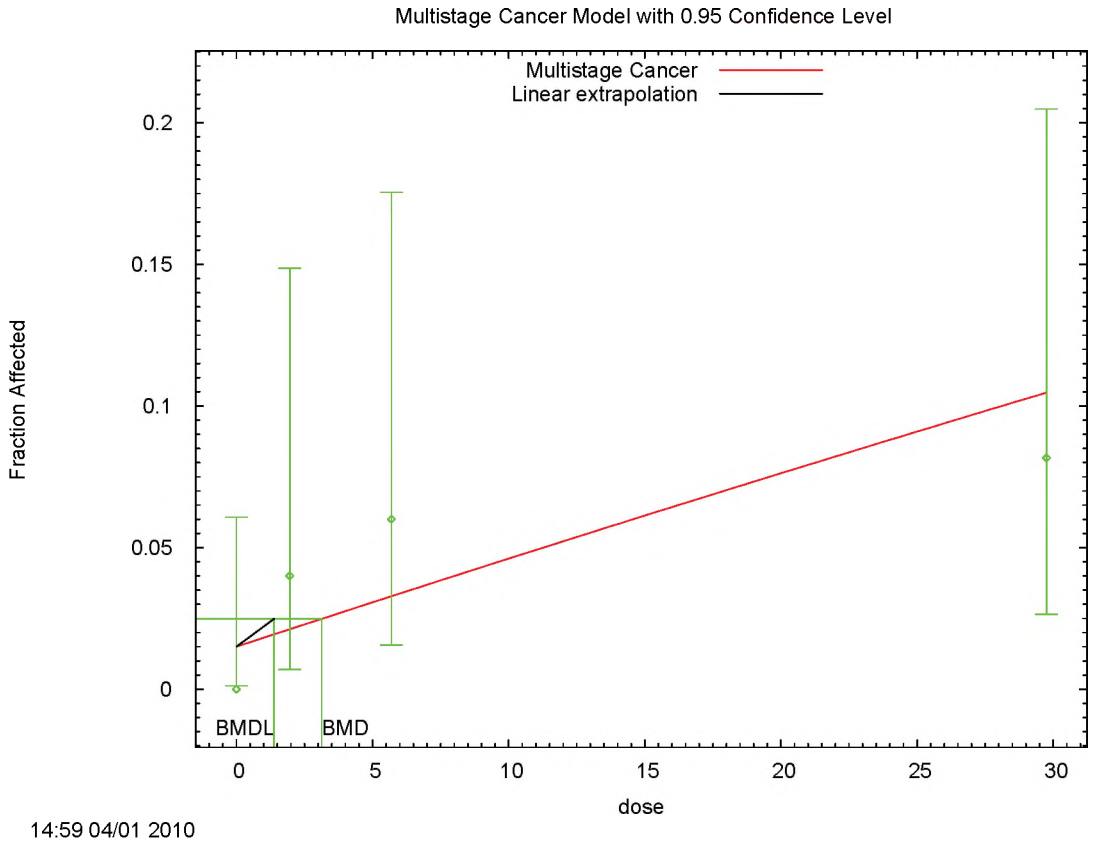
BMDL = 1.38047

BMDU = 2.18232e+006

Taken together, (1.38047, 2.18232e+006) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00724391

F.1.7.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.1.8. National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

F.1.8.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.218**	**135.190**	**1.169E+00**	**7.375E-01**	
Multistage Cancer, 2-Degree	2	0.491	133.447	5.578E+00	8.771E-01	
Multistage Cancer, 3-Degree	1	0.239	135.435	7.204E+00	8.786E-01	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.8.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\4\Blood\8_msc1_1Perc_liver_nod.(d)
         Gnuplot Plotting File:  C:\4\Blood\8_msc1_1Perc_liver_nod.plt
                                                 Thu Apr 01 16:00:00 2010
 ======================================================================

 Source - Table 10
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                    Background =    0.0261097
                       Beta(1) =    0.0102165
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.52
Beta(1)	-0.52	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0424738	*	*	*
Beta(1)	0.00859382	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-63.9149	4			
Fitted model	-65.5949	2	3.36005	2	0.1864
Reduced model	-74.0195	1	20.2092	3	0.0001536

AIC: 135.19

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0425	3.186	5.000	75	1.039
1.9574	0.0584	2.864	1.000	49	-1.135
5.6942	0.0882	4.410	3.000	50	-0.703
29.7519	0.2585	12.667	14.000	49	0.435

Chi^2 = 3.05 d.f. = 2 P-value = 0.2175

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.16948

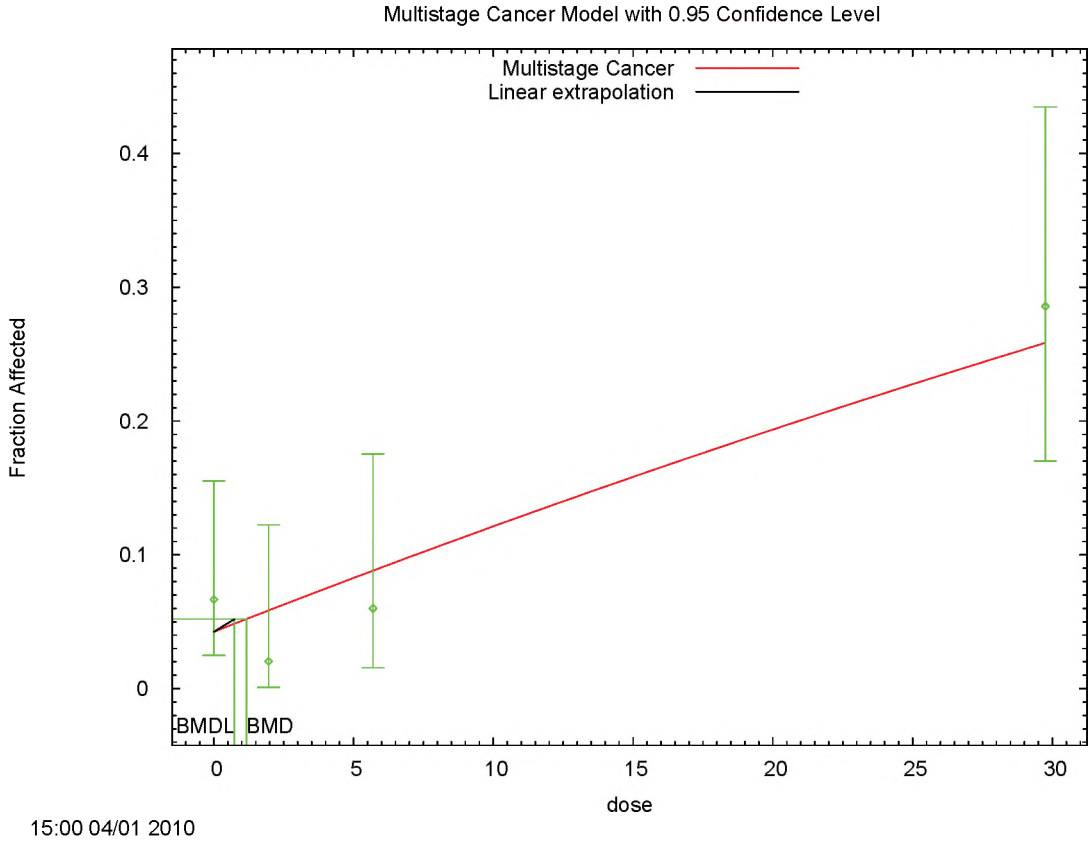
BMDL = 0.737535

BMDU = 2.17906

Taken together, (0.737535, 2.17906) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0135587

F.1.8.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

F.1.9. National Toxicology Program, 1982: Adrenal: Cortical Adenoma, or Carcinoma or Adenoma, NOS

F.1.9.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.337**	**203.824**	**1.611E+00**	**8.140E-01**	
Multistage Cancer, 2-Degree	2	0.470	203.033	6.652E+00	8.904E-01	
Multistage Cancer, 3-Degree	2	0.505	202.868	1.091E+01	9.100E-01	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.9.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Adrenal: Cortical Adenoma, or Carcinoma or Adenoma, NOS

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\4\Blood\9_msc1_1Perc_adre_cort_ad_carc.(d)
         Gnuplot Plotting File:  C:\4\Blood\9_msc1_1Perc_adre_cort_ad_carc.plt
                                                Thu Apr 01 16:06:15 2010
 ================================================================

 Source - Table 10
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
```

```
                      Background =     0.134165
                         Beta(1) =    0.0069662


            Asymptotic Correlation Matrix of Parameter Estimates

              Background      Beta(1)

Background            1        -0.54

   Beta(1)        -0.54            1


                                 Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background         0.139854            *               *                   *
        Beta(1)       0.00623778           *               *                   *

* - Indicates that this value is not calculated.


                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -98.7282         4
   Fitted model         -99.912         2       2.36764      2          0.3061
  Reduced model        -102.201         1       6.94636      3         0.07363

           AIC:         203.824


                                 Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.1399        10.209    11.000          73        0.267
    1.9574     0.1503         7.364     9.000          49        0.654
    5.6942     0.1699         8.324     5.000          49       -1.264
   29.7519     0.2855        13.135    14.000          46        0.282

 Chi^2 = 2.18      d.f. = 2        P-value = 0.3367


   Benchmark Dose Computation

Specified effect =           0.01

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         1.6112

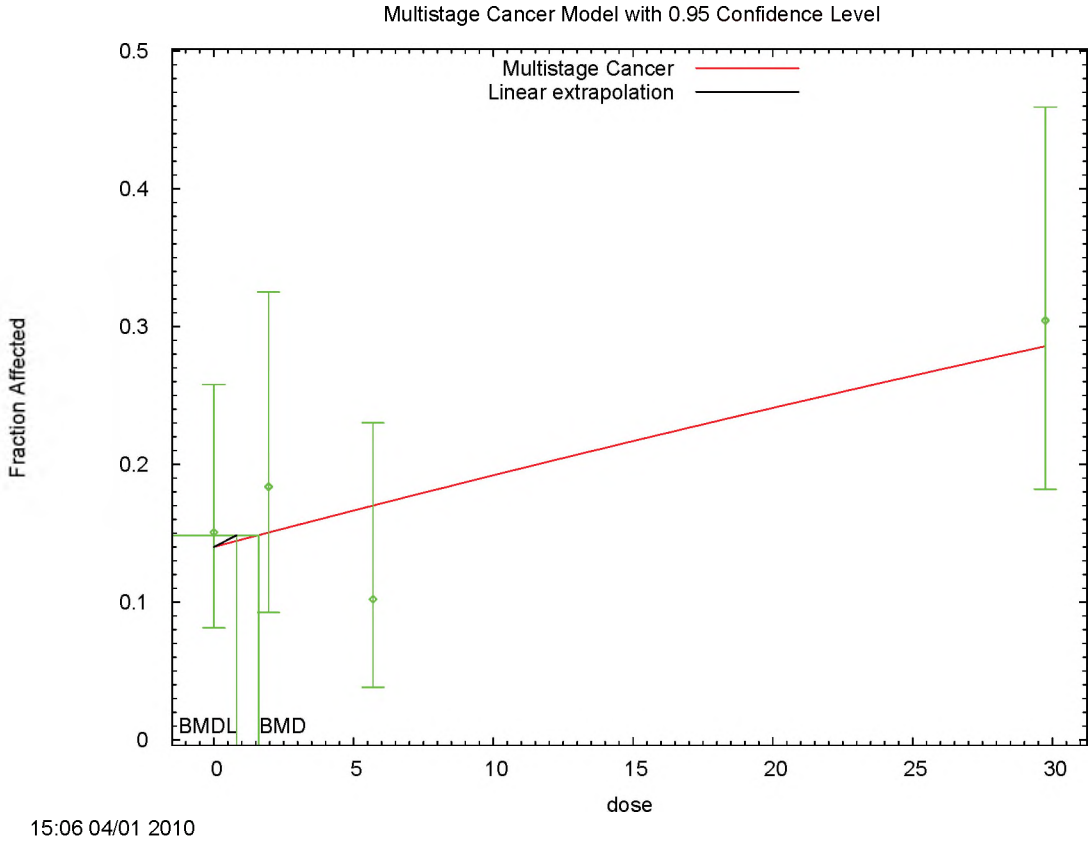
            BMDL =        0.81404

            BMDU =         370555

Taken together, (0.81404, 370555 ) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0122844
```

F.1.9.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Adrenal: Cortical Adenoma, or Carcinoma or Adenoma, NOS

F.1.10. National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma

F.1.10.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree[a]	**2**	**0.568**	**92.411**	**3.376E+00**	**1.553E+00**	
Multistage Cancer, 2-Degree	2	0.735	91.749	9.526E+00	1.690E+00	
Multistage Cancer, 3-Degree	2	0.773	91.626	1.385E+01	1.720E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.10.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\4\Blood\10_msc1_1Perc_thy_ad.(d)
        Gnuplot Plotting File:  C:\4\Blood\10_msc1_1Perc_thy_ad.plt
                                          Thu Apr 01 16:06:53 2010
=================================================================

Source - Table 10
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  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
               -beta1*dose^1)]

  The parameter betas are restricted to be positive


  Dependent variable = Mean
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                    Background =    0.0283212
                       Beta(1) =   0.00346762
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.54
Beta(1)	-0.54	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0332432	*	*	*
Beta(1)	0.00297726	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-43.5264	4			
Fitted model	-44.2053	2	1.35778	2	0.5072
Reduced model	-46.2299	1	5.40699	3	0.1443

AIC: 92.4106

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0332	2.427	3.000	73	0.374
1.9574	0.0389	1.749	2.000	45	0.194
5.6942	0.0495	2.425	1.000	49	-0.939
29.7519	0.1152	5.414	6.000	47	0.268

Chi^2 = 1.13 d.f. = 2 P-value = 0.5682

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.3757

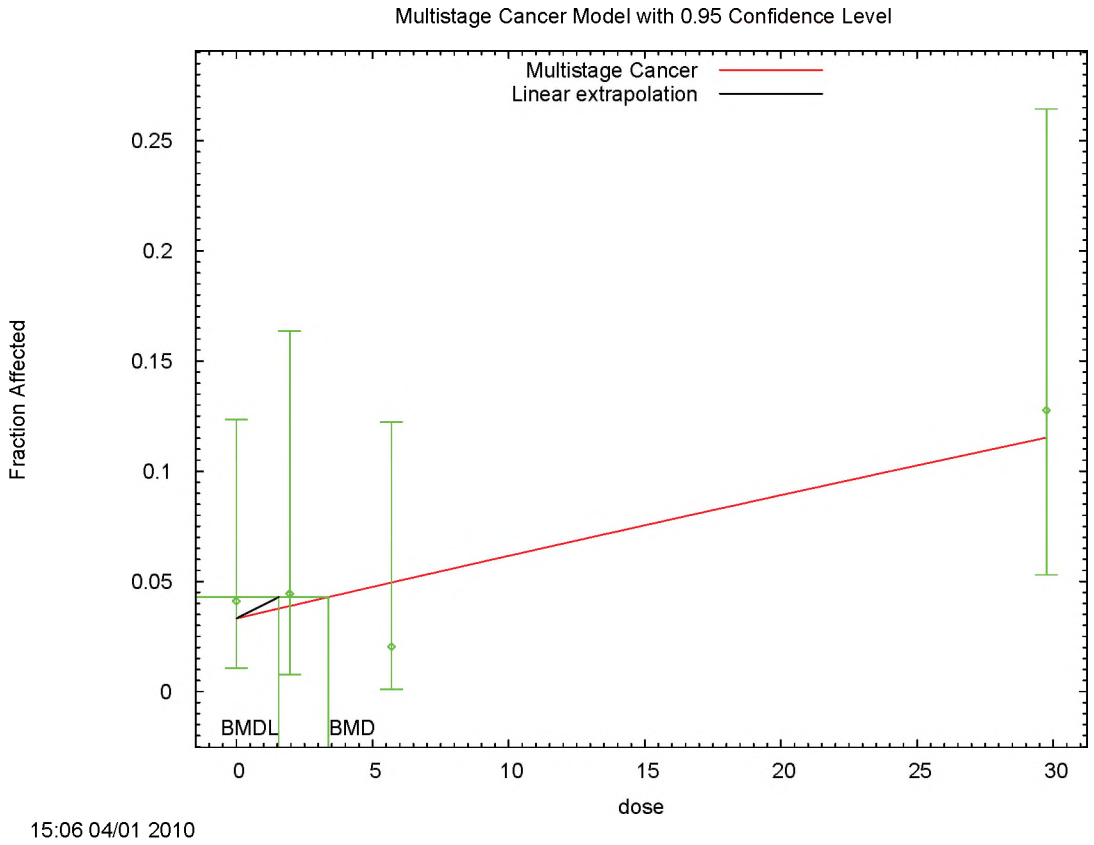
BMDL = 1.55287

BMDU = 306341

Taken together, (1.55287, 306341) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00643967

F.1.10.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma

F.1.11. National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

F.1.11.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.218**	**135.190**	**1.169E+00**	**7.375E-01**	
Multistage Cancer, 2-Degree	2	0.491	133.447	5.578E+00	8.771E-01	
Multistage Cancer, 3-Degree	1	0.239	135.435	7.204E+00	8.786E-01	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.11.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\4\Blood\11_msc1_1Perc_liver_nod.(d)
         Gnuplot Plotting File:  C:\4\Blood\11_msc1_1Perc_liver_nod.plt
                                                 Thu Apr 01 16:07:28 2010
 ================================================================

 Source - Table 9
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                    Background =            0
                       Beta(1) =   0.00219894
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00163808	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-11.3484	4			
Fitted model	-12.0522	1	1.40767	3	0.7037
Reduced model	-15.9189	1	9.14109	3	0.02747

AIC: 26.1044

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	74	0.000
1.9569	0.0032	0.160	0.000	50	-0.401
5.7027	0.0093	0.465	0.000	50	-0.685
29.8723	0.0478	2.388	3.000	50	0.406

Chi^2 = 0.79 d.f. = 3 P-value = 0.8507

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 6.13543

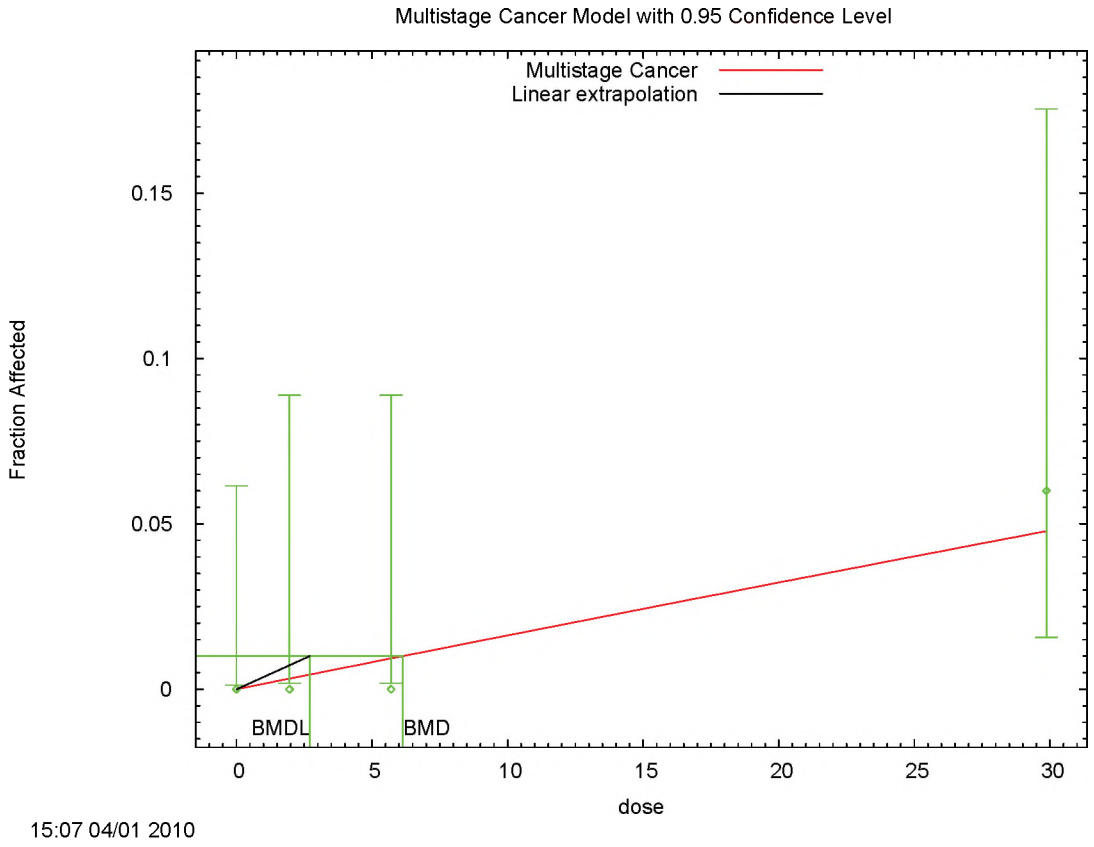
BMDL = 2.70101

BMDU = 18.9354

Taken together, (2.70101, 18.9354) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00370232

F.1.11.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

F.1.12. National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma or Carcinoma

F.1.12.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.057**	**149.263**	**1.208E+00**	**6.984E-01**	
Multistage Cancer, 2-Degree	2	0.057	149.263	1.208E+00	6.984E-01	final ß=0
Multistage Cancer, 3-Degree	2	0.057	149.263	1.208E+00	6.984E-01	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.12.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma or Carcinoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\4\Blood\12_msc1_1Perc_thyroid.(d)
         Gnuplot Plotting File:  C:\4\Blood\12_msc1_1Perc_thyroid.plt
                                                 Thu Apr 01 16:08:03 2010
 ======================================================================

 Source - Table 9
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                    Background =    0.0768555
                       Beta(1) =   0.00606248
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.62
Beta(1)	-0.62	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0529006	*	*	*
Beta(1)	0.00831706	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-69.5946	4			
Fitted model	-72.6315	2	6.07383	2	0.04798
Reduced model	-77.5267	1	15.8643	3	0.001209

AIC: 149.263

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0529	3.650	1.000	69	-1.425
1.9569	0.0682	3.273	5.000	48	0.989
5.7027	0.0968	4.839	8.000	50	1.512
29.8723	0.2613	13.063	11.000	50	-0.664

Chi^2 = 5.74 d.f. = 2 P-value = 0.0568

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.2084

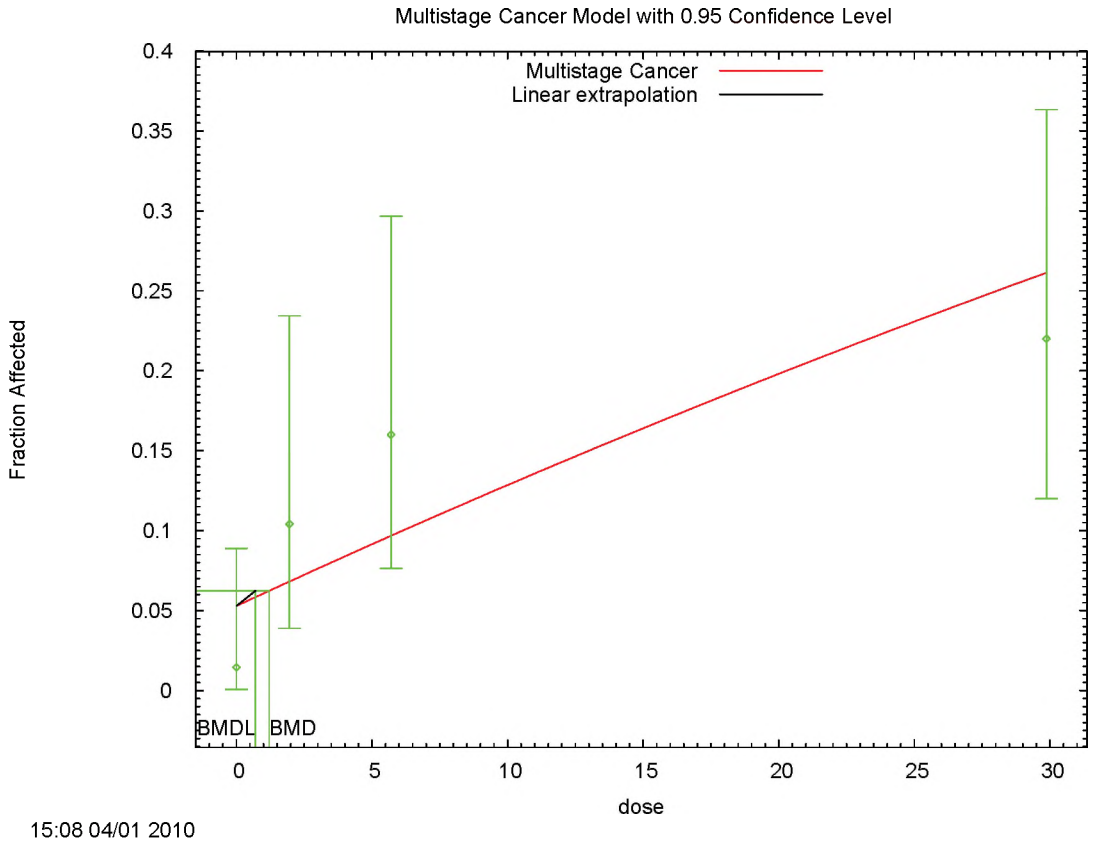
BMDL = 0.698436

BMDU = 2.89109

Taken together, (0.698436, 2.89109) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0143177

F.1.12.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma or Carcinoma

F.1.13. National Toxicology Program, 1982: Adrenal cortex: Adenoma

F.1.13.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.062**	**199.309**	**3.977E+00**	**1.223E+00**	
Multistage Cancer, 2-Degree	2	0.062	199.309	3.977E+00	1.223E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.062	199.309	3.977E+00	1.223E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.13.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Adrenal cortex: Adenoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\13_msc1_1Perc_adre_cort.(d)
         Gnuplot Plotting File:  C:\1\Blood\13_msc1_1Perc_adre_cort.plt
                                           Fri Apr 02 10:53:16 2010
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 Source - Table 9
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.163685
                        Beta(1) =   0.00144687
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.6
Beta(1)	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.146079	*	*	*
Beta(1)	0.00252696	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-94.8672	4			
Fitted model	-97.6546	2	5.57468	2	0.06158
Reduced model	-98.0432	1	6.35197	3	0.09569

AIC: 199.309

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1461	10.518	6.000	72	-1.507
1.9569	0.1503	7.515	9.000	50	0.588
5.7027	0.1583	7.756	12.000	49	1.661
29.8723	0.2082	10.200	9.000	49	-0.422

Chi^2 = 5.55 d.f. = 2 P-value = 0.0622

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

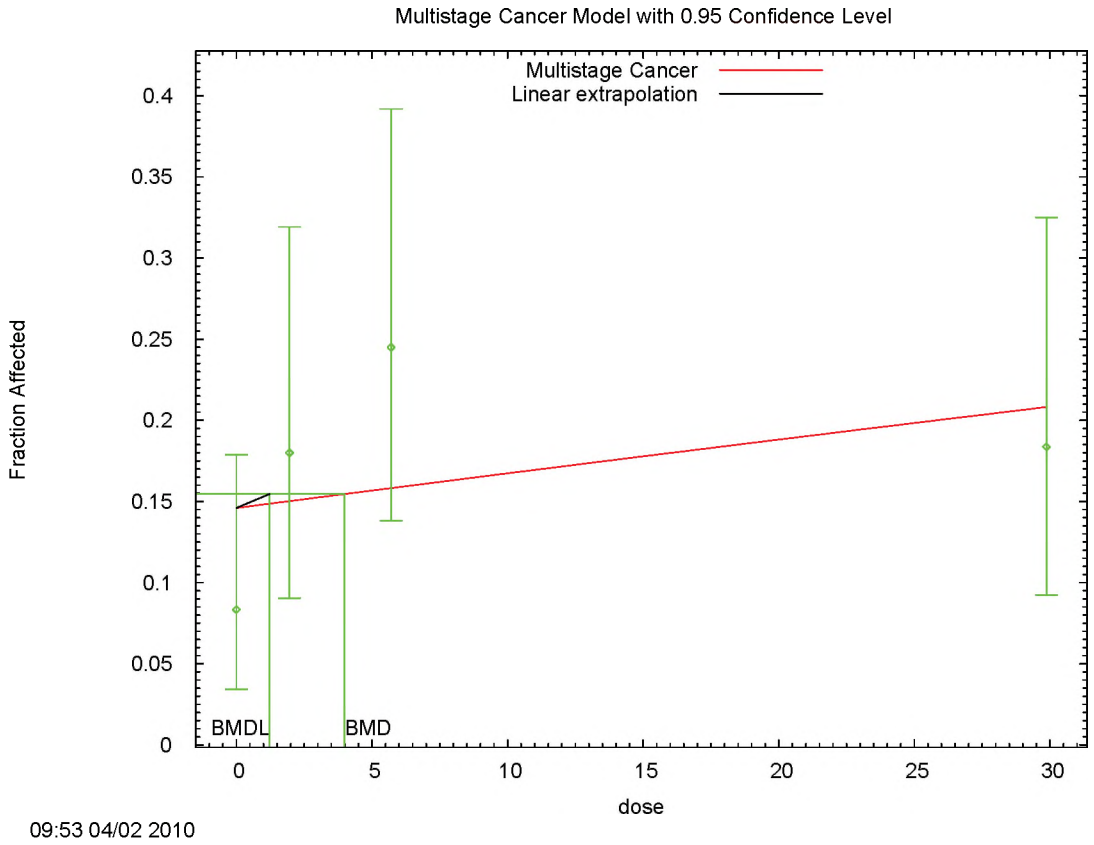
Confidence level = 0.95

BMD = 3.97724

BMDL = 1.22286

BMDU did not converge for BMR = 0.010000
BMDU calculation failed
BMDU = Inf

F.1.13.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Adrenal cortex: Adenoma

F.1.14. National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.1.14.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree[a]	**2**	**0.179**	**75.385**	**3.127E+00**	**1.380E+00**	
Multistage Cancer, 2-Degree	2	0.179	75.385	3.127E+00	1.380E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.179	75.385	3.127E+00	1.380E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.14.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\1\Blood\14_msc1_1Perc_subcu_fibro.(d)
        Gnuplot Plotting File:  C:\1\Blood\14_msc1_1Perc_subcu_fibro.plt
                                          Fri Apr 02 10:59:38 2010
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  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
               -beta1*dose^1)]

  The parameter betas are restricted to be positive


  Dependent variable = Mean
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                    Background =     0.010477
                       Beta(1) =   0.00314237
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.55
Beta(1)	-0.55	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0124357	*	*	*
Beta(1)	0.0029518	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-30.9876	4			
Fitted model	-31.0692	2	0.163345	2	0.9216
Reduced model	-34.3291	1	6.68308	3	0.08272

AIC: 66.1385

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0124	0.920	1.000	74	0.084
1.9460	0.0181	0.905	1.000	50	0.101
5.8440	0.0293	1.408	1.000	48	-0.349
32.0560	0.1016	4.775	5.000	47	0.109

Chi^2 = 0.15 d.f. = 2 P-value = 0.9274

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.40481

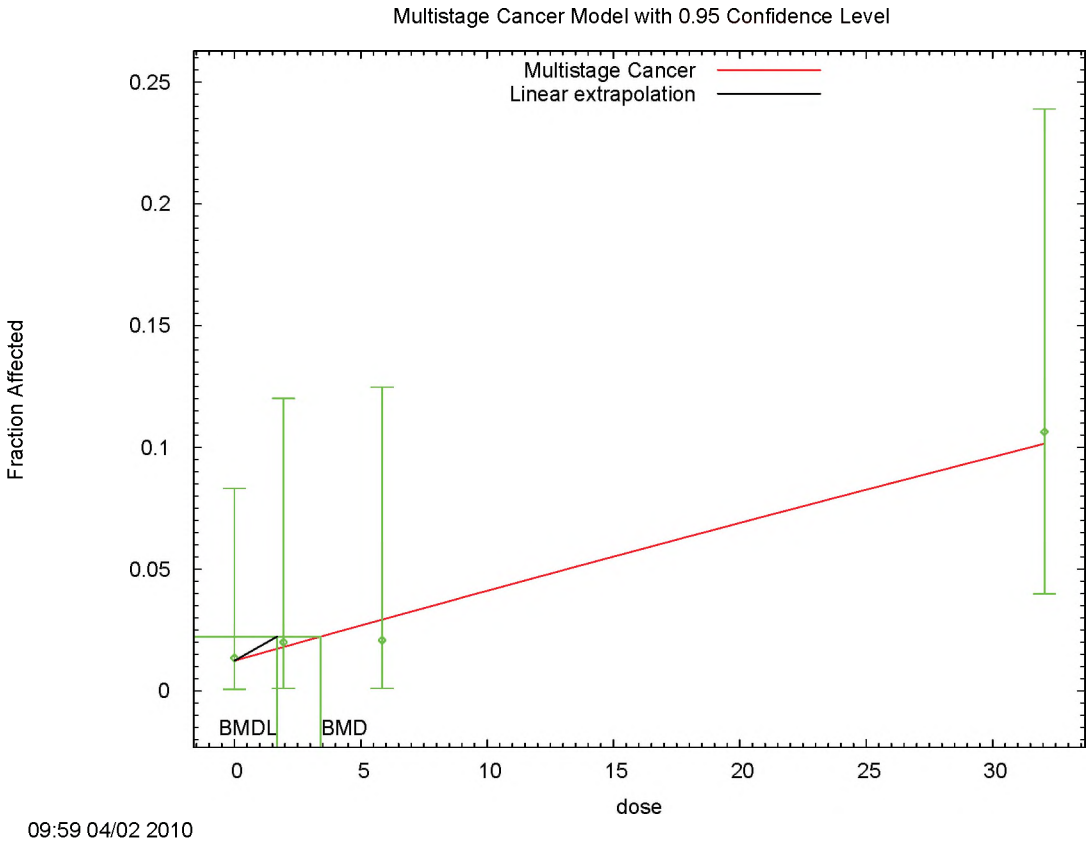
BMDL = 1.68615

BMDU = 11.3501

Taken together, (1.68615, 11.3501) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00593067

F.1.14.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.1.15. National Toxicology Program, 1982: Hematopoietio System: Lymphoma or Leukemia

F.1.15.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.977**	**261.445**	**1.145E+00**	**6.091E-01**	
Multistage Cancer, 2-Degree	1	0.869	263.426	1.704E+00	6.102E-01	
Multistage Cancer, 3-Degree	1	0.869	263.426	1.704E+00	6.102E-01	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.15.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Hematopoietio System: Lymphoma or Leukemia

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\15_msc1_1Perc_mice_f_lymphoma.(d)
         Gnuplot Plotting File:  C:\1\Blood\15_msc1_1Perc_mice_f_lymphoma.plt
                                                 Fri Apr 02 11:00:07 2010
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 Table 15 page 64 Hematopoietic System Lymphoma or Leukemia
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                    Background =      0.23423
                       Beta(1) =   0.00892991
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.54
Beta(1)	-0.54	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.236159	*	*	*
Beta(1)	0.00877894	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-128.699	4			
Fitted model	-128.723	2	0.0465401	2	0.977
Reduced model	-131.412	1	5.42487	3	0.1432

AIC: 261.445

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2362	17.476	18.000	74	0.143
1.9460	0.2491	12.455	12.000	50	-0.149
5.8440	0.2744	13.169	13.000	48	-0.055
32.0560	0.4235	19.905	20.000	47	0.028

Chi^2 = 0.05 d.f. = 2 P-value = 0.9770

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.14482

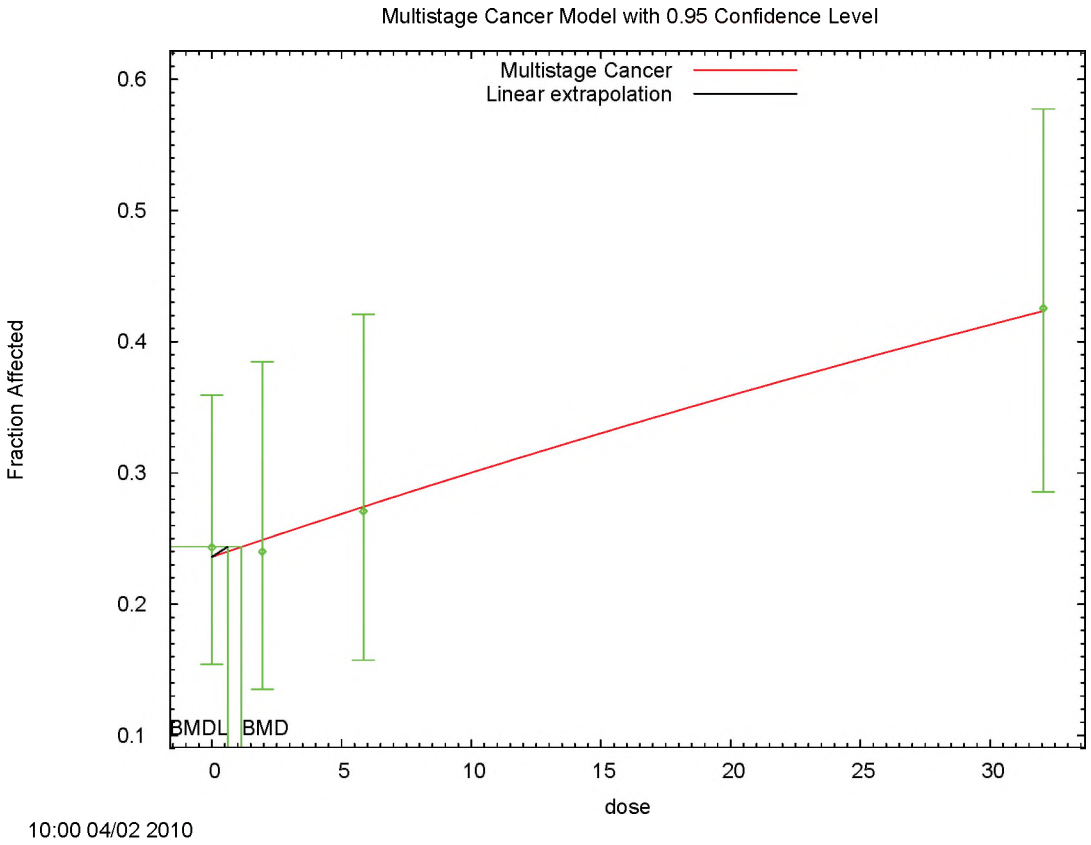
BMDL = 0.609084

BMDU = 4.29581

Taken together, (0.609084, 4.29581) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0164181

F.1.15.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Hematopoietio System: Lymphoma or Leukemia

F.1.16. National Toxicology Program, 1982: Liver: Hepatooellular Adenoma or Carcinoma

F.1.16.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.340**	**155.213**	**1.488E+00**	**8.265E-01**	
Multistage Cancer, 2-Degree	2	0.340	155.213	1.488E+00	8.265E-01	final ß=0
Multistage Cancer, 3-Degree	2	0.340	155.213	1.488E+00	8.265E-01	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.16.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Liver: Hepatooellular Adenoma or Carcinoma

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\1\Blood\16_msc1_1Perc_mf_LivAdenCarc.(d)
        Gnuplot Plotting File:  C:\1\Blood\16_msc1_1Perc_mf_LivAdenCarc.plt
                                          Fri Apr 02 11:04:11 2010
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  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
               -beta1*dose^1)]

  The parameter betas are restricted to be positive


  Dependent variable = Mean
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                Default Initial Parameter Values
                   Background =     0.080941
                      Beta(1) =   0.00583089
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.57
Beta(1)	-0.57	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0692161	*	*	*
Beta(1)	0.00675636	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-74.5177	4			
Fitted model	-75.6063	2	2.17736	2	0.3367
Reduced model	-79.6703	1	10.3053	3	0.01614

AIC: 155.213

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0692	5.053	3.000	73	-0.947
1.9460	0.0814	4.069	6.000	50	0.999
5.8440	0.1053	5.052	6.000	48	0.446
32.0560	0.2505	11.772	11.000	47	-0.260

Chi^2 = 2.16 d.f. = 2 P-value = 0.3395

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.48754

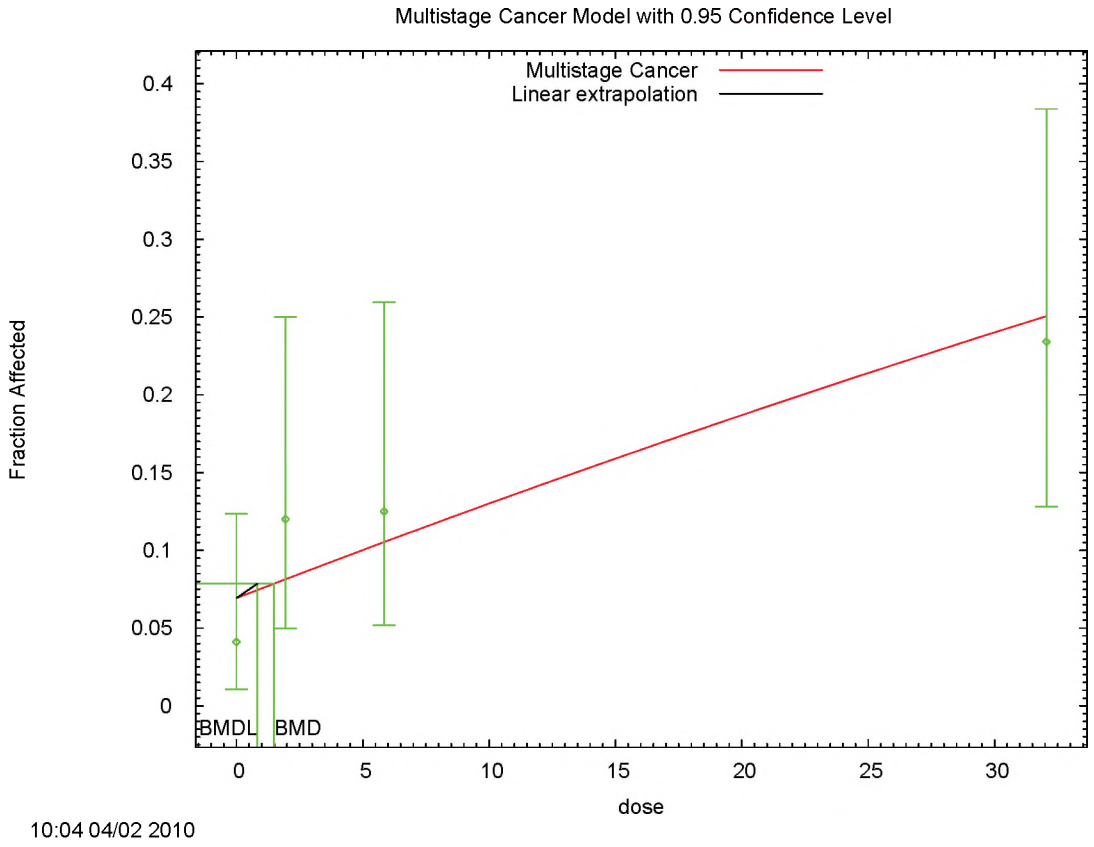
BMDL = 0.826482

BMDU = 3.9863

Taken together, (0.826482, 3.9863) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0120995

F.1.16.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Liver: Hepatooellular Adenoma or Carcinoma

F.1.17. National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.1.17.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree[a]	**2**	**0.179**	**75.385**	**3.127E+00**	**1.380E+00**	
Multistage Cancer, 2-Degree	2	0.179	75.385	3.127E+00	1.380E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.179	75.385	3.127E+00	1.380E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.17.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\1\Blood\17_msc1_1Perc_mice_f_thyroid_aden.(d)
        Gnuplot Plotting File:  C:\1\Blood\17_msc1_1Perc_mice_f_thyroid_aden.plt
                                          Fri Apr 02 11:04:39 2010
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  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
               -beta1*dose^1)]

  The parameter betas are restricted to be positive


  Dependent variable = Mean
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                Default Initial Parameter Values
                   Background =    0.0202346
                      Beta(1) =   0.00292833
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.58
Beta(1)	-0.58	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0153082	*	*	*
Beta(1)	0.00329742	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-32.0017	4			
Fitted model	-34.3904	2	4.77738	2	0.09175
Reduced model	-37.2405	1	10.4776	3	0.01491

AIC: 72.7807

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0153	1.056	0.000	69	-1.036
1.9460	0.0216	1.080	3.000	50	1.867
5.8440	0.0341	1.603	1.000	47	-0.484
32.0560	0.1141	5.248	5.000	46	-0.115

Chi^2 = 4.81 d.f. = 2 P-value = 0.0904

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.04794

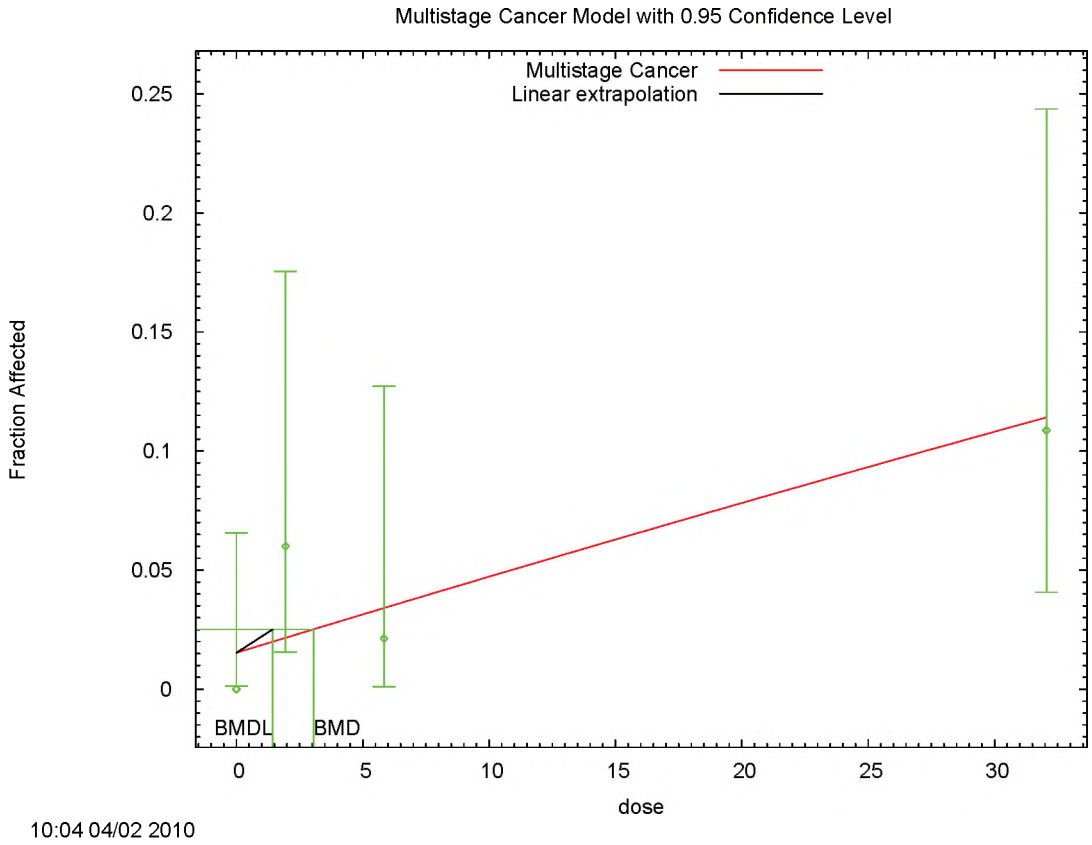
BMDL = 1.43569

BMDU = 138876

Taken together, (1.43569, 138876) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00696528

F.1.17.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.1.18. National Toxicology Program, 1982: Lung: Alveolar/Bronchiolar Adenoma or Carcinoma

F.1.18.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	2	0.088	168.342	6.499E-01	3.512E-01	
Multistage Cancer, 2-Degree [a]	**2**	**0.167**	**166.946**	**2.528E+00**	**4.135E-01**	
Multistage Cancer, 3-Degree	2	0.182	166.799	4.147E+00	4.230E-01	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.18.2. *Output for Selected Model: Multistage Cancer, 2-Degree*

National Toxicology Program, 1982: Lung: Alveolar/Bronchiolar Adenoma or Carcinoma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\18_msc2_1Perc_lung_aden_carc.(d)
         Gnuplot Plotting File:  C:\1\Blood\18_msc2_1Perc_lung_aden_carc.plt
                                                 Fri Apr 02 11:05:09 2010
 ================================================================

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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                    Background =    0.0868577
                       Beta(1) =            0
```

```
                        Beta(2) =   0.00165722


            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Beta(1)
                  have been estimated at a boundary point, or have been specified by the user,
                  and do not appear in the correlation matrix )

              Background      Beta(2)

Background            1        -0.46

   Beta(2)        -0.46            1


                                  Parameter Estimates

                                                                  95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0942466            *               *                   *
        Beta(1)                0            *               *                   *
        Beta(2)       0.00157255            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -79.5959         4
   Fitted model        -81.4729         2         3.754      2           0.153
  Reduced model        -85.3351         1       11.4782      3        0.009402

           AIC:         166.946


                                  Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
  ---------------------------------------------------------------------------
    0.0000     0.0942         6.692    10.000          71         1.344
    0.7665     0.0951         4.564     2.000          48        -1.262
    2.2711     0.1016         4.875     4.000          48        -0.418
   11.2437     0.2575        12.877    13.000          50         0.040

 Chi^2 = 3.57      d.f. = 2        P-value = 0.1674


   Benchmark Dose Computation

Specified effect =           0.01

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        2.52806

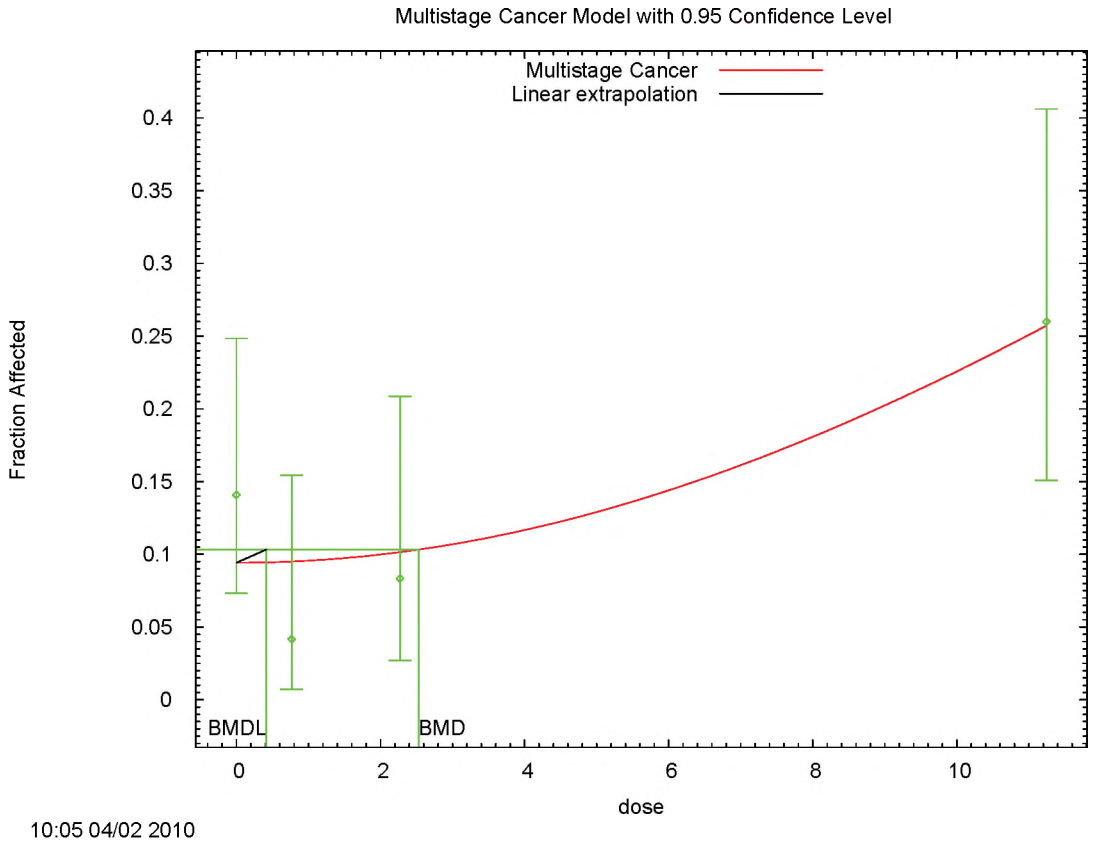
            BMDL =       0.413504

            BMDU =        4.19905

Taken together, (0.413504, 4.19905) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0241835
```

F.1.18.3. *Figure for Selected Model: Multistage Cancer, 2-Degree*



National Toxicology Program, 1982: Lung: Alveolar/Bronchiolar Adenoma or Carcinoma

F.1.19. National Toxicology Program, 1982: Liver: Hepatocellular Adenoma or Carcinoma

F.1.19.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.928**	**258.548**	**2.110E-01**	**1.378E-01**	
Multistage Cancer, 2-Degree	1	0.779	260.475	3.072E-01	1.385E-01	
Multistage Cancer, 3-Degree	1	0.790	260.468	2.934E-01	1.385E-01	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.19.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Liver: Hepatocellular Adenoma or Carcinoma

```
 ========================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\19_msc1_1Perc_mice_m_liver_aden_carc.(d)
         Gnuplot Plotting File:  C:\1\Blood\19_msc1_1Perc_mice_m_liver_aden_carc.plt
                                                 Fri Apr 02 11:05:36 2010
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.201679
                        Beta(1) =    0.0486492


           Asymptotic Correlation Matrix of Parameter Estimates
```

	Background	Beta(1)
Background	1	-0.53
Beta(1)	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.204258	*	*	*
Beta(1)	0.0476385	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-127.199	4			
Fitted model	-127.274	2	0.149955	2	0.9278
Reduced model	-135.589	1	16.7801	3	0.0007843

AIC: 258.548

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2043	14.911	15.000	73	0.026
0.7665	0.2328	11.407	12.000	49	0.201
2.2711	0.2859	14.007	13.000	49	-0.318
11.2437	0.5343	26.713	27.000	50	0.081

Chi^2 = 0.15 d.f. = 2 P-value = 0.9283

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.210971

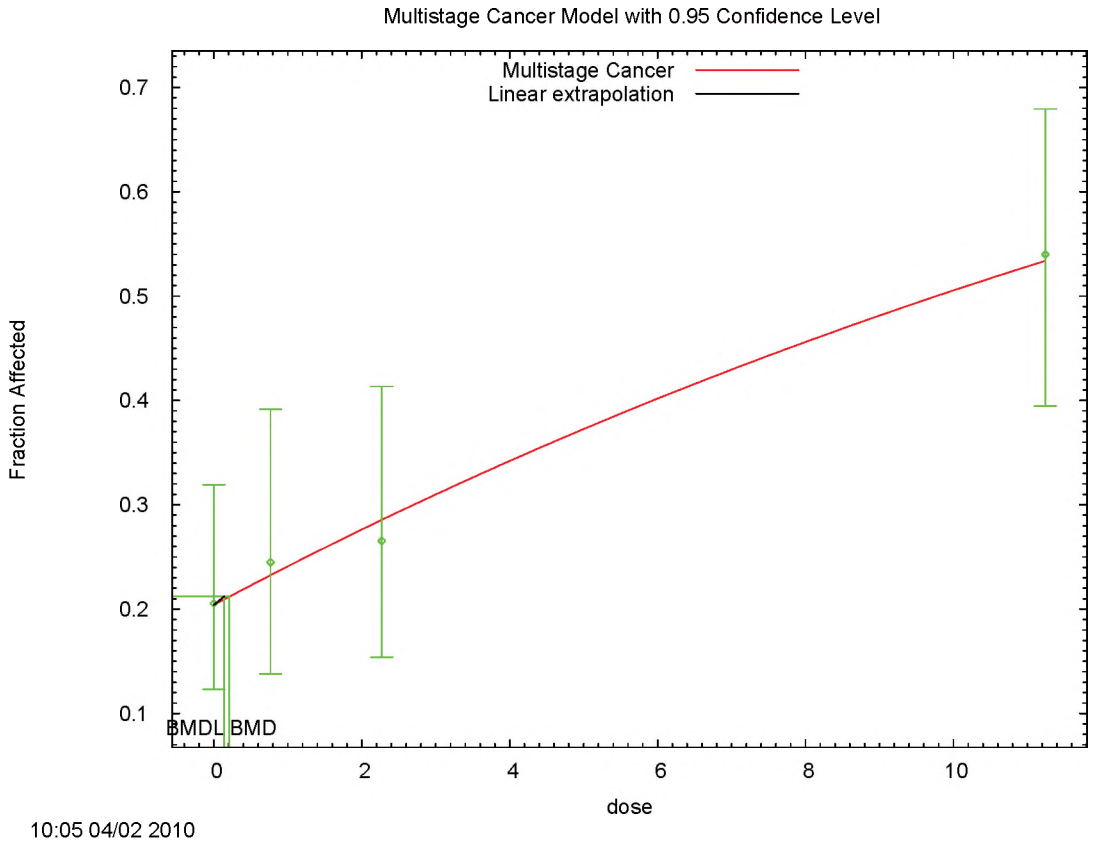
BMDL = 0.137771

BMDU = 0.383981

Taken together, (0.137771, 0.383981) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0725843

F.1.19.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Liver: Hepatocellular Adenoma or Carcinoma

F.1.20. National Toxicology Program, 2006: Liver: Cholangiocarcinoma

F.1.20.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	5	0.001	138.456	9.481E-01	7.114E-01	
Multistage Cancer, 2-Degree	5	0.405	119.374	4.263E+00	2.959E+00	
Multistage Cancer, 3-Degree [a]	**5**	**0.993**	**113.508**	**7.574E+00**	**4.133E+00**	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.20.2. *Output for Selected Model: Multistage Cancer, 3-Degree*

National Toxicology Program, 2006: Liver: Cholangiocarcinoma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\20_msc3_1Perc_liv_cho-carc.(d)
         Gnuplot Plotting File:  C:\1\Blood\20_msc3_1Perc_liv_cho-carc.plt
                                                Fri Apr 02 11:06:03 2010
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 2.44727e-005
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(3)
Beta(3)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	2.31301e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-55.408	6			
Fitted model	-55.7538	1	0.691671	5	0.9834
Reduced model	-96.9934	1	83.1708	5	<.0001

AIC: 113.508

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	49	0.000
2.5565	0.0004	0.019	0.000	48	-0.136
5.6937	0.0043	0.196	0.000	46	-0.444
9.7882	0.0215	1.073	1.000	50	-0.071
16.5688	0.0999	4.893	4.000	49	-0.426
29.6953	0.4543	24.078	25.000	53	0.254

Chi^2 = 0.47 d.f. = 5 P-value = 0.9933

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.57416

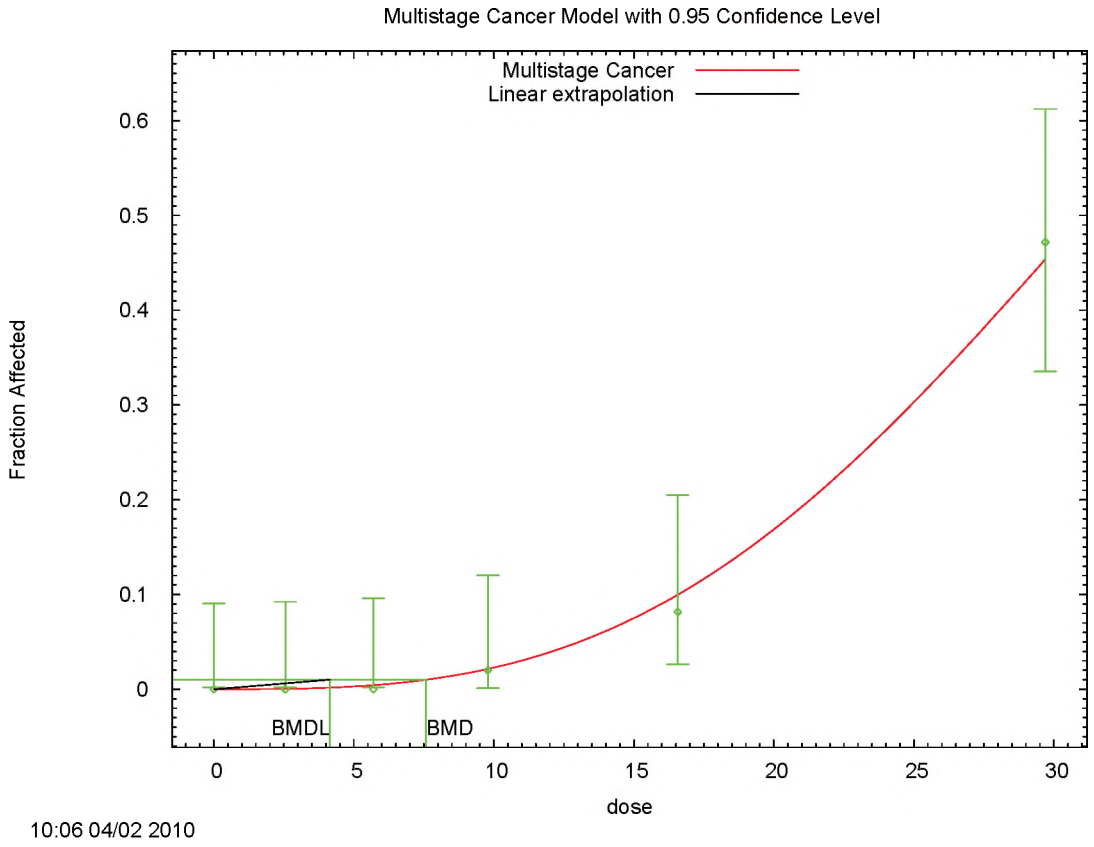
BMDL = 4.13304

BMDU = 8.42557

Taken together, (4.13304, 8.42557) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00241953

F.1.20.3. *Figure for Selected Model: Multistage Cancer, 3-Degree*



National Toxicology Program, 2006: Liver: Cholangiocarcinoma

F.1.21. National Toxicology Program, 2006: Liver: Hepatocellular adenoma

F.1.21.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	5	0.026	87.024	2.192E+00	1.455E+00	
Multistage Cancer, 2-Degree	5	0.509	76.982	6.602E+00	4.342E+00	
Multistage Cancer, 3-Degree [a]	**5**	**0.933**	**72.782**	**1.022E+01**	**6.527E+00**	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.21.2. *Output For Selected Model: Multistage Cancer, 3-Degree*

National Toxicology Program, 2006: Liver: Hepatocellular adenoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\21_msc3_1Perc_liv_hepat_ad.(d)
         Gnuplot Plotting File:  C:\1\Blood\21_msc3_1Perc_liv_hepat_ad.plt
                                                Fri Apr 02 11:06:32 2010
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 1.08896e-005

            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)
```

have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(3)
Beta(3)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	9.41228e-006	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-34.4075	6			
Fitted model	-35.3907	1	1.96648	5	0.8538
Reduced model	-56.3333	1	43.8515	5	<.0001

AIC: 72.7815

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	49	0.000
2.5565	0.0002	0.008	0.000	48	-0.087
5.6937	0.0017	0.080	0.000	46	-0.283
9.7882	0.0088	0.439	0.000	50	-0.666
16.5688	0.0419	2.054	1.000	49	-0.751
29.6953	0.2184	11.577	13.000	53	0.473

Chi^2 = 1.32 d.f. = 5 P-value = 0.9330

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 10.221

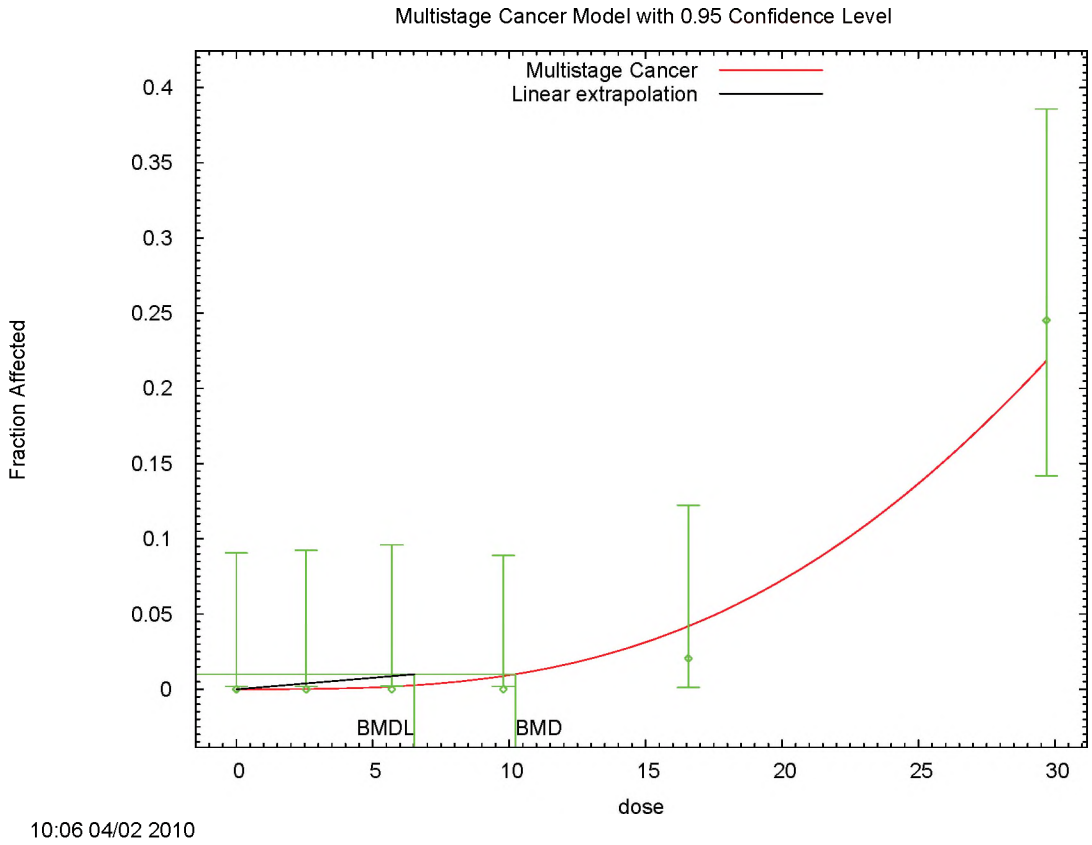
BMDL = 6.52683

BMDU = 11.9754

Taken together, (6.52683, 11.9754) is a 90 % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor = 0.00153214

F.1.21.3. *Figure For Selected Model: Multistage Cancer, 3-Degree*



National Toxicology Program, 2006: Liver: Hepatocellular adenoma

F.1.22. National Toxicology Program, 2006: Oral mucosa: squamous cell carcinoma

F.1.22.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**4**	**0.270**	**126.963**	**2.204E+00**	**1.389E+00**	
Multistage Cancer, 2-Degree	4	0.538	123.896	7.108E+00	2.158E+00	
Multistage Cancer, 3-Degree	4	0.565	123.295	1.103E+01	2.298E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.22.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 2006: Oral mucosa: squamous cell carcinoma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\22_msc1_1Perc_oral_carc.(d)
         Gnuplot Plotting File:  C:\1\Blood\22_msc1_1Perc_oral_carc.plt
                                              Fri Apr 02 11:07:00 2010
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =   0.00629243


           Asymptotic Correlation Matrix of Parameter Estimates
```

	Background	Beta(1)
Background	1	-0.67
Beta(1)	-0.67	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0139169	*	*	*
Beta(1)	0.00456055	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-57.5353	6			
Fitted model	-61.4815	2	7.89233	4	0.0956
Reduced model	-67.7782	1	20.4858	5	0.001013

AIC: 126.963

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0139	0.682	1.000	49	0.388
2.5565	0.0253	1.217	2.000	48	0.719
5.6937	0.0392	1.803	1.000	46	-0.610
9.7882	0.0570	2.848	0.000	50	-1.738
16.5688	0.0857	4.198	4.000	49	-0.101
29.6953	0.1388	7.357	10.000	53	1.050

Chi^2 = 5.17 d.f. = 4 P-value = 0.2700

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.20376

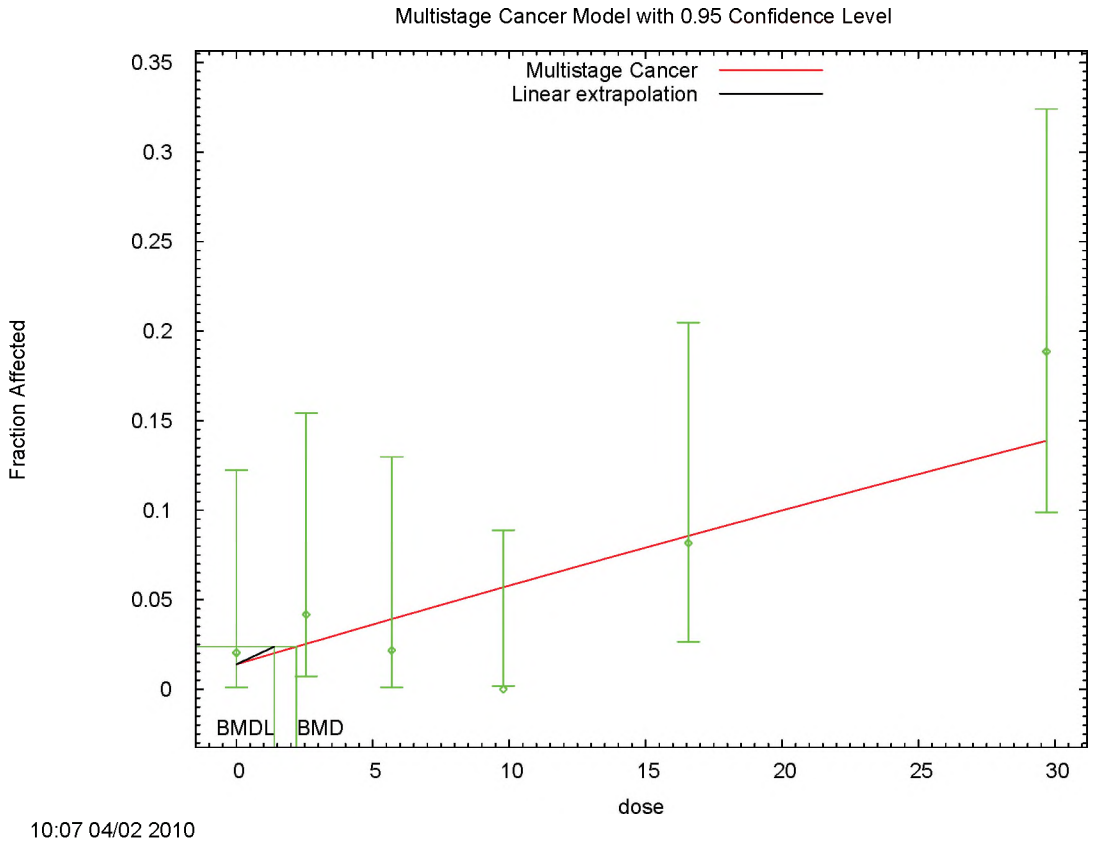
BMDL = 1.38901

BMDU = 4.3103

Taken together, (1.38901, 4.3103) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00719939

F.1.22.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 2006: Oral mucosa: squamous cell carcinoma

F.1.23. National Toxicology Program, 2006: Pancreas: adenoma or carcinoma

F.1.23.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**5**	**0.640**	**29.373**	**1.052E+01**	**4.630E+00**	
Multistage Cancer, 2-Degree	5	0.929	27.061	1.458E+01	7.227E+00	
Multistage Cancer, 3-Degree	5	0.986	25.972	1.739E+01	9.373E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.23.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 2006: Pancreas: adenoma or carcinoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\23_msc1_1Perc_panc_ad_carc.(d)
         Gnuplot Plotting File:  C:\1\Blood\23_msc1_1Perc_panc_ad_carc.plt
                                                Fri Apr 02 11:07:29 2010
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =   0.00191132


           Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

Beta(1)

Beta(1) 1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.000955662	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-11.4096	6			
Fitted model	-13.6865	1	4.55375	5	0.4727
Reduced model	-16.7086	1	10.598	5	0.05996

AIC: 29.373

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	48	-0.007
2.5565	0.0024	0.117	0.000	48	-0.343
5.6937	0.0054	0.250	0.000	46	-0.501
9.7882	0.0093	0.466	0.000	50	-0.686
16.5688	0.0157	0.754	0.000	48	-0.875
29.6953	0.0280	1.427	3.000	51	1.336

Chi^2 = 3.39 d.f. = 5 P-value = 0.6403

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 10.5166

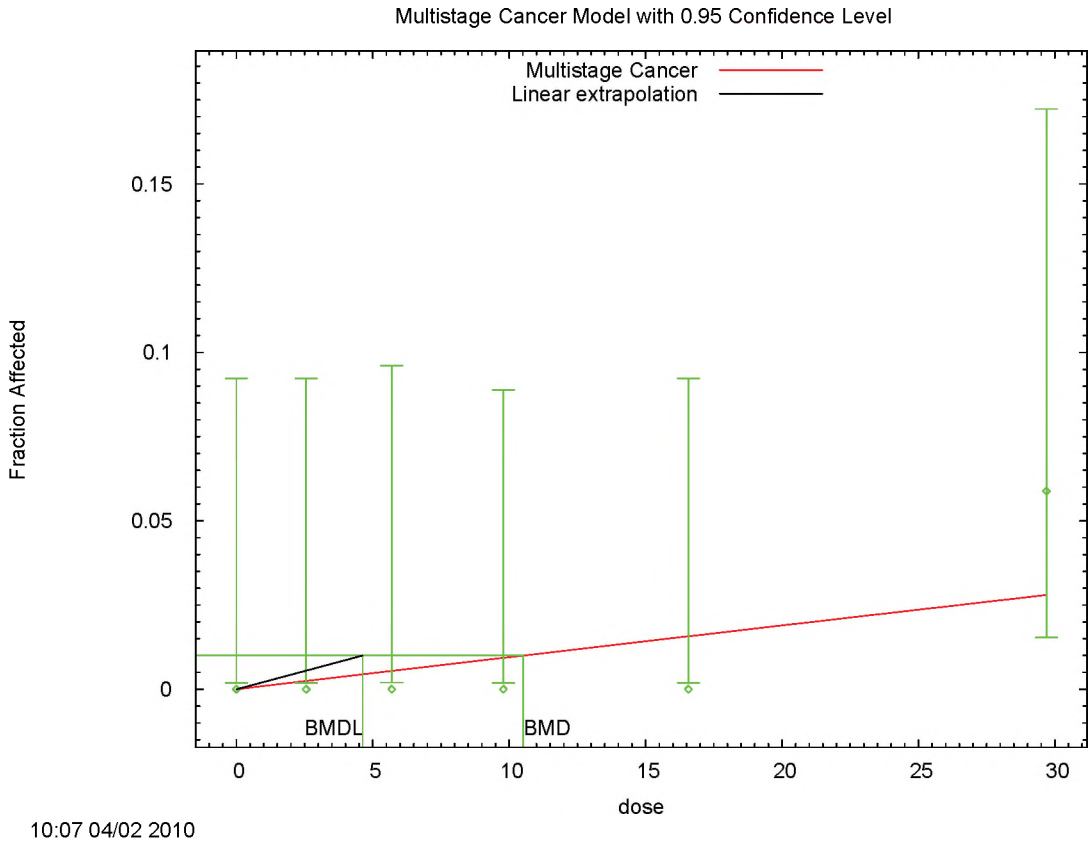
BMDL = 4.62967

BMDU = 32.8573

Taken together, (4.62967, 32.8573) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00215998

F.1.23.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 2006: Pancreas: adenoma or carcinoma

F.1.24. National Toxicology Program, 2006: Lung: Cystic keratinizing epithelioma

F.1.24.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	5	0.062	64.034	3.445E+00	2.084E+00	
Multistage Cancer, 2-Degree [a]	**5**	**0.507**	**56.943**	**8.304E+00**	**5.245E+00**	
Multistage Cancer, 3-Degree	5	0.845	53.558	1.193E+01	7.765E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.24.2. *Output for Selected Model: Multistage Cancer, 2-Degree*

National Toxicology Program, 2006: Lung: Cystic keratinizing epithelioma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\24_msc2_1Perc_lung_epith.(d)
         Gnuplot Plotting File:  C:\1\Blood\24_msc2_1Perc_lung_epith.plt
                                                 Fri Apr 02 11:07:57 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =           0
                        Beta(2) =  0.000216412
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.000145744	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-23.958	6			
Fitted model	-27.4714	1	7.02662	5	0.2187
Reduced model	-40.2069	1	32.4976	5	<.0001

AIC: 56.9427

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	49	0.000
2.5565	0.0010	0.046	0.000	48	-0.214
5.6937	0.0047	0.217	0.000	46	-0.467
9.7882	0.0139	0.679	0.000	49	-0.830
16.5688	0.0392	1.922	0.000	49	-1.414
29.6953	0.1206	6.271	9.000	52	1.162

Chi^2 = 4.30 d.f. = 5 P-value = 0.5067

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 8.30415

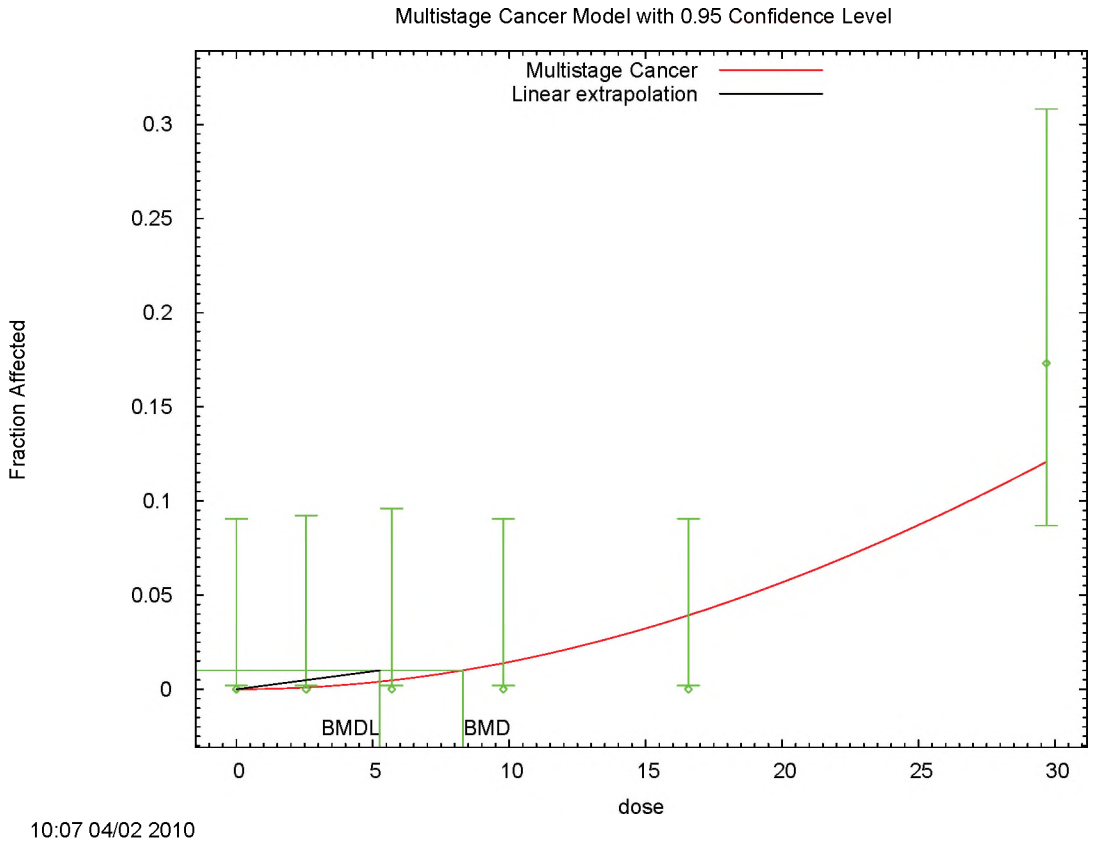
BMDL = 5.24499

BMDU = 11.2298

Taken together, (5.24499, 11.2298) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00190658

F.1.24.3. *Figure for Selected Model: Multistage Cancer, 2-Degree*



National Toxicology Program, 2006: Lung: Cystic keratinizing epithelioma

F.1.25. Toth et al., 1979: Liver: Tumors

F.1.25.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**1**	**0.293**	**155.740**	**3.684E-01**	**2.096E-01**	
Multistage Cancer, 2-Degree	1	0.293	155.740	3.684E-01	2.096E-01	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.25.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Toth et al., 1979: Liver: Tumors

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\25_msc1_1Perc_adr_cor_1yr.(d)
         Gnuplot Plotting File:  C:\1\Blood\25_msc1_1Perc_adr_cor_1yr.plt
                                              Fri Apr 02 11:08:26 2010
 ================================================================

 Table 1
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 1
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.234952
                        Beta(1) =    0.0269892


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)
```

Background	1	-0.55
Beta(1)	-0.55	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.235297	*	*	*
Beta(1)	0.0272796	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-75.3127	3			
Fitted model	-75.8702	2	1.11506	1	0.291
Reduced model	-79.4897	1	8.35401	2	0.01534

AIC: 155.74

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2353	8.941	7.000	38	-0.742
0.5732	0.2472	10.875	13.000	44	0.743
14.2123	0.4811	21.167	21.000	44	-0.050

Chi^2 = 1.11 d.f. = 1 P-value = 0.2931

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.368419

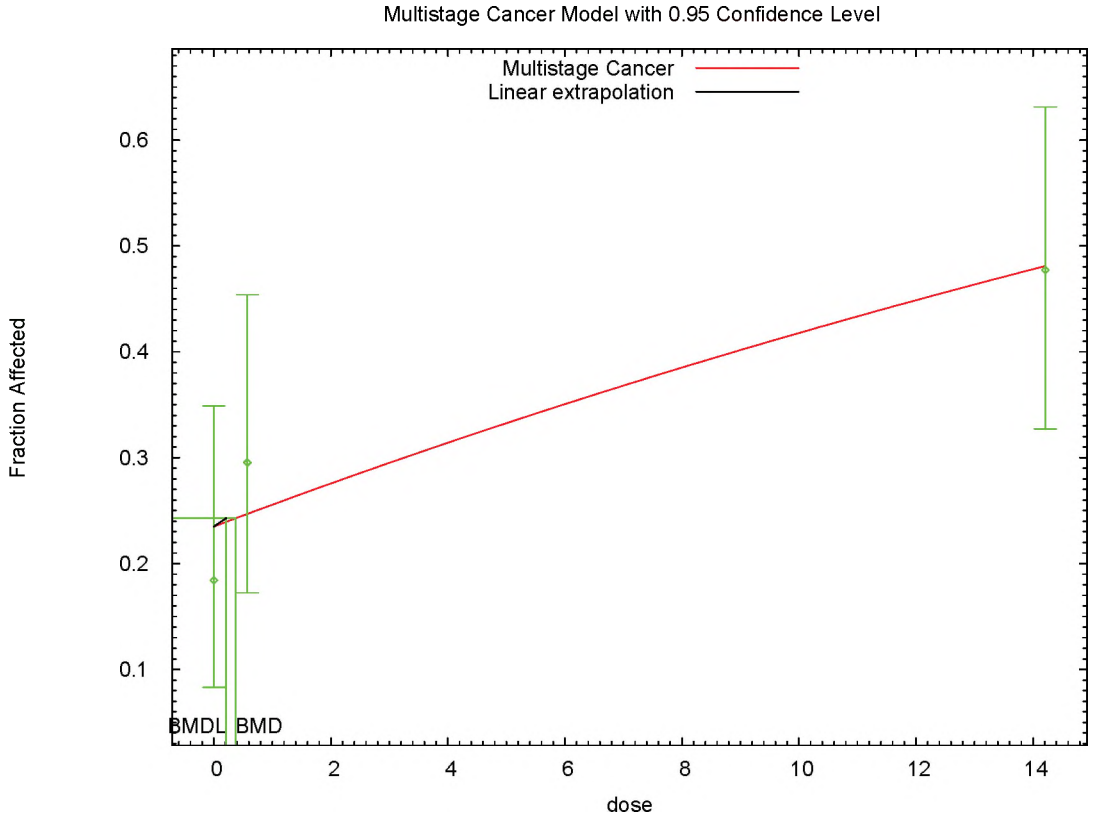
BMDL = 0.209642

BMDU = 1.01064

Taken together, (0.209642, 1.01064) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0477004

F.1.25.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Toth et al., 1979: Liver: Tumors

F.1.26. Della Porta et al., 1987: Table 4, B6C3 mice, male, hepatocellular carcinoma

F.1.26.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	1	0.036	165.333	9.239E-01	6.933E-01	
Multistage Cancer, 2-Degree [a]	**1**	**0.525**	**161.217**	**7.143E+00**	**1.170E+00**	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.26.2. *Output for Selected Model: Multistage Cancer, 2-Degree*

Della Porta et al., 1987: Table 4, B6C3 mice, male, hepatocellular carcinoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\94_DPorta_1987_Male_Hep_Carc_MultiCanc2_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\94_DPorta_1987_Male_Hep_Carc_MultiCanc2_1.plt
                                                Fri Apr 02 13:52:21 2010
 ======================================================================

 Table 4, B6C3 mice, Male, Hepatocellular carcinoma
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0865895
                        Beta(1) =            0
                        Beta(2) =  0.000211877


            Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.64
Beta(2)	-0.64	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.107218	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.00019698	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-78.4036	3			
Fitted model	-78.6083	2	0.409345	1	0.5223
Reduced model	-94.7394	1	32.6717	2	<.0001

AIC: 161.217

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1072	4.610	5.000	43	0.192
37.9990	0.3282	16.740	15.000	51	-0.519
67.7695	0.6387	31.936	33.000	50	0.313

Chi^2 = 0.40 d.f. = 1 P-value = 0.5249

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.14298

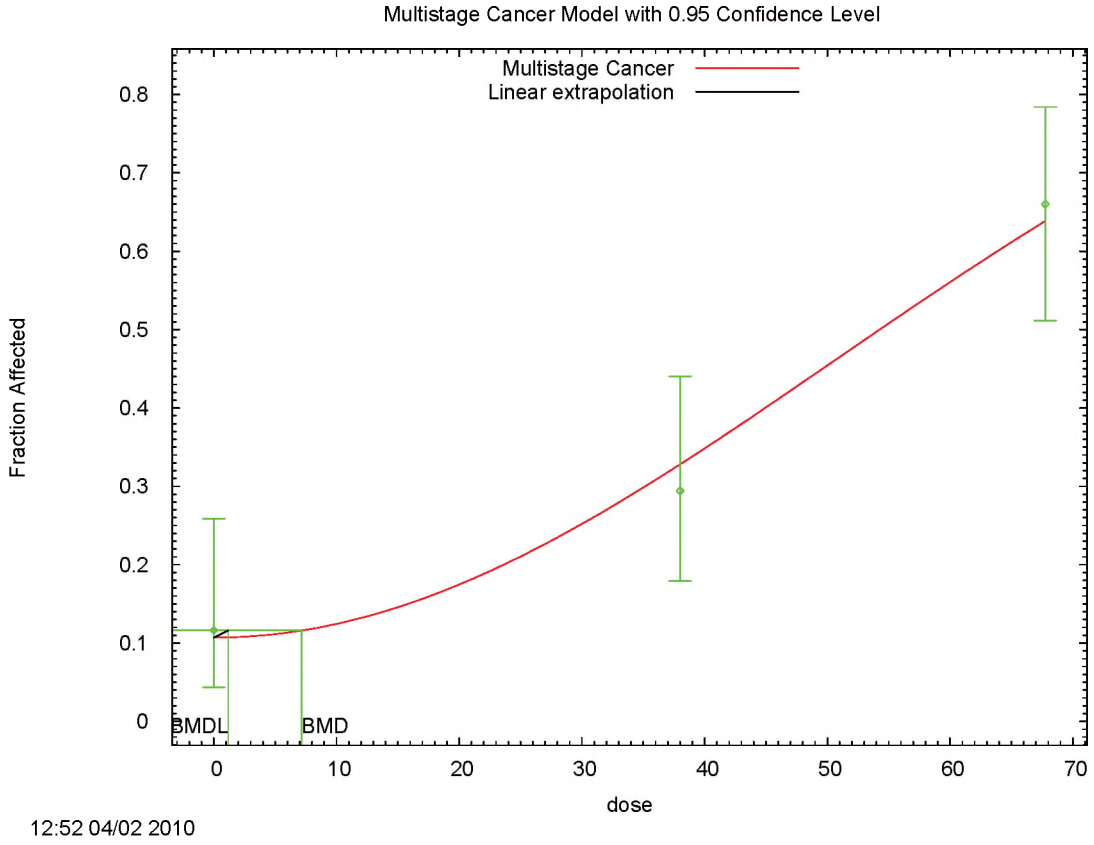
BMDL = 1.16991

BMDU = 8.58118

Taken together, (1.16991, 8.58118) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0085477

F.1.26.3. *Figure for Selected Model: Multistage Cancer, 2-Degree*



Della Porta et al., 1987: Table 4, B6C3 mice, male, hepatocellular carcinoma

F.1.27. Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular adenoma

F.1.27.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	1	0.380	99.614	3.599E+00	2.186E+00	
Multistage Cancer, 2-Degree [a]	**1**	**0.863**	**98.833**	**1.449E+01**	**2.342E+00**	

[a] Best-fitting model, BMDS output presented in this appendix

F.1.27.2. *Output for Selected Model: Multistage Cancer, 2-Degree*

Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular adenoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\95_DPorta_1987_Female_Hep_Aden_MultiCanc2_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\95_DPorta_1987_Female_Hep_Aden_MultiCanc2_1.plt
                                                 Fri Apr 02 13:52:51 2010
 ======================================================================

 Table 4, B6C3 mice, Female, Hepatocellular adenoma
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0364319
                        Beta(1) =            0
                        Beta(2) = 4.92861e-005


            Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.69
Beta(2)	-0.69	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0392633	*	*	*
Beta(1)	0	*	*	*
Beta(2)	4.78928e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-47.4015	3			
Fitted model	-47.4165	2	0.0299957	1	0.8625
Reduced model	-51.6367	1	8.47042	2	0.01448

AIC: 98.8329

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0393	1.924	2.000	49	0.056
37.5865	0.1021	4.289	4.000	42	-0.147
66.9741	0.2250	10.800	11.000	48	0.069

Chi^2 = 0.03 d.f. = 1 P-value = 0.8634

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 14.4862

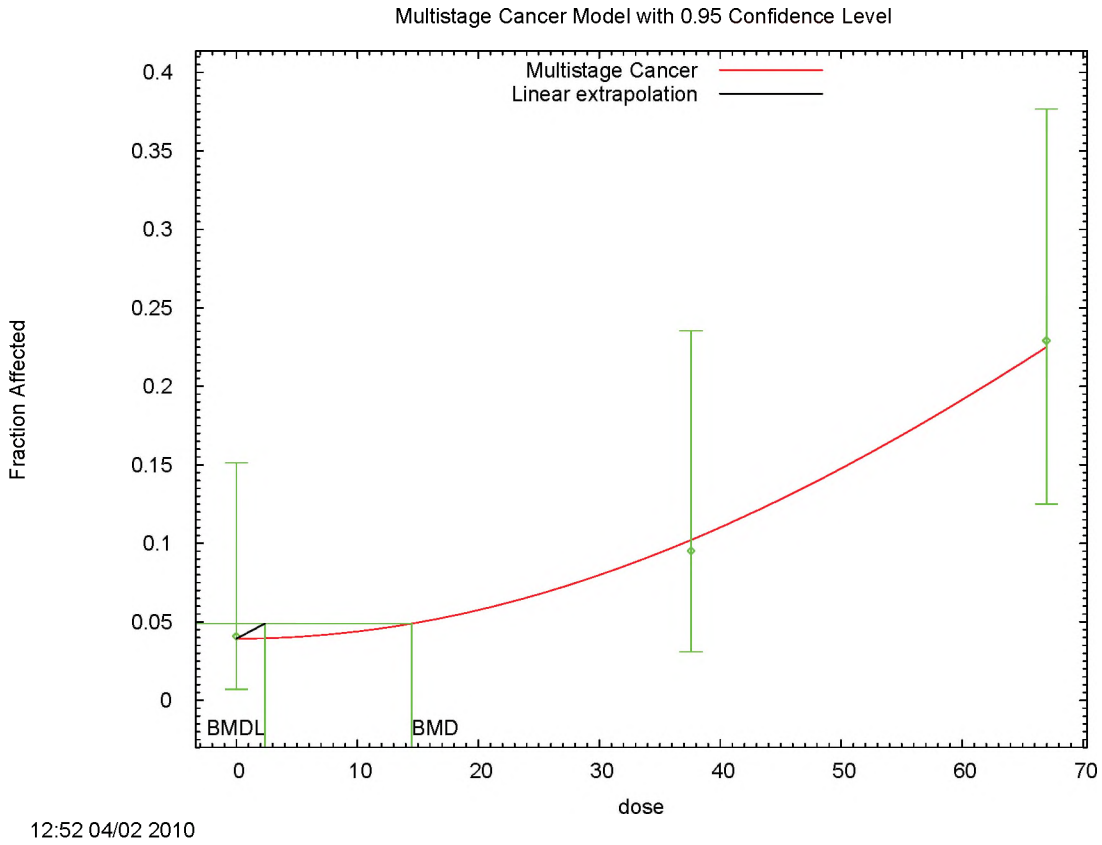
BMDL = 2.3421

BMDU = 22.1663

Taken together, (2.3421 , 22.1663) is a 90 % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor = 0.00426967

F.1.27.3. *Figure for Selected Model: Multistage Cancer, 2-Degree*



Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular adenoma

F.1.28. Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular carcinoma

F.1.28.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**1**	**0.019**	**115.539**	**2.302E+00**	**1.545E+00**	
Multistage Cancer, 2-Degree	1	0.019	115.539	2.302E+00	1.545E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.1.28.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular carcinoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\Blood\96_DPorta_1987_Female_Hep_Carc_MultiCanc1_1.(d)
         Gnuplot Plotting File:  C:\1\Blood\96_DPorta_1987_Female_Hep_Carc_MultiCanc1_1.plt
                                                Fri Apr 02 13:53:20 2010
 ======================================================================

 Table 4, B6C3 mice, Female, Hepatocellular carcinoma
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0787329
                        Beta(1) =   0.00304814


            Asymptotic Correlation Matrix of Parameter Estimates

              Background      Beta(1)
```

Background	1	-0.8
Beta(1)	-0.8	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0268873	*	*	*
Beta(1)	0.00436529	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-53.1726	3			
Fitted model	-55.7697	2	5.19425	1	0.02266
Reduced model	-60.7146	1	15.084	2	0.0005303

AIC: 115.539

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0269	1.317	1.000	49	-0.280
37.5865	0.1741	7.314	12.000	42	1.907
66.9741	0.2736	13.131	9.000	48	-1.338

Chi^2 = 5.50 d.f. = 1 P-value = 0.0190

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.30233

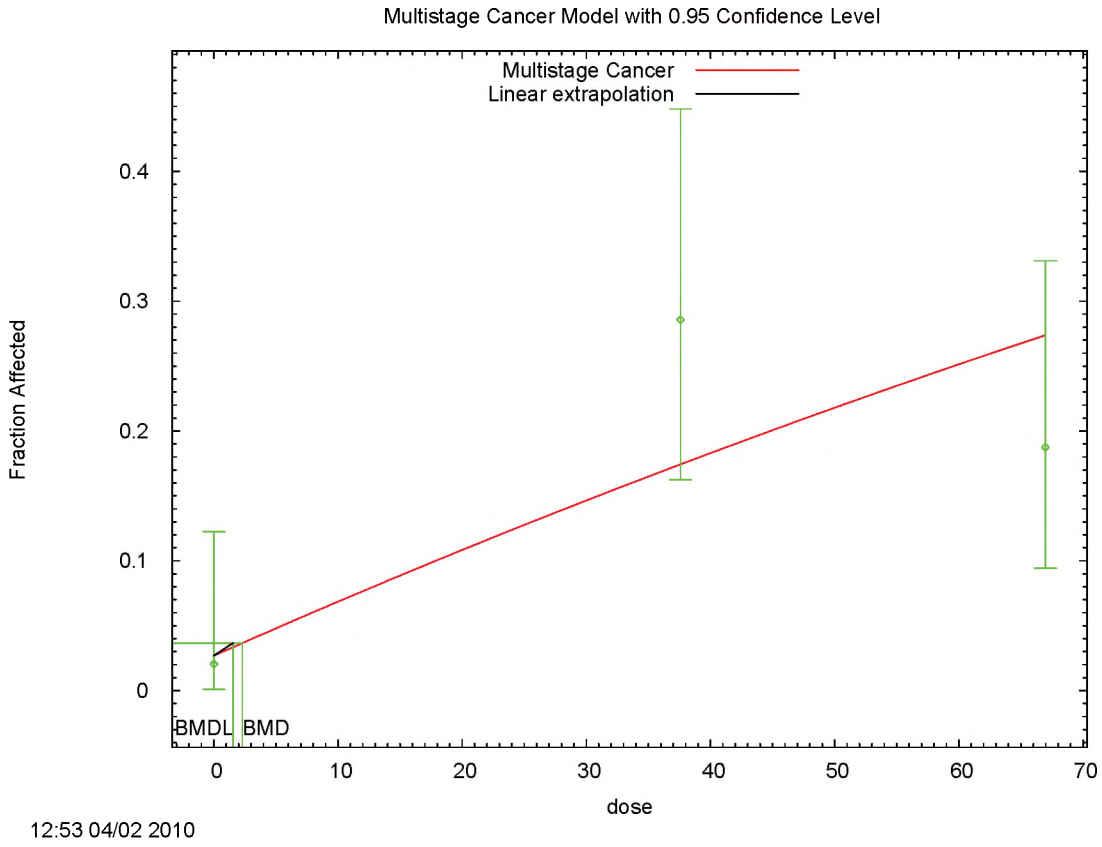
BMDL = 1.54479

BMDU = 4.37768

Taken together, (1.54479, 4.37768) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00647339

F.1.28.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular carcinoma

F.2. ADMINISTERED DOSE BMDS RESULTS

F.2.1. Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

F.2.1.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**3**	**0.928**	**30.745**	**1.344E+01**	**6.515E+00**	
Multistage Cancer, 2-Degree	3	0.998	29.961	3.490E+01	7.216E+00	
Multistage Cancer, 3-Degree	3	1.000	29.885	4.941E+01	7.297E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.1.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\1_msc1_1Perc_palate_nasal.(d)
         Gnuplot Plotting File:  C:\Canc\1_msc1_1Perc_palate_nasal.plt
                                                 Thu Apr 01 12:47:40 2010
 ======================================================================

 Source - Table 4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
```

```
                    Background =            0
                       Beta(1) =  0.000858074


            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Background
                  have been estimated at a boundary point, or have been specified by the user,
                  and do not appear in the correlation matrix )

                 Beta(1)

    Beta(1)            1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
      Background                0            *                *                   *
         Beta(1)       0.00074801            *                *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
      Full model        -13.9385         4
     Fitted model       -14.3726         1      0.868297      3          0.8331
   Reduced model        -20.2589         1       12.6409      3        0.005481

            AIC:         30.7452


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
     0.0000     0.0000         0.000     0.000          85        0.000
     1.0000     0.0007         0.037     0.000          50       -0.193
    10.0000     0.0075         0.373     0.000          50       -0.613
   100.0000     0.0721         3.604     4.000          50        0.217

 Chi^2 = 0.46      d.f. = 3        P-value = 0.9276


    Benchmark Dose Computation

Specified effect =           0.01

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        13.4361

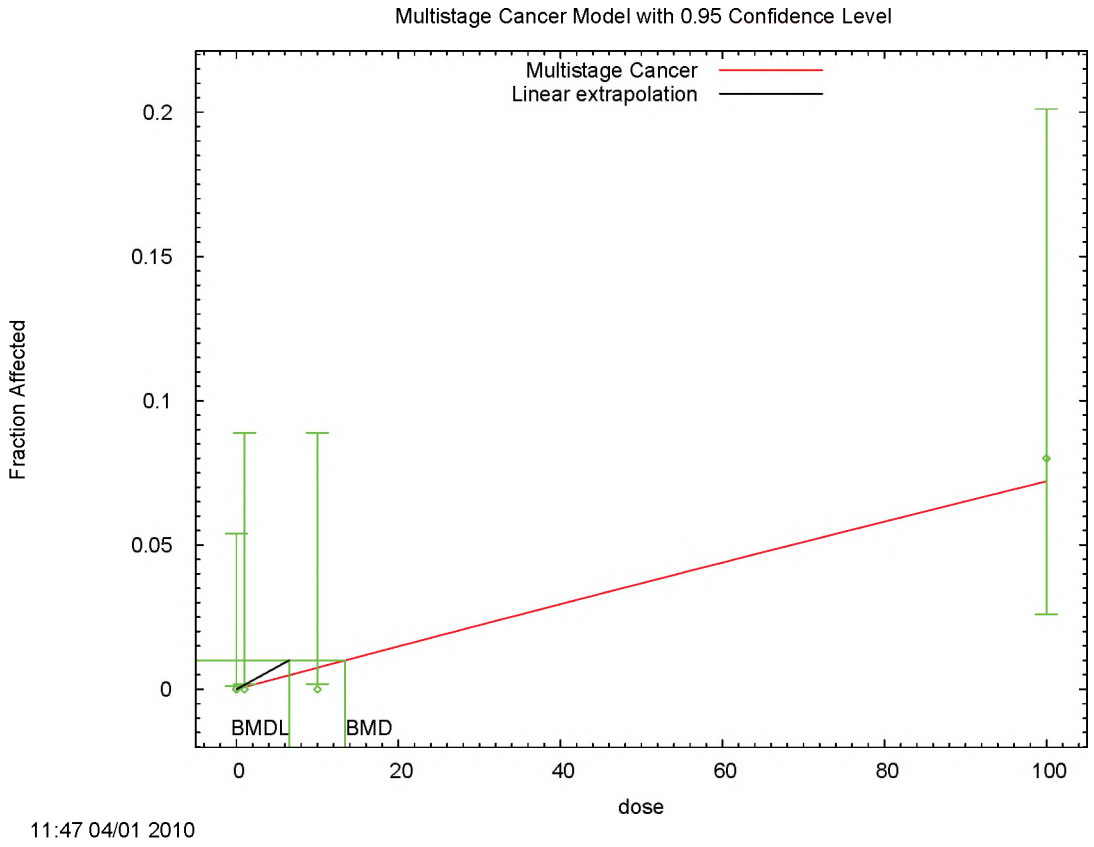
             BMDL =        6.51522

             BMDU =         34.829

Taken together, (6.51522, 34.829 ) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00153487
```

F.2.1.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

F.2.2. Kociba et al., 1978: Stratified squamous cell carcinoma of tongue

F.2.2.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.451**	**48.368**	**1.742E+01**	**7.146E+00**	
Multistage Cancer, 2-Degree	2	0.451	48.368	1.742E+01	7.146E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.451	48.368	1.742E+01	7.146E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.2.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Stratified squamous cell carcinoma of tongue

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\2_msc1_1Perc_tongue.(d)
         Gnuplot Plotting File:  C:\Canc\2_msc1_1Perc_tongue.plt
                                                Thu Apr 01 12:48:16 2010
 ======================================================================

 Source - Table 4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0113883
                        Beta(1) =  0.000508703
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.52
Beta(1)	-0.52	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.00809154	*	*	*
Beta(1)	0.000576915	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-21.1523	4			
Fitted model	-22.1838	2	2.06309	2	0.3565
Reduced model	-24.1972	1	6.08976	3	0.1073

AIC: 48.3677

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0081	0.688	0.000	85	-0.833
1.0000	0.0087	0.433	1.000	50	0.865
10.0000	0.0138	0.690	1.000	50	0.376
100.0000	0.0637	3.185	3.000	50	-0.107

Chi^2 = 1.59 d.f. = 2 P-value = 0.4506

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 17.4208

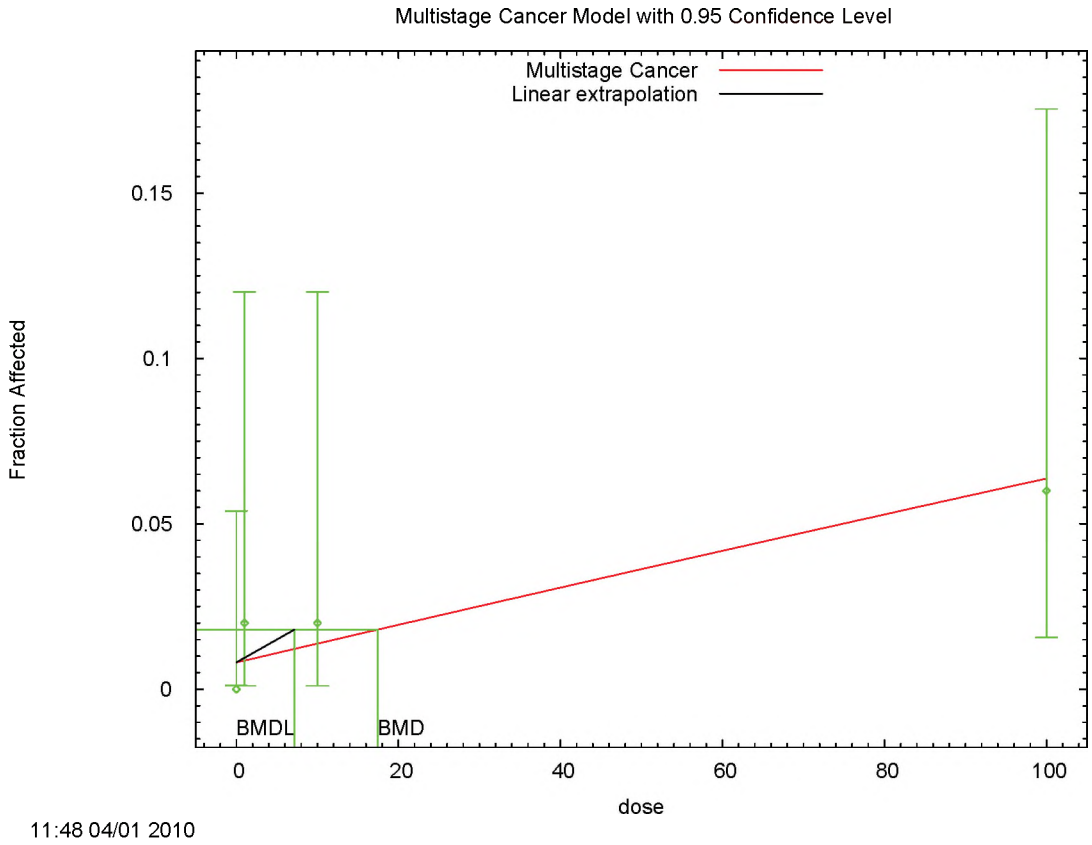
BMDL = 7.14637

BMDU = 3.20359e+006

Taken together, (7.14637, 3.20359e+006) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00139931

F.2.2.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Stratified squamous cell carcinoma of tongue

F.2.3. Kociba et al., 1978: Adenoma of adrenal cortex

F.2.3.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**3**	**0.376**	**53.518**	**7.587E+00**	**4.317E+00**	
Multistage Cancer, 2-Degree	3	0.376	53.518	7.587E+00	4.317E+00	final ß=0
Multistage Cancer, 3-Degree	3	0.376	53.518	7.587E+00	4.317E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.3.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Adenoma of adrenal cortex

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\3_msc1_1Perc_adre_adenoma.(d)
         Gnuplot Plotting File:  C:\Canc\3_msc1_1Perc_adre_adenoma.plt
                                                 Thu Apr 01 12:48:52 2010
 ======================================================================

 Source - Table 5
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =   0.00927818
                        Beta(1) =   0.00098105
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00132464	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-24.6514	4			
Fitted model	-25.759	1	2.2152	3	0.529
Reduced model	-31.4904	1	13.6781	3	0.003378

AIC: 53.5179

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	85	0.000
1.0000	0.0013	0.066	0.000	50	-0.257
10.0000	0.0132	0.658	2.000	50	1.666
100.0000	0.1241	6.203	5.000	50	-0.516

Chi^2 = 3.11 d.f. = 3 P-value = 0.3755

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.58722

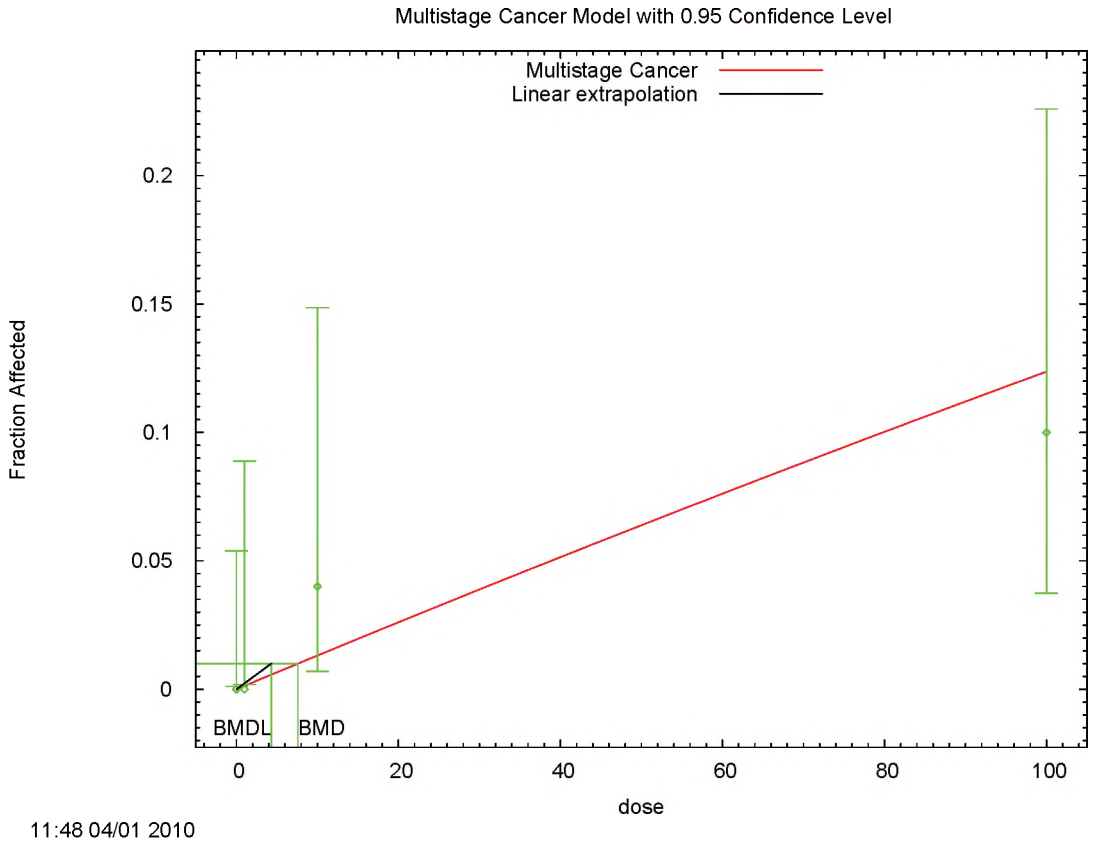
BMDL = 4.31737

BMDU = 17.638

Taken together, (4.31737, 17.638) is a 90 % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor = 0.00231623

F.2.3.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Adenoma of adrenal cortex

F.2.4. Kociba et al., 1978: Hepatocellular adenoma(s) or carcinoma(s)

F.2.4.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.034**	**146.199**	**1.769E+00**	**1.225E+00**	
Multistage Cancer, 2-Degree	2	0.034	146.199	1.768E+00	1.225E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.034	146.199	1.768E+00	1.225E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.4.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Hepatocellular adenoma(s) or carcinoma(s)

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\4_msc1_1Perc_liver_ad_carc.(d)
         Gnuplot Plotting File:  C:\Canc\4_msc1_1Perc_liver_ad_carc.plt
                                                Thu Apr 01 12:49:25 2010
 ======================================================================

 Source - Table 1 in Goodman and Sauer 1992
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0591902
                        Beta(1) =   0.00458516
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.47
Beta(1)	-0.47	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0328755	*	*	*
Beta(1)	0.00568299	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-68.2561	4			
Fitted model	-71.0993	2	5.68634	2	0.05824
Reduced model	-89.1983	1	41.8843	3	<.0001

AIC: 146.199

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0329	2.827	2.000	86	-0.500
1.0000	0.0384	1.918	1.000	50	-0.676
10.0000	0.0863	4.315	9.000	50	2.359
100.0000	0.4521	20.346	18.000	45	-0.703

Chi^2 = 6.77 d.f. = 2 P-value = 0.0339

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.7685

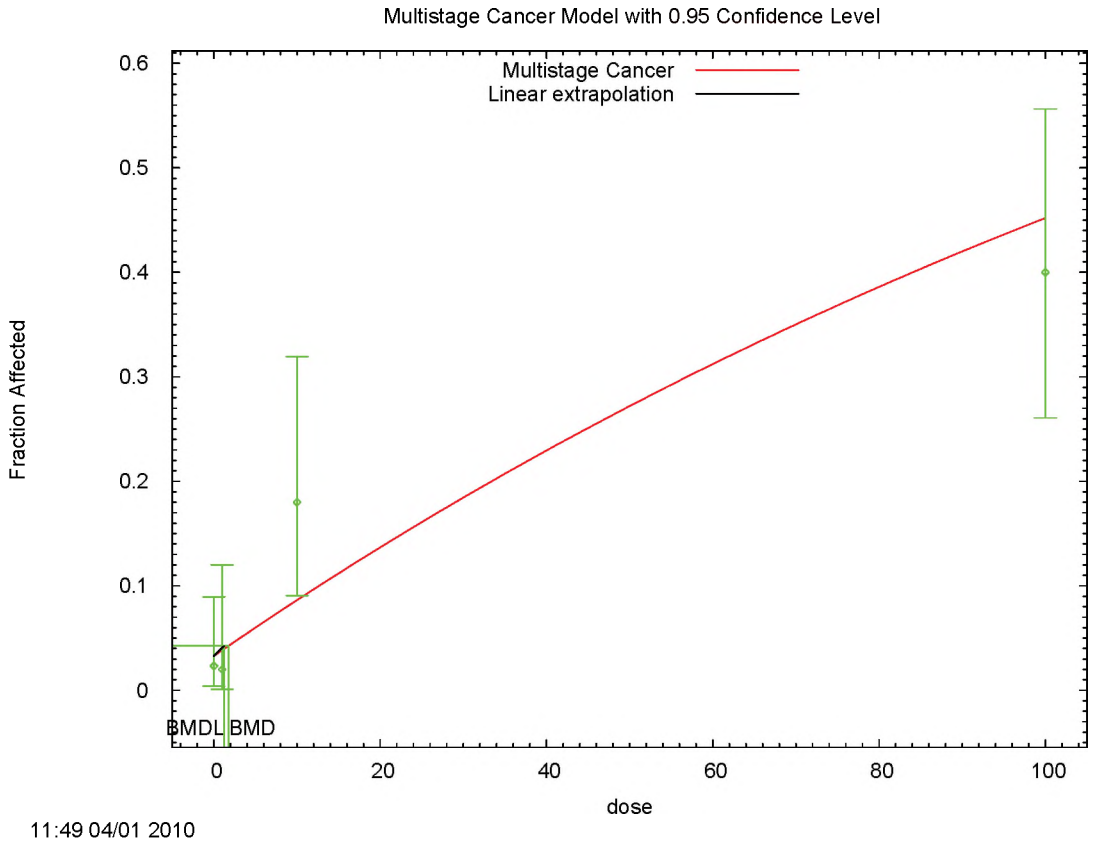
BMDL = 1.22517

BMDU = 2.77641

Taken together, (1.22517, 2.77641) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00816214

F.2.4.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Hepatocellular adenoma(s) or carcinoma(s)

F.2.5. Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

F.2.5.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**3**	**0.928**	**30.745**	**1.344E+01**	**6.515E+00**	
Multistage Cancer, 2-Degree	3	0.998	29.961	3.490E+01	7.216E+00	
Multistage Cancer, 3-Degree	3	1.000	29.885	4.941E+01	7.297E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.5.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\Canc\5_msc1_1Perc_nasal.(d)
        Gnuplot Plotting File:  C:\Canc\5_msc1_1Perc_nasal.plt
                                          Thu Apr 01 12:49:59 2010
=================================================================

Source - Table 5
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  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
               -beta1*dose^1)]

  The parameter betas are restricted to be positive


  Dependent variable = Mean
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                Default Initial Parameter Values
                   Background =   0.00343283
                      Beta(1) =  0.000825276
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.000953868	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-18.7562	4			
Fitted model	-19.0532	1	0.594034	3	0.8978
Reduced model	-24.1972	1	10.882	3	0.01238

AIC: 40.1064

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	86	0.000
1.0000	0.0010	0.048	0.000	50	-0.218
10.0000	0.0095	0.475	1.000	50	0.766
100.0000	0.0910	4.458	4.000	49	-0.227

Chi^2 = 0.69 d.f. = 3 P-value = 0.8764

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 10.5364

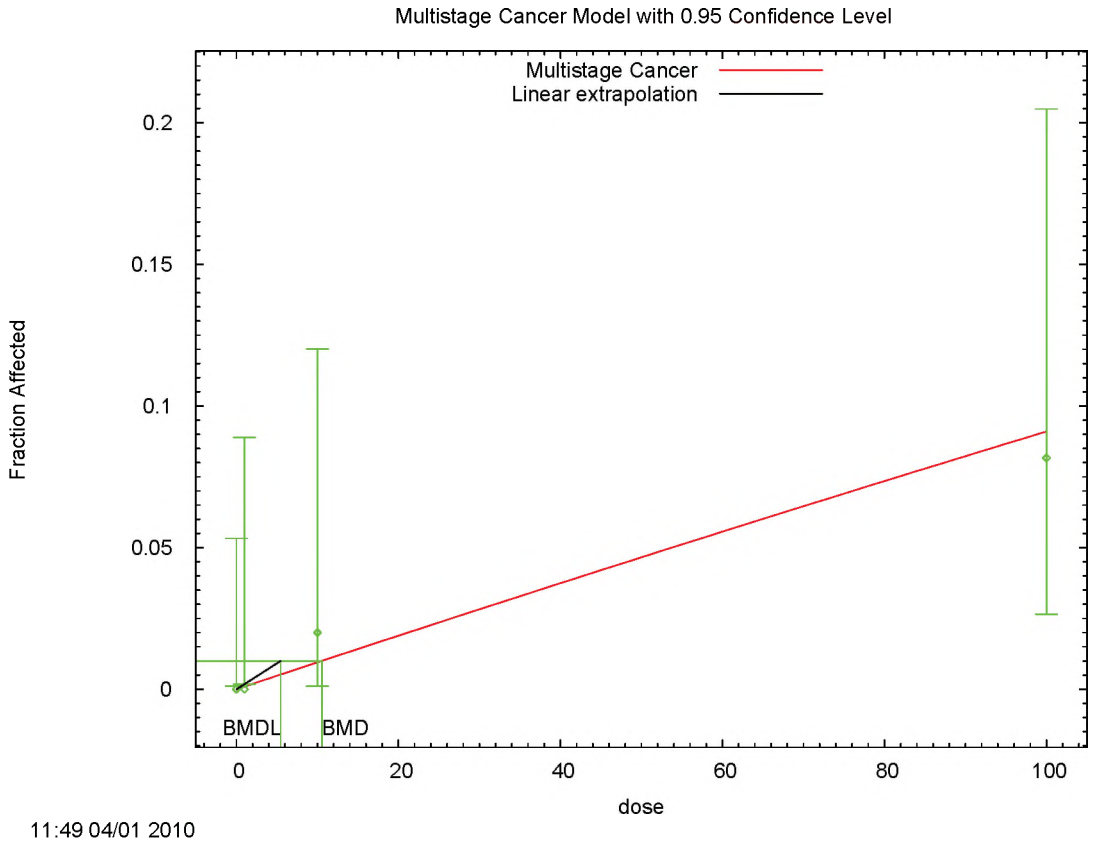
BMDL = 5.46907

BMDU = 25.864

Taken together, (5.46907, 25.864) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00182846

F.2.5.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Stratified squamous cell carcinoma of hard palate or nasal turbinates

F.2.6. Kociba et al., 1978: Keratinizing squamous cell carcinoma of lung

F.2.6.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**3**	**0.837**	**43.792**	**7.311E+00**	**4.159E+00**	
Multistage Cancer, 2-Degree	3	0.994	42.346	2.568E+01	4.917E+00	
Multistage Cancer, 3-Degree	3	1.000	42.207	4.026E+01	5.022E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.6.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Kociba et al., 1978: Keratinizing squamous cell carcinoma of lung

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\6_msc1_1Perc_kera_carc.(d)
         Gnuplot Plotting File:  C:\Canc\6_msc1_1Perc_kera_carc.plt
                                                Thu Apr 01 12:50:34 2010
 ================================================================

 Source - Table 5
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =   0.00158635
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.0013747	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-20.0957	4			
Fitted model	-20.8959	1	1.60041	3	0.6593
Reduced model	-31.4904	1	22.7894	3	<.0001

AIC: 43.7918

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	86	0.000
1.0000	0.0014	0.069	0.000	50	-0.262
10.0000	0.0137	0.683	0.000	50	-0.832
100.0000	0.1284	6.294	7.000	49	0.302

Chi^2 = 0.85 d.f. = 3 P-value = 0.8370

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.31091

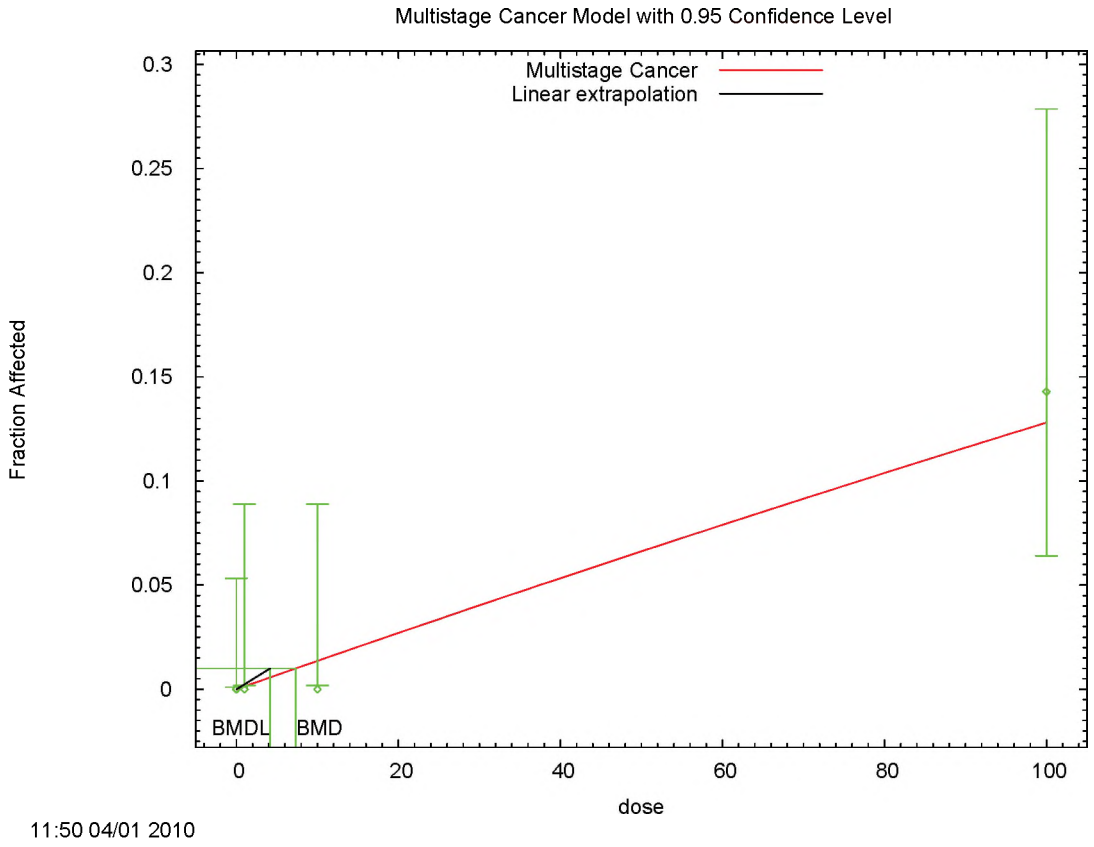
BMDL = 4.15929

BMDU = 14.6306

Taken together, (4.15929, 14.6306) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00240426

F.2.6.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Kociba et al., 1978: Keratinizing squamous cell carcinoma of lung

F.2.7. National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.2.7.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.146**	**76.377**	**9.761E+00**	**3.964E+00**	
Multistage Cancer, 2-Degree	2	0.146	76.377	9.761E+00	3.964E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.146	76.377	9.761E+00	3.964E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.7.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\7_msc1_1Perc_sub_fibro.(d)
         Gnuplot Plotting File:  C:\Canc\7_msc1_1Perc_sub_fibro.plt
                                                 Thu Apr 01 12:51:07 2010
 ======================================================================

 Source - Table 10
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.030595
                        Beta(1) =  0.000799545
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.54
Beta(1)	-0.54	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0231556	*	*	*
Beta(1)	0.00102962	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-33.5998	4			
Fitted model	-36.1883	2	5.17698	2	0.07513
Reduced model	-37.7465	1	8.29346	3	0.04032

AIC: 76.3766

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0232	1.737	0.000	75	-1.333
1.4000	0.0246	1.228	2.000	50	0.705
7.1000	0.0303	1.514	3.000	50	1.227
71.0000	0.0920	4.509	4.000	49	-0.252

Chi^2 = 3.84 d.f. = 2 P-value = 0.1463

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 9.76124

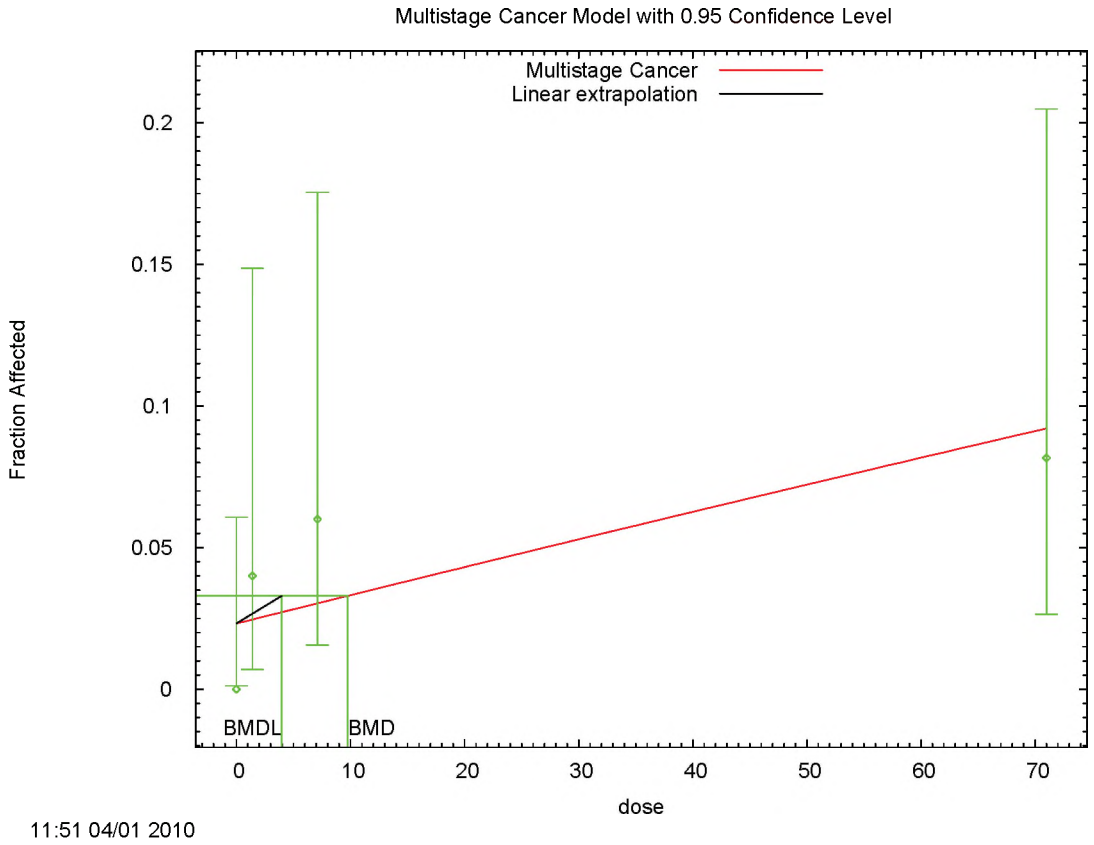
BMDL = 3.96354

BMDU = 1.03301e+006

Taken together, (3.96354, 1.03301e+006) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.002523

F.2.7.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.2.8. National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

F.2.8.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.398**	**133.832**	**2.554E+00**	**1.600E+00**	
Multistage Cancer, 2-Degree	2	0.503	133.436	1.334E+01	1.652E+00	
Multistage Cancer, 3-Degree	2	0.503	133.436	1.334E+01	1.652E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.8.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\8_msc1_1Perc_liver_nod.(d)
         Gnuplot Plotting File:  C:\Canc\8_msc1_1Perc_liver_nod.plt
                                              Thu Apr 01 12:51:41 2010
 ================================================================

 Source - Table 10
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0383072
                        Beta(1) =   0.00417257
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.47
Beta(1)	-0.47	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0451327	*	*	*
Beta(1)	0.00393556	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-63.9149	4			
Fitted model	-64.916	2	2.00214	2	0.3675
Reduced model	-74.0195	1	20.2092	3	0.0001536
AIC:	133.832				

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0451	3.385	5.000	75	0.898
1.4000	0.0504	2.469	1.000	49	-0.959
7.1000	0.0714	3.572	3.000	50	-0.314
71.0000	0.2779	13.618	14.000	49	0.122

Chi^2 = 1.84 d.f. = 2 P-value = 0.3984

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.55373

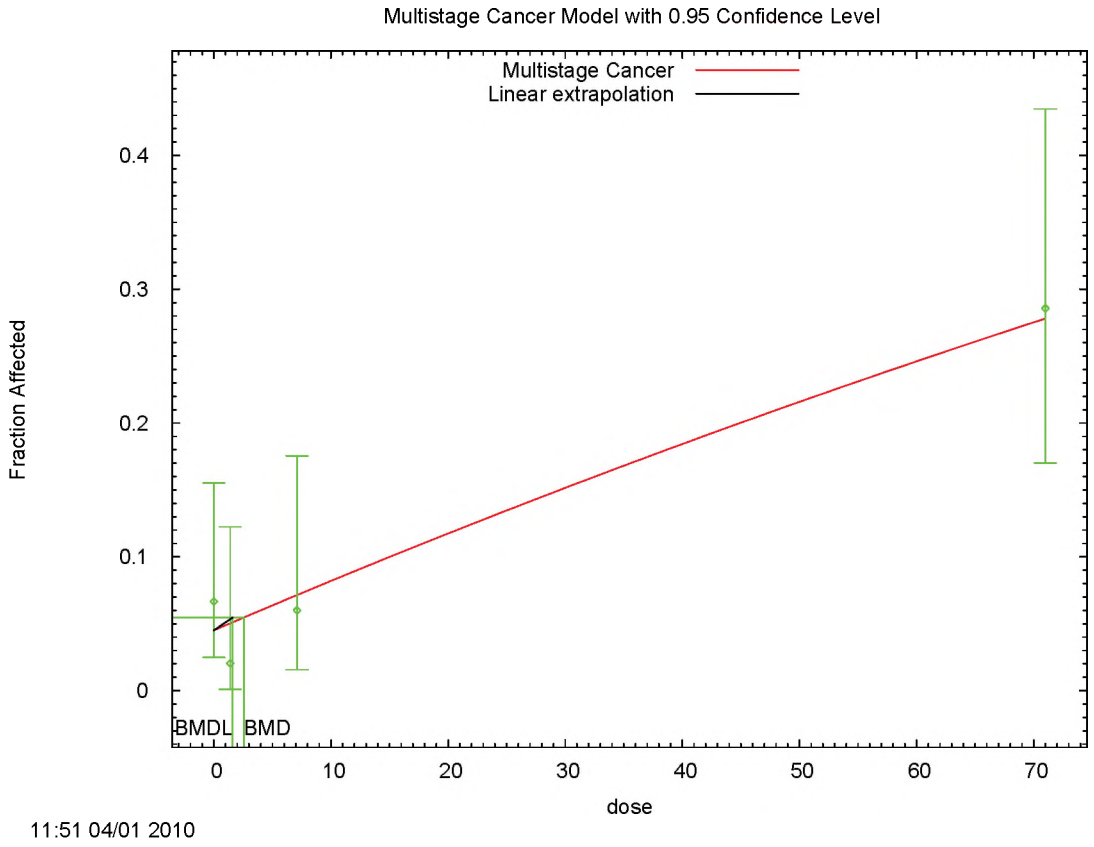
BMDL = 1.59983

BMDU = 4.74206

Taken together, (1.59983, 4.74206) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00625067

F.2.8.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

F.2.9. National Toxicology Program, 1982: Adrenal: Cortical Adenoma, or Carcinoma or Adenoma, NOS

F.2.9.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.405**	**203.380**	**3.672E+00**	**1.871E+00**	
Multistage Cancer, 2-Degree	2	0.501	202.885	1.577E+01	1.974E+00	
Multistage Cancer, 3-Degree	2	0.513	202.832	2.600E+01	1.986E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.9.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Adrenal: Cortical Adenoma, or Carcinoma or Adenoma, NOS

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\9_msc1_1Perc_adre_cort_ad_carc.(d)
         Gnuplot Plotting File:  C:\Canc\9_msc1_1Perc_adre_cort_ad_carc.plt
                                                Thu Apr 01 12:53:57 2010
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 Source - Table 10
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.140663
```

Beta(1) = 0.00289845

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.48
Beta(1)	-0.48	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.143284	*	*	*
Beta(1)	0.00273674	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-98.7282	4			
Fitted model	-99.6898	2	1.92318	2	0.3823
Reduced model	-102.201	1	6.94636	3	0.07363

AIC: 203.38

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1433	10.460	11.000	73	0.180
1.4000	0.1466	7.181	9.000	49	0.735
7.1000	0.1598	7.829	5.000	49	-1.103
71.0000	0.2946	13.551	14.000	46	0.145

Chi^2 = 1.81 d.f. = 2 P-value = 0.4046

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.67237

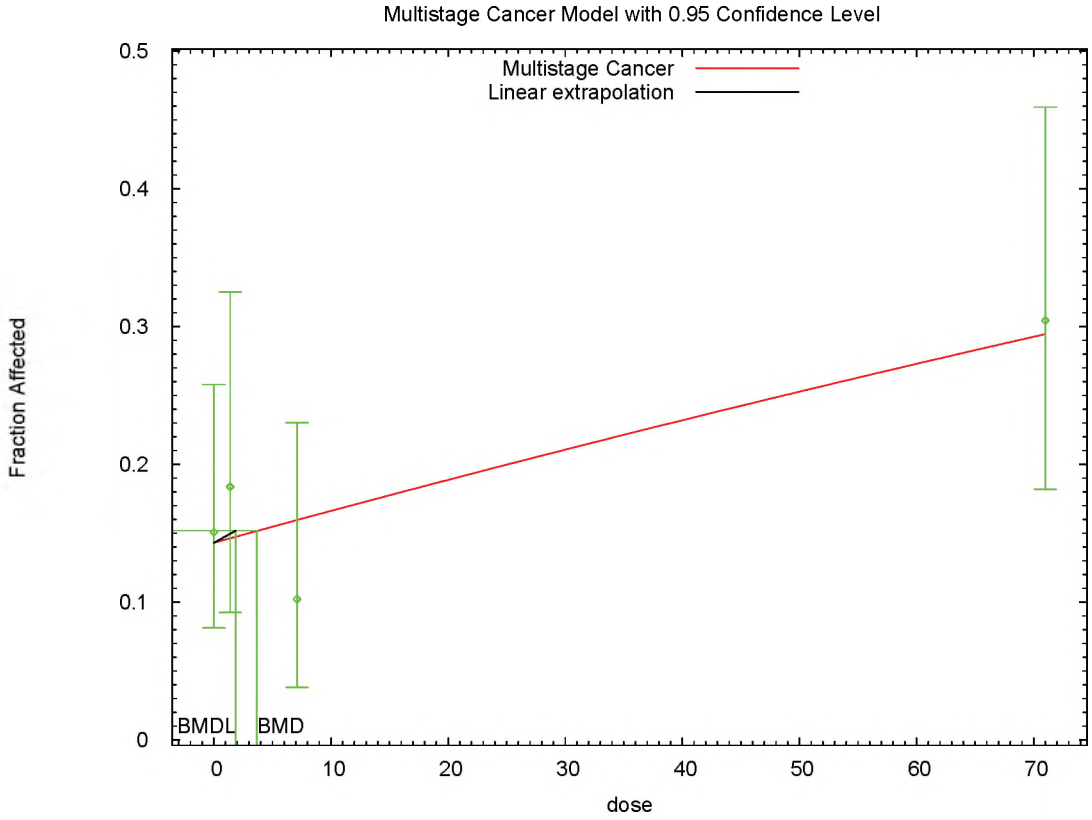
BMDL = 1.87133

BMDU = 15.4002

Taken together, (1.87133, 15.4002) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00534381

F.2.9.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Adrenal: Cortical Adenoma, or Carcinoma or Adenoma, NOS

F.2.10. National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma

F.2.10.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.661**	**92.020**	**7.571E+00**	**3.488E+00**	
Multistage Cancer, 2-Degree	2	0.769	91.639	2.257E+01	3.656E+00	
Multistage Cancer, 3-Degree	2	0.781	91.601	3.302E+01	3.675E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.10.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\10_msc1_1Perc_thy_ad.(d)
         Gnuplot Plotting File:  C:\Canc\10_msc1_1Perc_thy_ad.plt
                                                Thu Apr 01 12:54:31 2010
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 Source - Table 10
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.032089
                        Beta(1) =   0.00143599
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.5
Beta(1)	-0.5	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0345958	*	*	*
Beta(1)	0.00132742	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-43.5264	4			
Fitted model	-44.0098	2	0.966786	2	0.6167
Reduced model	-46.2299	1	5.40699	3	0.1443

AIC: 92.0196

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0346	2.525	3.000	73	0.304
1.4000	0.0364	1.637	2.000	45	0.289
7.1000	0.0437	2.139	1.000	49	-0.796
71.0000	0.1214	5.707	6.000	47	0.131

Chi^2 = 0.83 d.f. = 2 P-value = 0.6614

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.57131

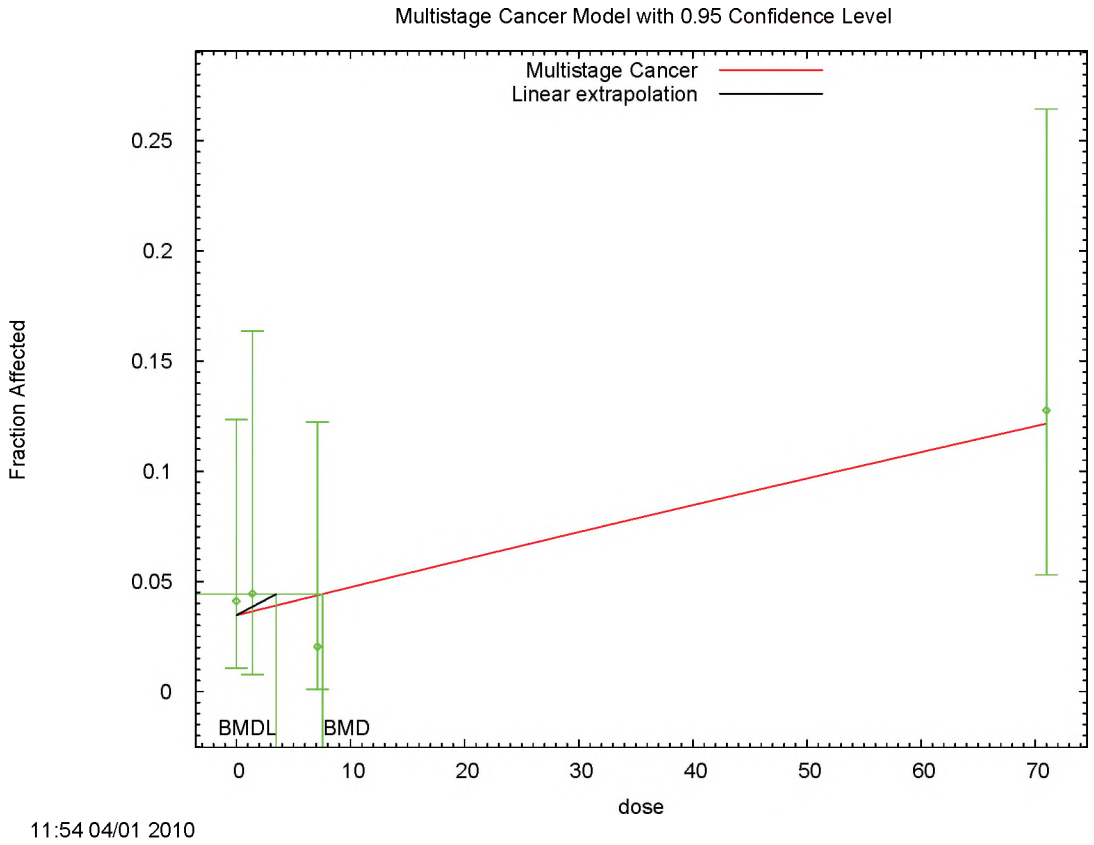
BMDL = 3.48815

BMDU = 964541

Taken together, (3.48815, 964541) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00286685

F.2.10.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma

F.2.11. National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

F.2.11.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.398**	**133.832**	**2.554E+00**	**1.600E+00**	
Multistage Cancer, 2-Degree	2	0.503	133.436	1.334E+01	1.652E+00	
Multistage Cancer, 3-Degree	2	0.503	133.436	1.334E+01	1.652E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.11.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\11_msc1_1Perc_liver_nod.(d)
         Gnuplot Plotting File:  C:\Canc\11_msc1_1Perc_liver_nod.plt
                                                Thu Apr 01 12:55:05 2010
 ======================================================================

 Source - Table 9
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =  0.000900399
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.000775683	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-11.3484	4			
Fitted model	-11.6976	1	0.698469	3	0.8736
Reduced model	-15.9189	1	9.14109	3	0.02747

AIC: 25.3952

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	74	0.000
1.4000	0.0011	0.054	0.000	50	-0.233
7.1000	0.0055	0.275	0.000	50	-0.525
71.0000	0.0536	2.679	3.000	50	0.201

Chi^2 = 0.37 d.f. = 3 P-value = 0.9462

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 12.9568

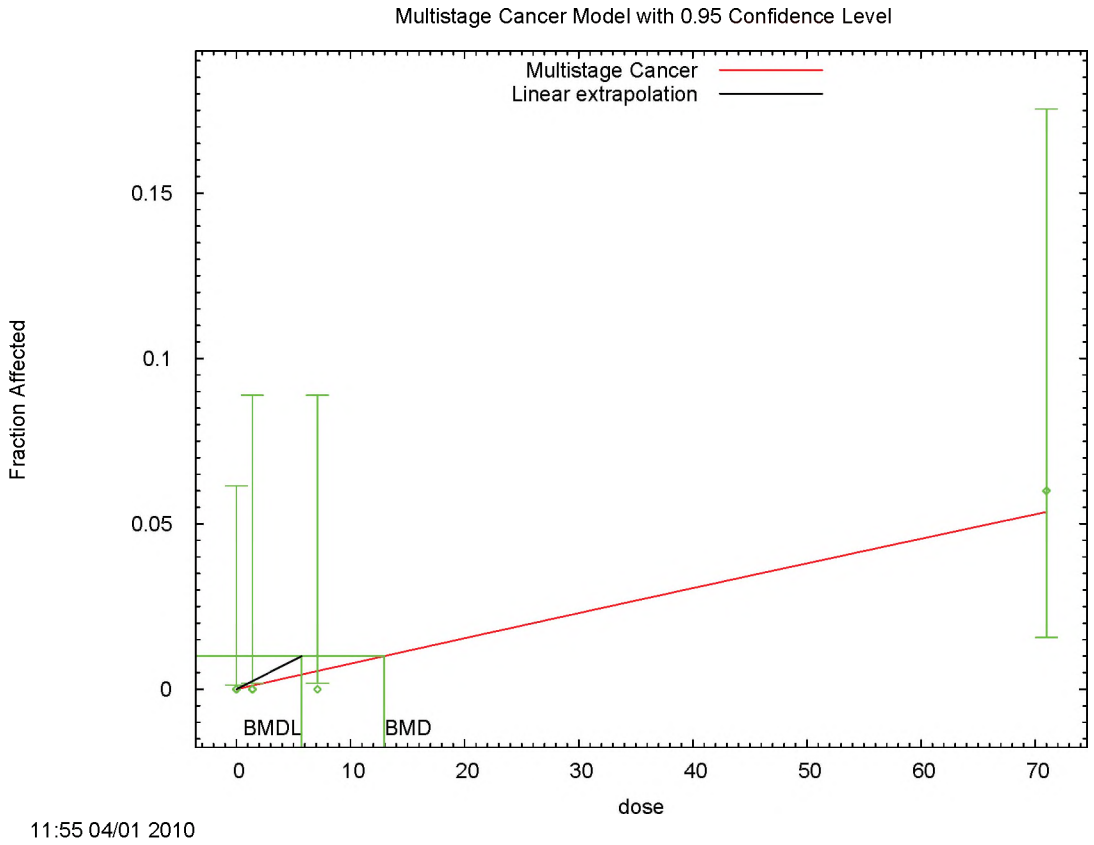
BMDL = 5.70369

BMDU = 39.9878

Taken together, (5.70369, 39.9878) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00175325

F.2.11.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Liver: Neoplastic Nodule or Hepatocellular Carcinoma

F.2.12. National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma or Carcinoma

F.2.12.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.028**	**151.224**	**3.521E+00**	**1.916E+00**	
Multistage Cancer, 2-Degree	2	0.028	151.224	3.521E+00	1.916E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.028	151.224	3.521E+00	1.916E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.12.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma or Carcinoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\12_msc1_1Perc_thyroid.(d)
         Gnuplot Plotting File:  C:\Canc\12_msc1_1Perc_thyroid.plt
                                                Thu Apr 01 12:55:38 2010
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 Source - Table 9
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0867382
                        Beta(1) =   0.00232055
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.53
Beta(1)	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0704713	*	*	*
Beta(1)	0.00285481	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-69.5946	4			
Fitted model	-73.6119	2	8.03468	2	0.018
Reduced model	-77.5267	1	15.8643	3	0.001209

AIC: 151.224

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0705	4.863	1.000	69	-1.817
1.4000	0.0742	3.561	5.000	48	0.793
7.1000	0.0891	4.456	8.000	50	1.759
71.0000	0.2410	12.051	11.000	50	-0.347

Chi^2 = 7.14 d.f. = 2 P-value = 0.0281

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.5205

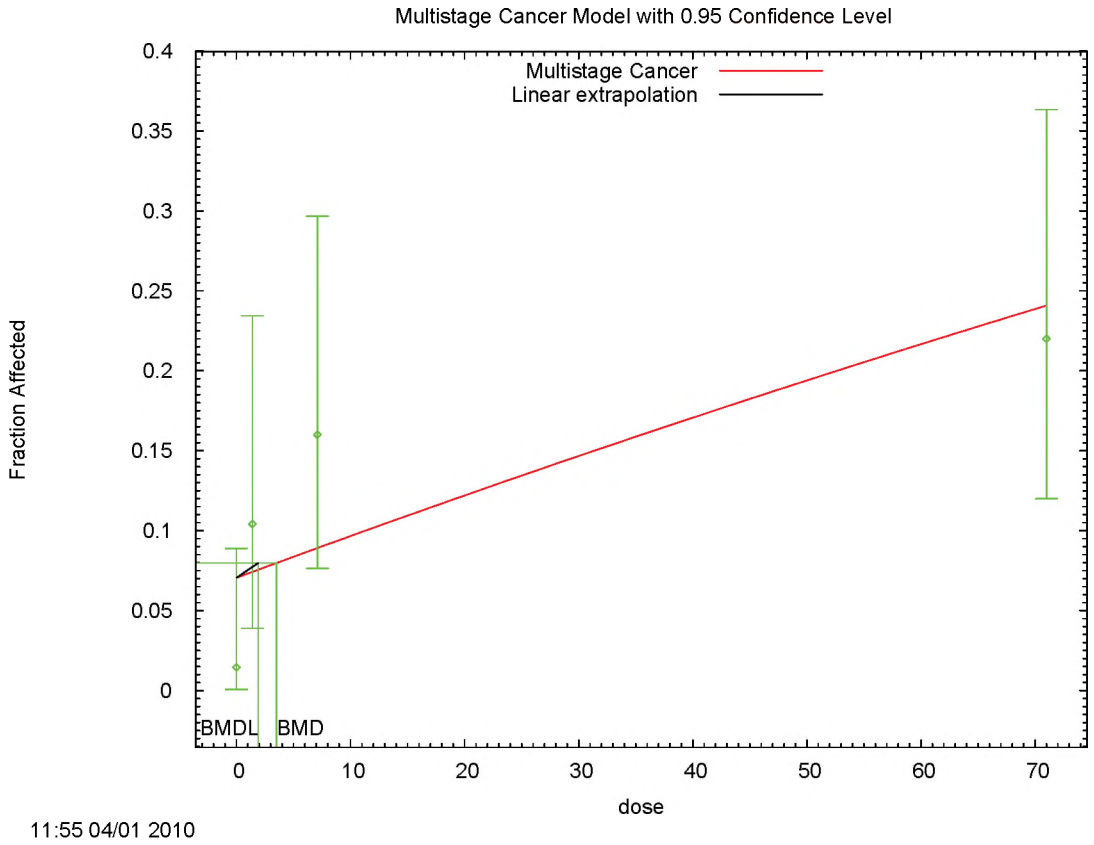
BMDL = 1.91558

BMDU = 9.76663

Taken together, (1.91558, 9.76663) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00522034

F.2.12.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Thyroid: Follicular-Cell Adenoma or Carcinoma

F.2.13. National Toxicology Program, 1982: Adrenal cortex: Adenoma

F.2.13.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.054**	**199.672**	**1.400E+01**	**3.444E+00**	
Multistage Cancer, 2-Degree	2	0.054	199.672	1.400E+01	3.444E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.054	199.672	1.400E+01	3.444E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.13.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Adrenal cortex: Adenoma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\13_msc1_1Perc_adre_cort.(d)
         Gnuplot Plotting File:  C:\Canc\13_msc1_1Perc_adre_cort.plt
                                                Thu Apr 01 12:56:10 2010
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 Source - Table 9
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.168444
                        Beta(1) =  0.000395949
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.53
Beta(1)	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.153096	*	*	*
Beta(1)	0.000718012	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-94.8672	4			
Fitted model	-97.8359	2	5.93732	2	0.05137
Reduced model	-98.0432	1	6.35197	3	0.09569

AIC: 199.672

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1531	11.023	6.000	72	-1.644
1.4000	0.1539	7.697	9.000	50	0.510
7.1000	0.1574	7.713	12.000	49	1.682
71.0000	0.1952	9.564	9.000	49	-0.203

Chi^2 = 5.83 d.f. = 2 P-value = 0.0541

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

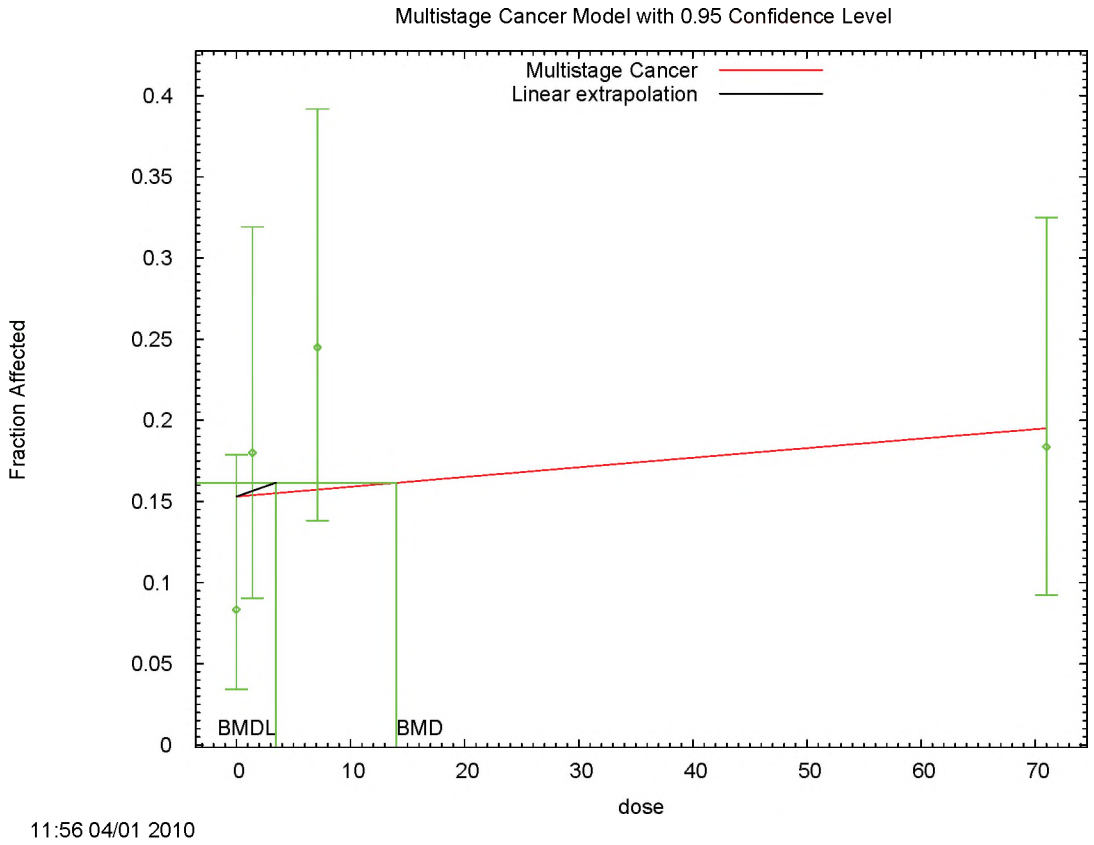
Confidence level = 0.95

BMD = 13.9974

BMDL = 3.4443

BMDU did not converge for BMR = 0.010000
BMDU calculation failed
BMDU = Inf

F.2.13.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Adrenal cortex: Adenoma

F.2.14. National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.2.14.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.146**	**76.377**	**9.761E+00**	**3.964E+00**	
Multistage Cancer, 2-Degree	2	0.146	76.377	9.761E+00	3.964E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.146	76.377	9.761E+00	3.964E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.14.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\14_msc1_1Perc_subcu_fibro.(d)
         Gnuplot Plotting File:  C:\Canc\14_msc1_1Perc_subcu_fibro.plt
                                                 Thu Apr 01 12:56:41 2010
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0143554
                        Beta(1) =  0.000341874
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.5
Beta(1)	-0.5	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0145028	*	*	*
Beta(1)	0.000338561	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-30.9876	4			
Fitted model	-31.0199	2	0.0645971	2	0.9682
Reduced model	-34.3291	1	6.68308	3	0.08272

AIC: 66.0397

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0145	1.073	1.000	74	-0.071
5.7000	0.0164	0.820	1.000	50	0.200
28.6000	0.0240	1.152	1.000	48	-0.143
286.0000	0.1055	4.956	5.000	47	0.021

Chi^2 = 0.07 d.f. = 2 P-value = 0.9675

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 29.6855

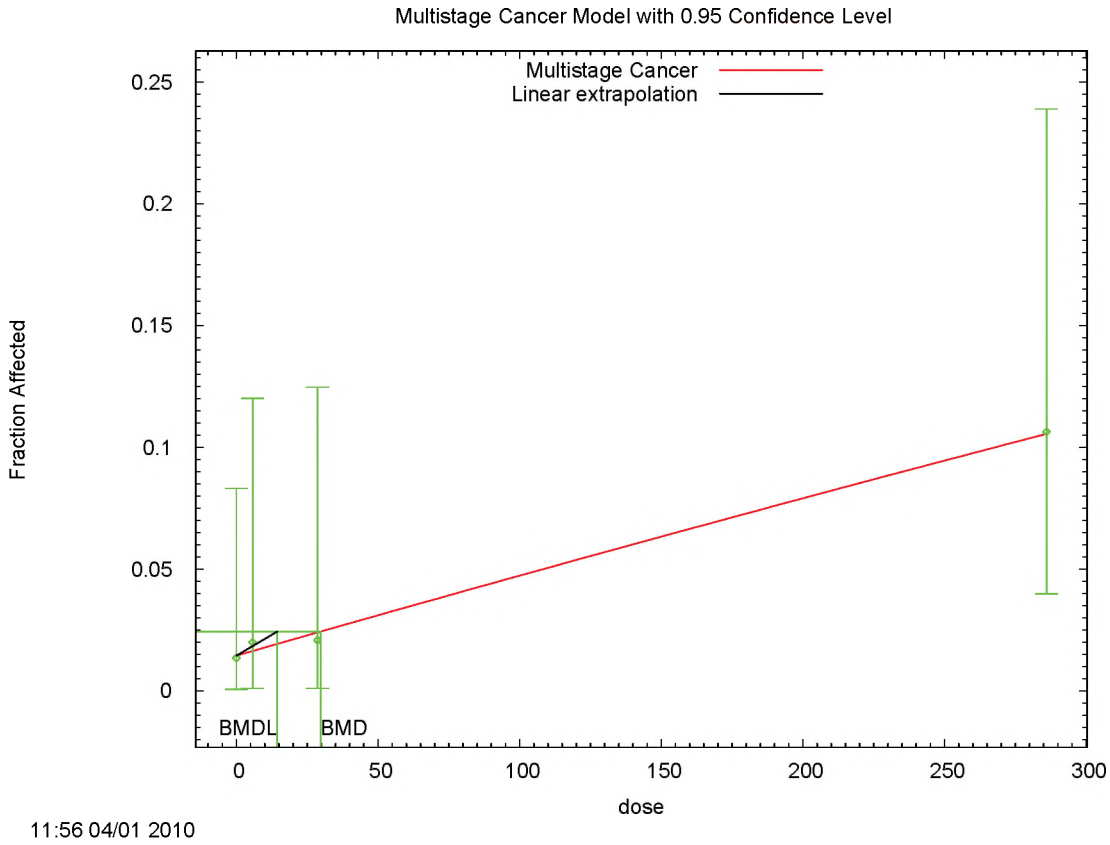
BMDL = 14.3524

BMDU = 100.382

Taken together, (14.3524, 100.382) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000696747

F.2.14.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.2.15. National Toxicology Program, 1982: Hematopoietio System: Lymphoma or Leukemia

F.2.15.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.987**	**261.425**	**1.034E+01**	**5.456E+00**	
Multistage Cancer, 2-Degree	2	0.987	261.425	1.034E+01	5.456E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.987	261.425	1.034E+01	5.456E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.15.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Hematopoietio System: Lymphoma or Leukemia

```
 ========================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\15_msc1_1Perc_mice_f_lymphoma.(d)
         Gnuplot Plotting File:  C:\Canc\15_msc1_1Perc_mice_f_lymphoma.plt
                                                Thu Apr 01 12:57:14 2010
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 Table 15 page 64 Hematopoietic System Lymphoma or Leukemia
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.242959
                        Beta(1) =  0.000967723
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.48
Beta(1)	-0.48	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.242712	*	*	*
Beta(1)	0.000971954	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-128.699	4			
Fitted model	-128.712	2	0.0264819	2	0.9868
Reduced model	-131.412	1	5.42487	3	0.1432
AIC:	261.425				

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2427	17.961	18.000	74	0.011
5.7000	0.2469	12.345	12.000	50	-0.113
28.6000	0.2635	12.647	13.000	48	0.116
286.0000	0.4265	20.045	20.000	47	-0.013

Chi^2 = 0.03 d.f. = 2 P-value = 0.9868

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 10.3403

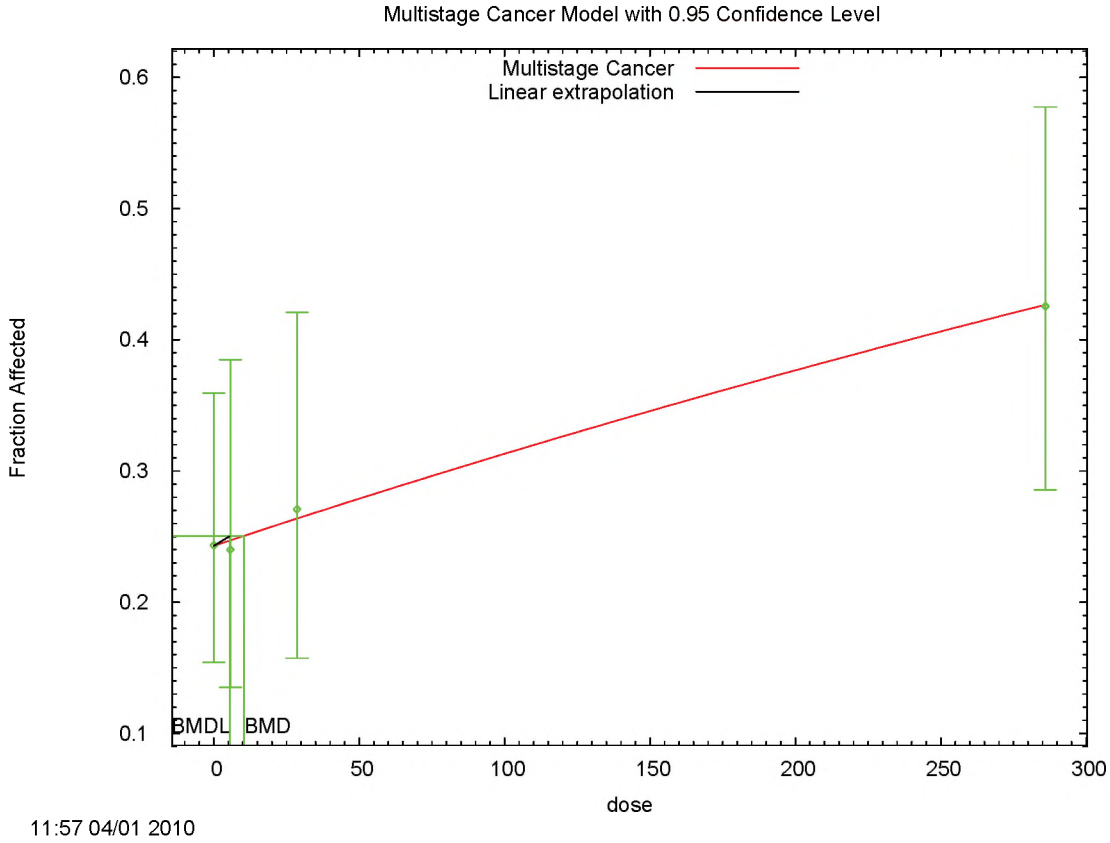
BMDL = 5.45599

BMDU = 38.9139

Taken together, (5.45599, 38.9139) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00183285

F.2.15.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Hematopoietio System: Lymphoma or Leukemia

F.2.16. National Toxicology Program, 1982: Liver: Hepatooellular Adenoma or Carcinoma

F.2.16.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.244**	**156.001**	**1.458E+01**	**7.829E+00**	
Multistage Cancer, 2-Degree	2	0.244	156.001	1.458E+01	7.829E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.244	156.001	1.458E+01	7.829E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.16.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Liver: Hepatooellular Adenoma or Carcinoma

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         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\16_msc1_1Perc_mice_f_liv_aden_carc.(d)
         Gnuplot Plotting File:  C:\Canc\16_msc1_1Perc_mice_f_liv_aden_carc.plt
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0888873
                        Beta(1) =  0.000616931
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.5
Beta(1)	-0.5	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0788077	*	*	*
Beta(1)	0.000689385	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-74.5177	4			
Fitted model	-76.0006	2	2.96597	2	0.227
Reduced model	-79.6703	1	10.3053	3	0.01614

AIC: 156.001

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0788	5.753	3.000	73	-1.196
5.7000	0.0824	4.121	6.000	50	0.966
28.6000	0.0968	4.646	6.000	48	0.661
286.0000	0.2436	11.452	11.000	47	-0.153

Chi^2 = 2.82 d.f. = 2 P-value = 0.2436

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 14.5787

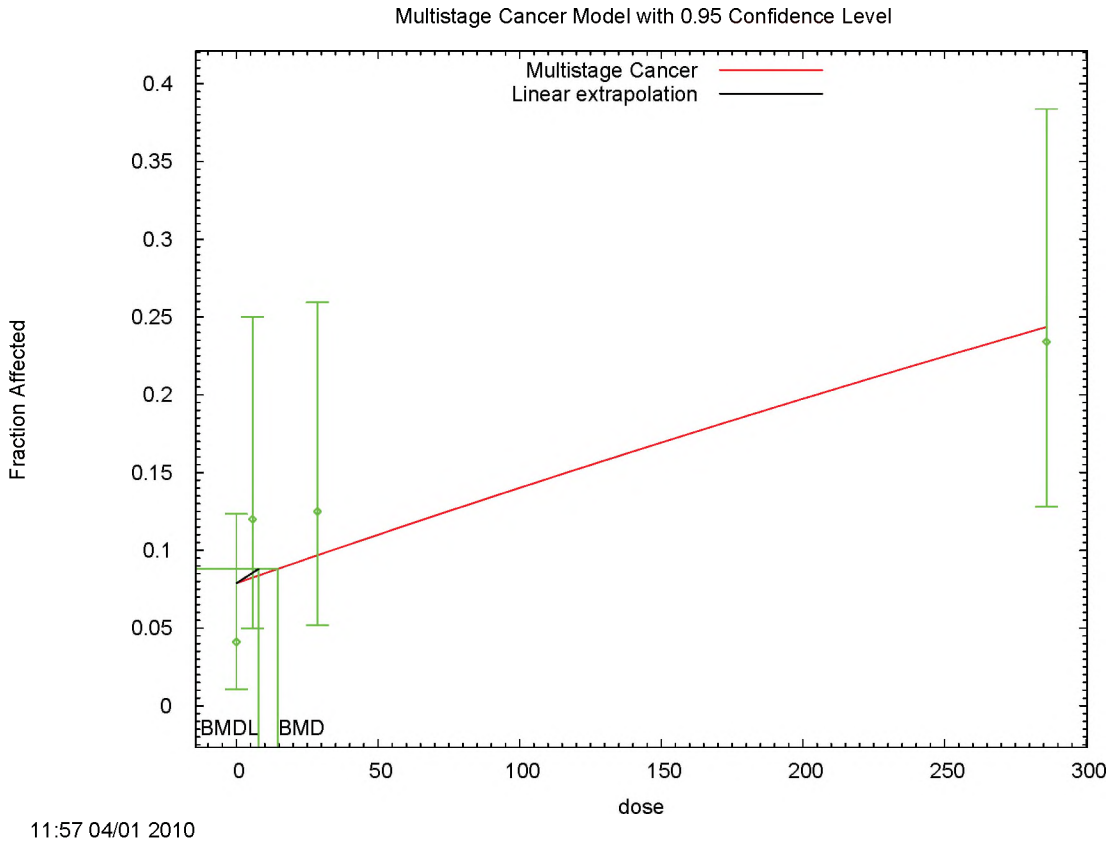
BMDL = 7.82902

BMDU = 42.4536

Taken together, (7.82902, 42.4536) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0012773

F.2.16.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Liver: Hepatooellular Adenoma or Carcinoma

F.2.17. National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.2.17.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.146**	**76.377**	**9.761E+00**	**3.964E+00**	
Multistage Cancer, 2-Degree	2	0.146	76.377	9.761E+00	3.964E+00	final ß=0
Multistage Cancer, 3-Degree	2	0.146	76.377	9.761E+00	3.964E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.17.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

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         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\17_msc1_1Perc_mice_f_thyroid_aden.(d)
         Gnuplot Plotting File:  C:\Canc\17_msc1_1Perc_mice_f_thyroid_aden.plt
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =      0.02405
                        Beta(1) =  0.000315564
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.51
Beta(1)	-0.51	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0207192	*	*	*
Beta(1)	0.000331835	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-32.0017	4			
Fitted model	-34.6122	2	5.22112	2	0.07349
Reduced model	-37.2405	1	10.4776	3	0.01491

AIC: 73.2245

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0207	1.430	0.000	69	-1.208
5.7000	0.0226	1.128	3.000	50	1.782
28.6000	0.0300	1.409	1.000	47	-0.350
286.0000	0.1094	5.032	5.000	46	-0.015

Chi^2 = 4.76 d.f. = 2 P-value = 0.0927

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 30.2871

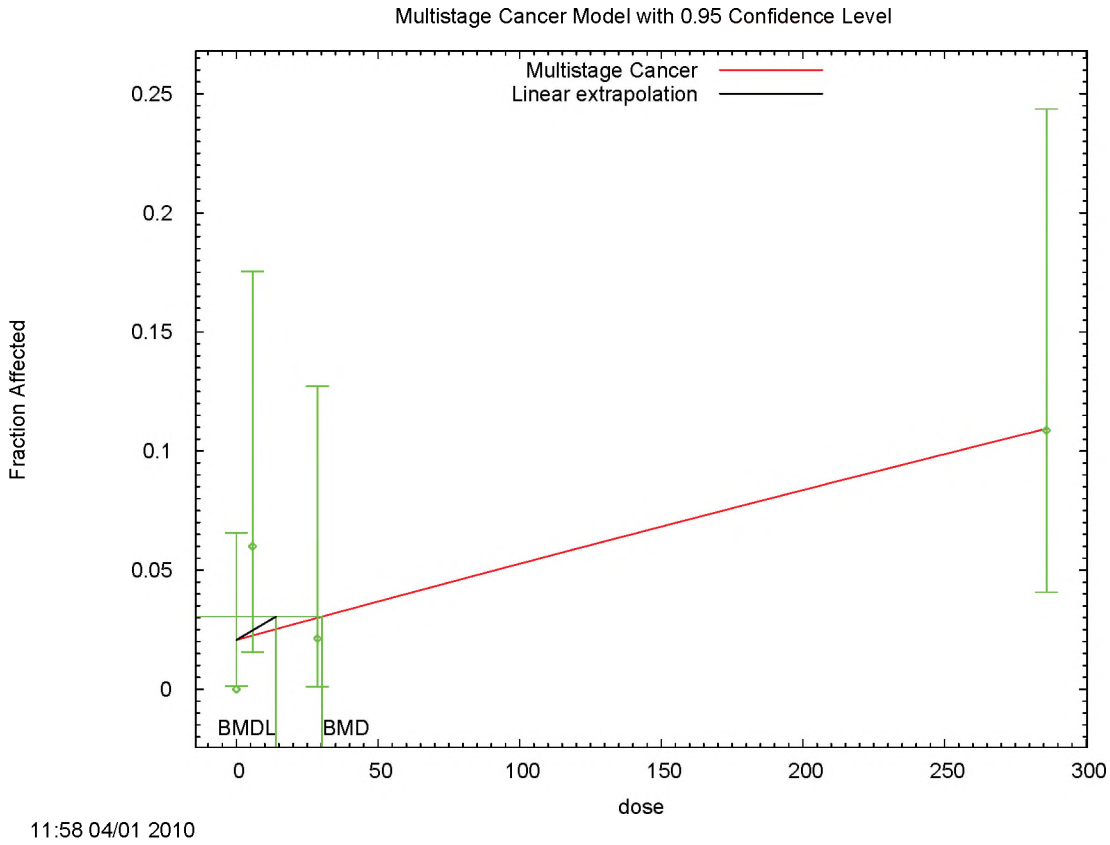
BMDL = 13.993

BMDU = 130.014

Taken together, (13.993 , 130.014) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000714641

F.2.17.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Subcutaneous Tissue: Fibrosarcoma

F.2.18. National Toxicology Program, 1982: Lung: Alveolar/Bronchiolar Adenoma or Carcinoma

F.2.18.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	2	0.138	167.341	3.706E+00	2.026E+00	
Multistage Cancer, 2-Degree [a]	**2**	**0.181**	**166.805**	**1.590E+01**	**2.139E+00**	
Multistage Cancer, 3-Degree	2	0.185	166.777	2.618E+01	2.145E+00	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.18.2. *Output for Selected Model: Multistage Cancer, 2-Degree*

National Toxicology Program, 1982: Lung: Alveolar/Bronchiolar Adenoma or Carcinoma

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 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\18_msc2_1Perc_lung_aden_carc.(d)
         Gnuplot Plotting File:  C:\Canc\18_msc2_1Perc_lung_aden_carc.plt
                                                Thu Apr 01 12:58:55 2010
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0889033
                        Beta(1) =            0
```

Beta(2) = 4.12413e-005

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.45
Beta(2)	-0.45	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0953987	*	*	*
Beta(1)	0	*	*	*
Beta(2)	3.97322e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-79.5959	4			
Fitted model	-81.4024	2	3.61287	2	0.1642
Reduced model	-85.3351	1	11.4782	3	0.009402

AIC: 166.805

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0954	6.773	10.000	71	1.304
1.4000	0.0955	4.583	2.000	48	-1.268
7.1000	0.0972	4.666	4.000	48	-0.325
71.0000	0.2596	12.979	13.000	50	0.007

Chi^2 = 3.41 d.f. = 2 P-value = 0.1814

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 15.9045

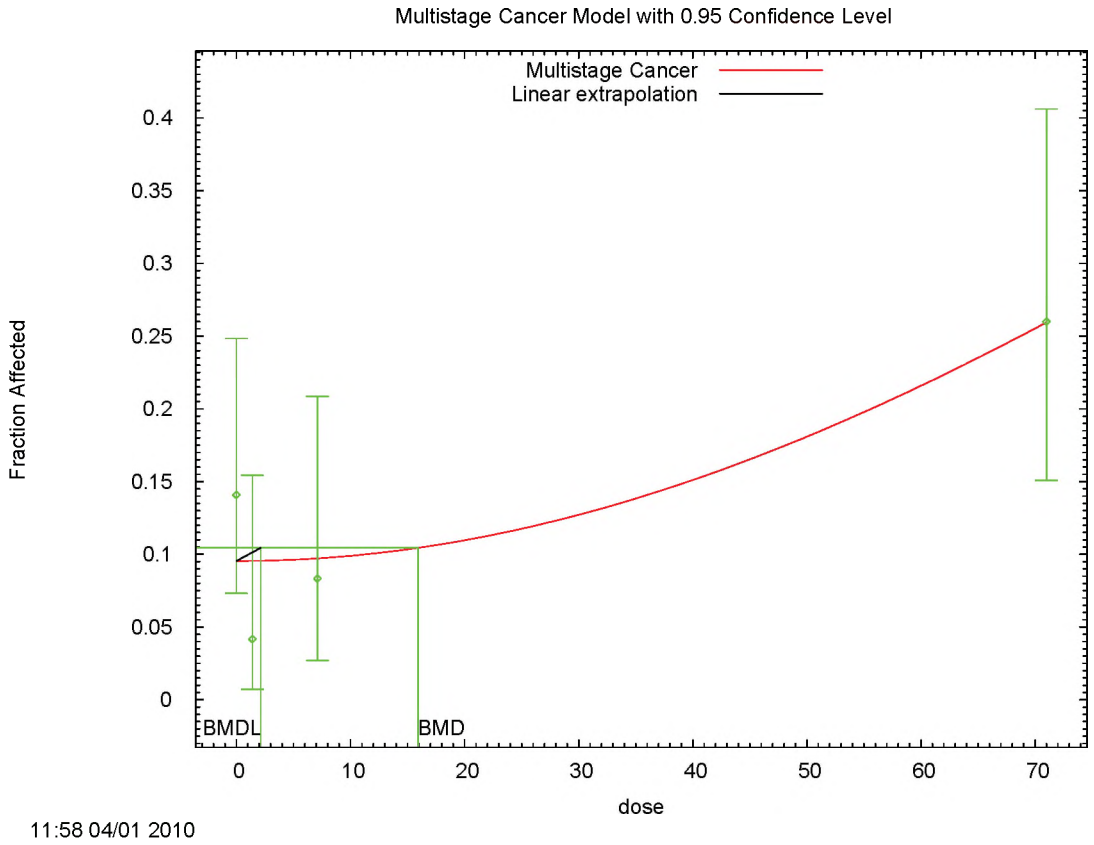
BMDL = 2.1388

BMDU = 26.2712

Taken together, (2.1388 , 26.2712) is a 90 % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor = 0.00467551

F.2.18.3. *Figure for Selected Model: Multistage Cancer, 2-Degree*



National Toxicology Program, 1982: Lung: Alveolar/Bronchiolar Adenoma or Carcinoma

F.2.19. National Toxicology Program, 1982: Liver: Hepatocellular Adenoma or Carcinoma

F.2.19.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**2**	**0.916**	**258.572**	**1.338E+00**	**8.620E-01**	
Multistage Cancer, 2-Degree	2	0.916	258.572	1.338E+00	8.620E-01	final ß=0
Multistage Cancer, 3-Degree	2	0.916	258.572	1.338E+00	8.620E-01	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.19.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 1982: Liver: Hepatocellular Adenoma or Carcinoma

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 ========================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\19_msc1_1Perc_mice_m_liver_aden_carc.(d)
         Gnuplot Plotting File:  C:\Canc\19_msc1_1Perc_mice_m_liver_aden_carc.plt
                                                 Thu Apr 01 12:59:28 2010
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =      0.22264
                        Beta(1) =    0.0074005
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.46
Beta(1)	-0.46	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.219315	*	*	*
Beta(1)	0.00750879	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-127.199	4			
Fitted model	-127.286	2	0.174343	2	0.9165
Reduced model	-135.589	1	16.7801	3	0.0007843

AIC: 258.572

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2193	16.010	15.000	73	-0.286
1.4000	0.2275	11.146	12.000	49	0.291
7.1000	0.2598	12.732	13.000	49	0.087
71.0000	0.5419	27.096	27.000	50	-0.027

Chi^2 = 0.17 d.f. = 2 P-value = 0.9164

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.33848

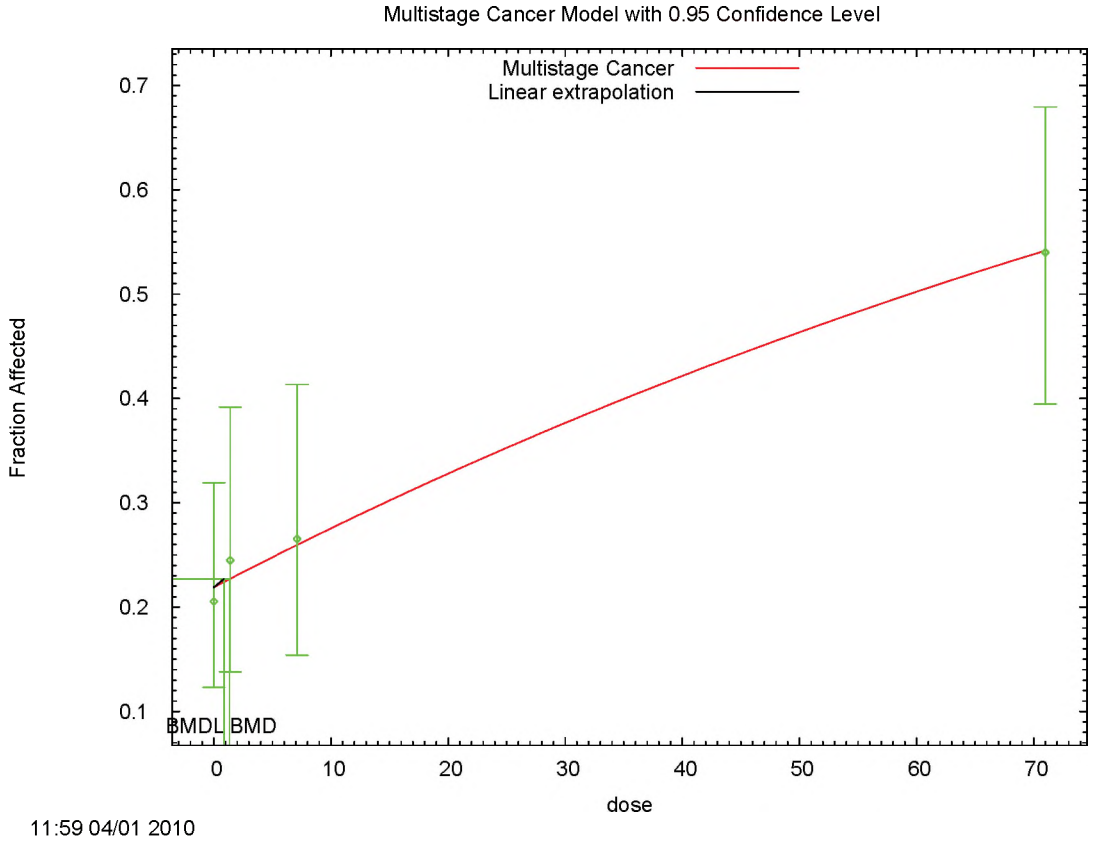
BMDL = 0.861975

BMDU = 2.4671

Taken together, (0.861975, 2.4671) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0116013

F.2.19.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 1982: Liver: Hepatocellular Adenoma or Carcinoma

F.2.20. National Toxicology Program, 2006: Liver: Cholangiocarcinoma

F.2.20.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	5	0.024	129.070	1.872E+00	1.404E+00	
Multistage Cancer, 2-Degree	5	0.947	114.349	9.440E+00	5.290E+00	
Multistage Cancer, 3-Degree [a]	**4**	**0.995**	**115.158**	**1.310E+01**	**4.468E+00**	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.20.2. *Output for Selected Model: Multistage Cancer, 3-Degree*

National Toxicology Program, 2006: Liver: Cholangiocarcinoma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\20_msc3_1Perc_liv_cho-carc.(d)
         Gnuplot Plotting File:  C:\Canc\20_msc3_1Perc_liv_cho-carc.plt
                                                 Thu Apr 01 13:00:03 2010
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =  0.000561481
                        Beta(2) = 1.74365e-005
                        Beta(3) = 1.40248e-006
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(2)	Beta(3)
Beta(2)	1	-0.99
Beta(3)	-0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	4.35927e-005	*	*	*
Beta(3)	1.14186e-006	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-55.408	6			
Fitted model	-55.5789	2	0.34181	4	0.987
Reduced model	-96.9934	1	83.1708	5	<.0001

AIC: 115.158

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	49	0.000
2.1400	0.0002	0.010	0.000	48	-0.101
7.1400	0.0026	0.121	0.000	46	-0.349
15.7000	0.0150	0.752	1.000	50	0.288
32.9000	0.0841	4.121	4.000	49	-0.062
71.4000	0.4716	24.994	25.000	53	0.002

Chi^2 = 0.22 d.f. = 4 P-value = 0.9945

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 13.1014

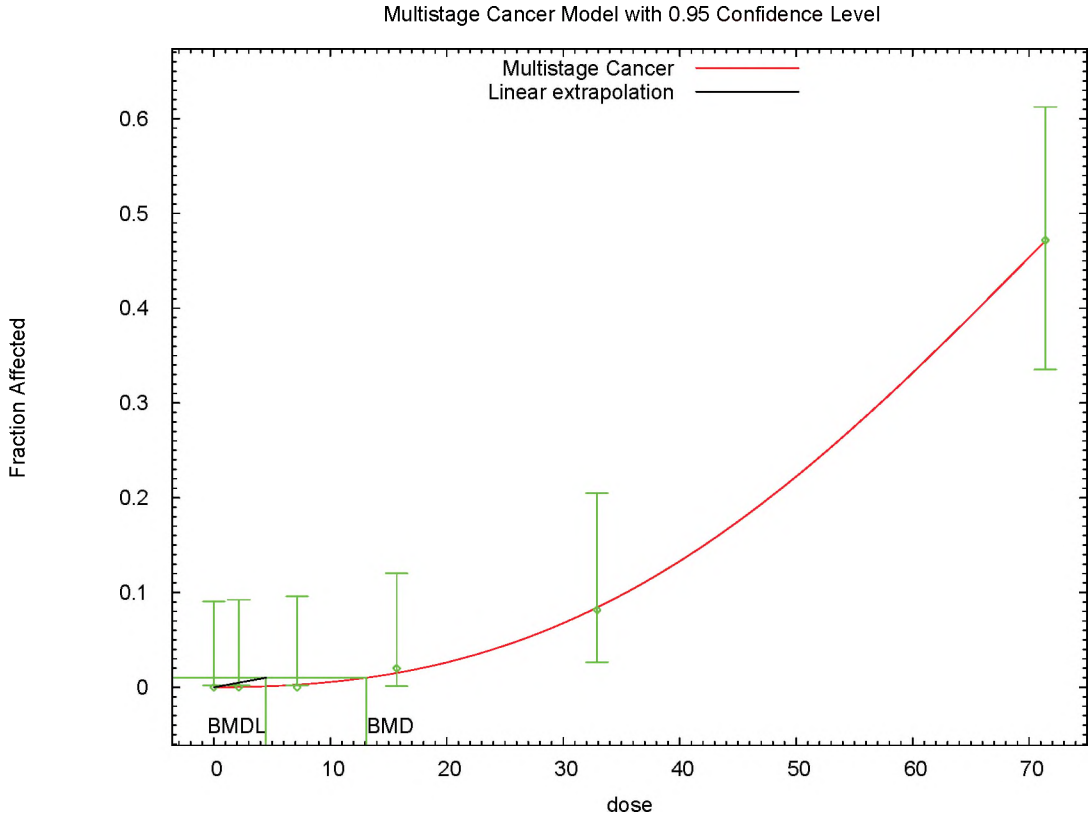
BMDL = 4.46755

BMDU = 19.1783

Taken together, (4.46755, 19.1783) is a 90 % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor = 0.00223836

F.2.20.3. *Figure for Selected Model: Multistage Cancer, 3-Degree*



National Toxicology Program, 2006: Liver: Cholangiocarcinoma

F.2.21. National Toxicology Program, 2006: Liver: Hepatocellular adenoma

F.2.21.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	5	0.131	82.310	4.393E+00	2.915E+00	
Multistage Cancer, 2-Degree	5	0.857	73.656	1.475E+01	8.618E+00	
Multistage Cancer, 3-Degree [a]	**5**	**0.999**	**71.216**	**2.379E+01**	**1.153E+01**	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.21.2. *Output for Selected Model: Multistage Cancer, 3-Degree*

National Toxicology Program, 2006: Liver: Hepatocellular adenoma

```
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         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\21_msc3_1Perc_liv_hepat_ad.(d)
         Gnuplot Plotting File:  C:\Canc\21_msc3_1Perc_liv_hepat_ad.plt
                                                 Thu Apr 01 13:00:36 2010
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 7.77141e-007
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(3)
Beta(3)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	7.46408e-007	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-34.4075	6			
Fitted model	-34.6078	1	0.40065	5	0.9953
Reduced model	-56.3333	1	43.8515	5	<.0001

AIC: 71.2156

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	49	0.000
2.1400	0.0000	0.000	0.000	48	-0.019
7.1400	0.0003	0.012	0.000	46	-0.112
15.7000	0.0029	0.144	0.000	50	-0.380
32.9000	0.0262	1.285	1.000	49	-0.255
71.4000	0.2379	12.609	13.000	53	0.126

Chi^2 = 0.24 d.f. = 5 P-value = 0.9986

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 23.7904

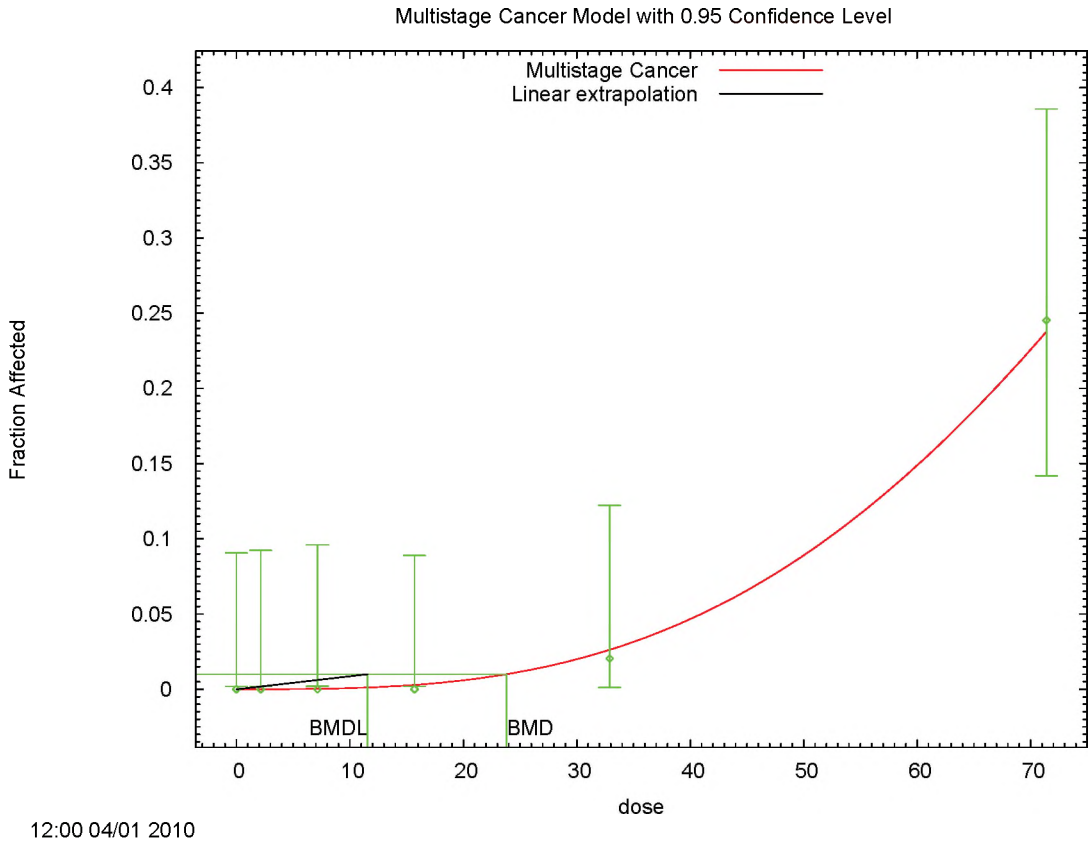
BMDL = 11.5343

BMDU = 27.8755

Taken together, (11.5343, 27.8755) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000866978

F.2.21.3. *Figure for Selected Model: Multistage Cancer, 3-Degree*



National Toxicology Program, 2006: Liver: Hepatocellular adenoma

F.2.22. National Toxicology Program, 2006: Oral mucosa: squamous cell carcinoma

F.2.22.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**4**	**0.386**	**125.484**	**4.751E+00**	**2.956E+00**	
Multistage Cancer, 2-Degree	4	0.587	123.245	1.635E+01	3.845E+00	
Multistage Cancer, 3-Degree	4	0.587	123.245	1.635E+01	3.844E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.22.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 2006: Oral mucosa: squamous cell carcinoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\22_msc1_1Perc_oral_carc.(d)
         Gnuplot Plotting File:  C:\Canc\22_msc1_1Perc_oral_carc.plt
                                                 Thu Apr 01 13:01:11 2010
 ======================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =   0.00607545
                        Beta(1) =   0.00265195
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.6
Beta(1)	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0171416	*	*	*
Beta(1)	0.00211536	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-57.5353	6			
Fitted model	-60.7418	2	6.41293	4	0.1704
Reduced model	-67.7782	1	20.4858	5	0.001013

AIC: 125.484

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0171	0.840	1.000	49	0.176
2.1400	0.0216	1.036	2.000	48	0.958
7.1400	0.0319	1.466	1.000	46	-0.391
15.7000	0.0492	2.462	0.000	50	-1.609
32.9000	0.0832	4.078	4.000	49	-0.040
71.4000	0.1549	8.211	10.000	53	0.679

Chi^2 = 4.15 d.f. = 4 P-value = 0.3855

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.75111

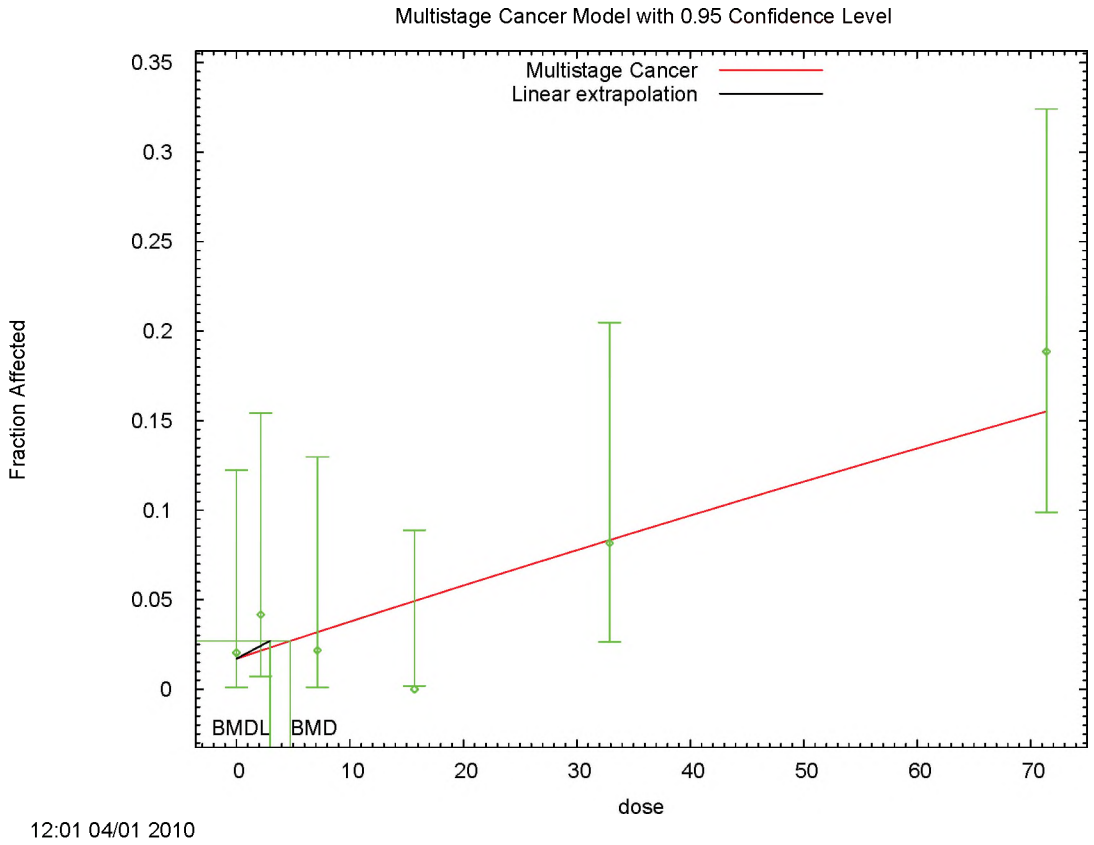
BMDL = 2.9556

BMDU = 9.19454

Taken together, (2.9556 , 9.19454) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0033834

F.2.22.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 2006: Oral mucosa: squamous cell carcinoma

F.2.23. National Toxicology Program, 2006: Pancreas: adenoma or carcinoma

F.2.23.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**5**	**0.796**	**28.316**	**2.120E+01**	**9.335E+00**	
Multistage Cancer, 2-Degree	5	0.977	26.230	3.270E+01	1.389E+01	
Multistage Cancer, 3-Degree	5	0.997	25.427	4.057E+01	1.755E+01	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.23.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

National Toxicology Program, 2006: Pancreas: adenoma or carcinoma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\23_msc1_1Perc_panc_ad_carc.(d)
         Gnuplot Plotting File:  C:\Canc\23_msc1_1Perc_panc_ad_carc.plt
                                                 Thu Apr 01 13:01:43 2010
 ================================================================

 0
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =  0.000817541


           Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

Beta(1)

Beta(1) 1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.000474004	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-11.4096	6			
Fitted model	-13.1581	1	3.49702	5	0.6238
Reduced model	-16.7086	1	10.598	5	0.05996

AIC: 28.3163

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	48	0.000
2.1400	0.0010	0.049	0.000	48	-0.221
7.1400	0.0034	0.155	0.000	46	-0.395
15.7000	0.0074	0.371	0.000	50	-0.611
32.9000	0.0155	0.743	0.000	48	-0.869
71.4000	0.0333	1.697	3.000	51	1.017

Chi^2 = 2.37 d.f. = 5 P-value = 0.7964

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 21.2031

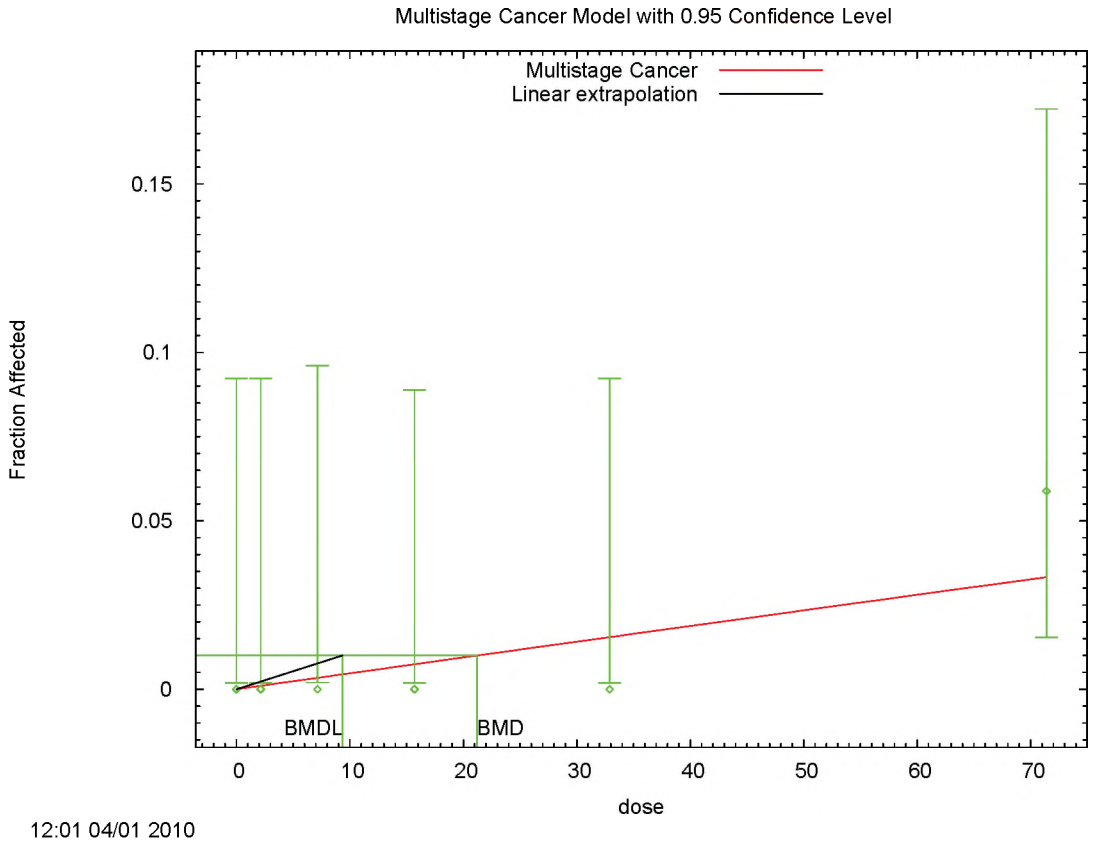
BMDL = 9.33481

BMDU = 65.4351

Taken together, (9.33481, 65.4351) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00107126

F.2.23.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



National Toxicology Program, 2006: Pancreas: adenoma or carcinoma

F.2.24. National Toxicology Program, 2006: Lung: Cystic keratinizing epithelioma

F.2.24.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	5	0.192	60.806	6.922E+00	4.187E+00	
Multistage Cancer, 2-Degree [a]	**5**	**0.771**	**54.363**	**1.858E+01**	**1.069E+01**	
Multistage Cancer, 3-Degree	5	0.961	51.847	2.778E+01	1.556E+01	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.24.2. *Output for Selected Model: Multistage Cancer, 2-Degree*

National Toxicology Program, 2006: Lung: Cystic keratinizing epithelioma

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\24_msc2_1Perc_lung_epith.(d)
         Gnuplot Plotting File:  C:\Canc\24_msc2_1Perc_lung_epith.plt
                                                 Thu Apr 01 13:02:19 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =           0
                        Beta(2) = 3.77591e-005
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	2.91011e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-23.958	6			
Fitted model	-26.1815	1	4.44693	5	0.487
Reduced model	-40.2069	1	32.4976	5	<.0001

AIC: 54.363

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	49	0.000
2.1400	0.0001	0.006	0.000	48	-0.080
7.1400	0.0015	0.068	0.000	46	-0.261
15.7000	0.0071	0.350	0.000	49	-0.594
32.9000	0.0310	1.519	0.000	49	-1.252
71.4000	0.1379	7.170	9.000	52	0.736

Chi^2 = 2.54 d.f. = 5 P-value = 0.7708

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 18.5839

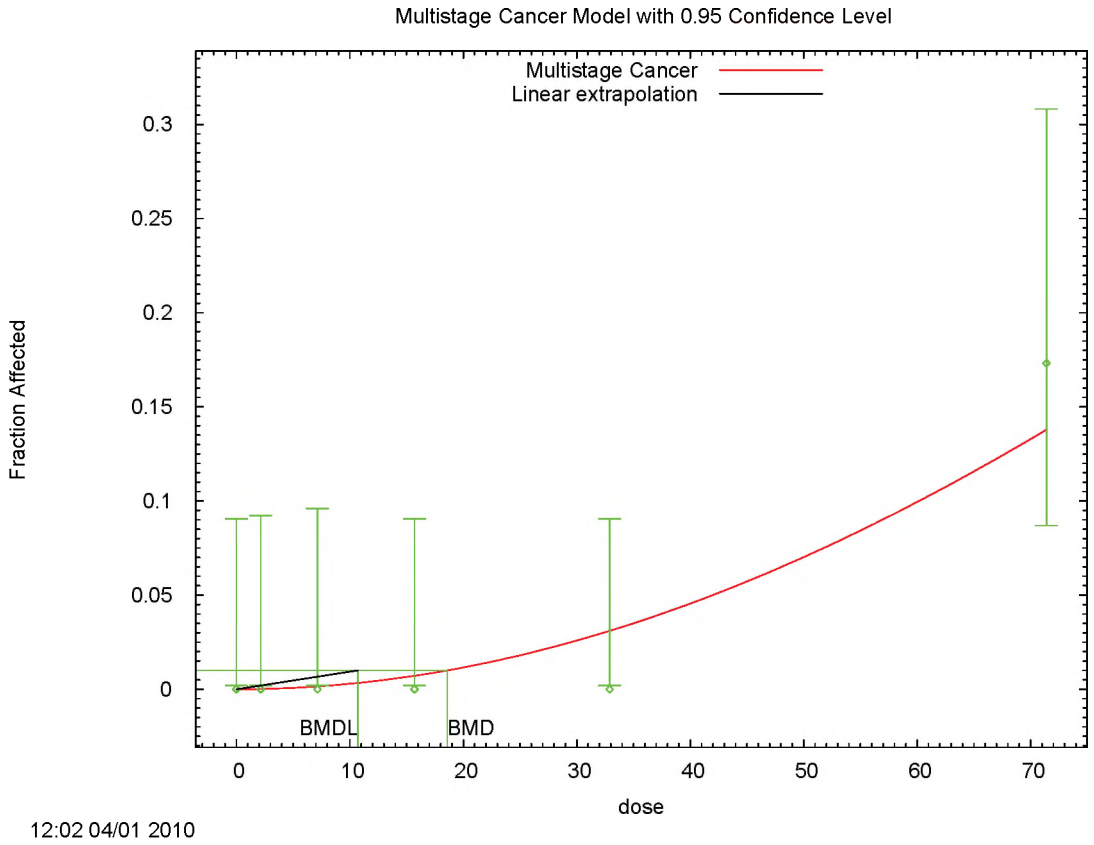
BMDL = 10.6878

BMDU = 25.1324

Taken together, (10.6878, 25.1324) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000935646

F.2.24.3. *Figure for Selected Model: Multistage Cancer, 2-Degree*



National Toxicology Program, 2006: Lung: Cystic keratinizing epithelioma

F.2.25. Toth et al., 1979: Liver: Tumors

F.2.25.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**1**	**0.254**	**155.946**	**2.689E+00**	**1.522E+00**	
Multistage Cancer, 2-Degree	1	0.254	155.946	2.689E+00	1.522E+00	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.25.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Toth et al., 1979: Liver: Tumors

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Canc\25_msc1_1Perc_adr_cor_1yr.(d)
         Gnuplot Plotting File:  C:\Canc\25_msc1_1Perc_adr_cor_1yr.plt
                                                Thu Apr 01 13:10:25 2010
 ======================================================================

 Table 1
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Mean
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.240176
                        Beta(1) =   0.00374745


            Asymptotic Correlation Matrix of Parameter Estimates

              Background      Beta(1)
```

Background	1	-0.53
Beta(1)	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.2418	*	*	*
Beta(1)	0.00373791	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-75.3127	3			
Fitted model	-75.9728	2	1.3201	1	0.2506
Reduced model	-79.4897	1	8.35401	2	0.01534
AIC:	155.946				

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2418	9.188	7.000	38	-0.829
1.0000	0.2446	10.764	13.000	44	0.784
100.0000	0.4783	21.044	21.000	44	-0.013

Chi^2 = 1.30 d.f. = 1 P-value = 0.2537

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.68876

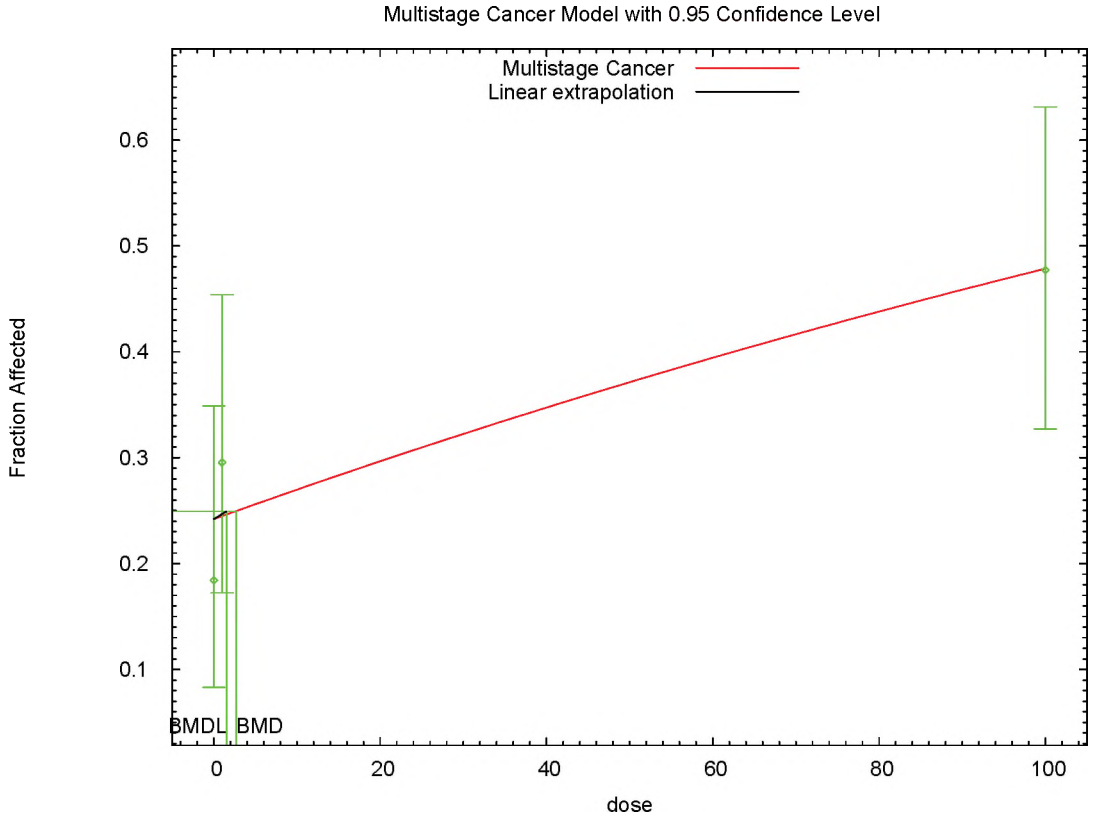
BMDL = 1.52183

BMDU = 7.54263

Taken together, (1.52183, 7.54263) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00657103

F.2.25.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Toth et al., 1979: Liver: Tumors

F.2.26. Della Porta et al., 1987: Table 4, B6C3 mice, male, hepatocellular carcinoma

F.2.26.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree	1	0.073	164.110	9.255E+00	6.946E+00	
Multistage Cancer, 2-Degree [a]	**1**	**0.899**	**160.823**	**7.359E+01**	**9.825E+00**	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.26.2. *Output for Selected Model: Multistage Cancer, 2-Degree*

Della Porta et al., 1987: Table 4, B6C3 mice, male, hepatocellular carcinoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\94_DPorta_1987_Male_Hep_Carc_MultiCanc2_1.(d)
         Gnuplot Plotting File:  C:\1\94_DPorta_1987_Male_Hep_Carc_MultiCanc2_1.plt
                                                 Fri Apr 02 13:58:02 2010
 ======================================================================

 Table 4, B6C3 mice, Male, Hepatocellular carcinoma
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.110507
                        Beta(1) =            0
                        Beta(2) = 1.88069e-006


            Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.62
Beta(2)	-0.62	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.114031	*	*	*
Beta(1)	0	*	*	*
Beta(2)	1.8559e-006	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-78.4036	3			
Fitted model	-78.4116	2	0.0160146	1	0.8993
Reduced model	-94.7394	1	32.6717	2	<.0001

AIC: 160.823

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1140	4.903	5.000	43	0.046
357.1429	0.3008	15.340	15.000	51	-0.104
714.2857	0.6563	32.815	33.000	50	0.055

Chi^2 = 0.02 d.f. = 1 P-value = 0.8994

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 73.5891

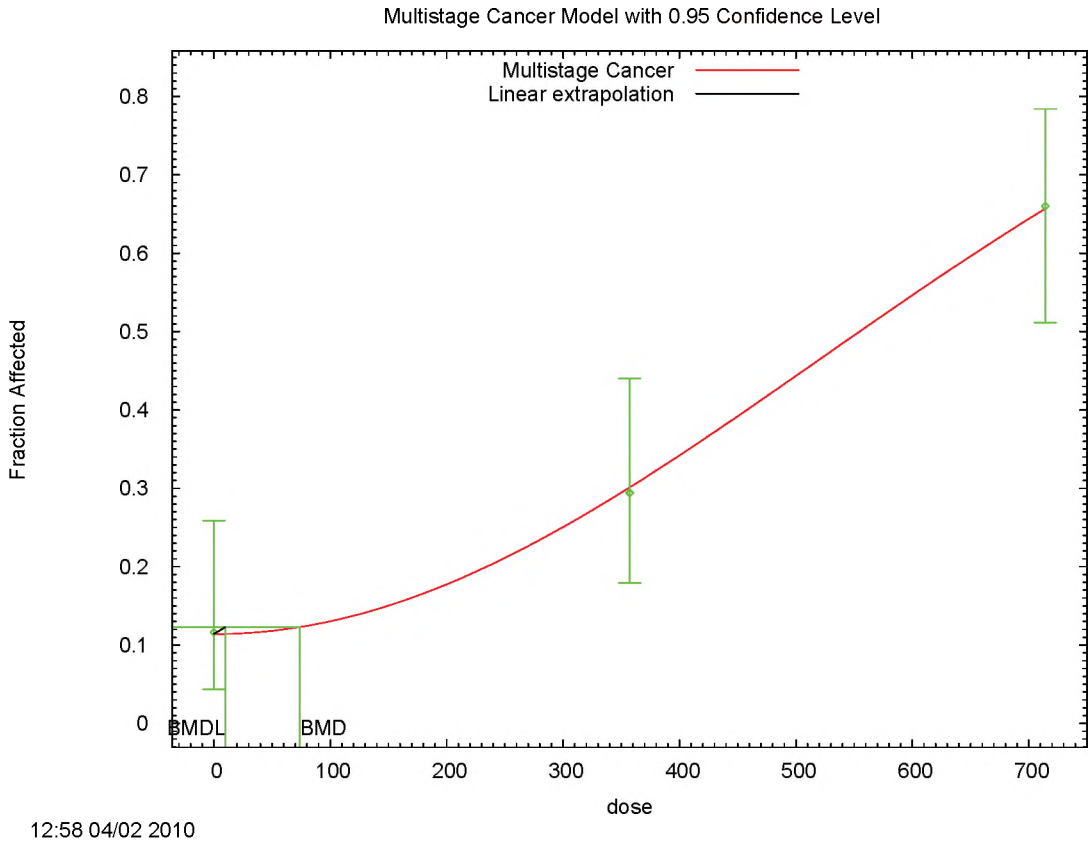
BMDL = 9.82517

BMDU = 88.9247

Taken together, (9.82517, 88.9247) is a 90 % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor = 0.00101779

F.2.26.3. *Figure for Selected Model: Multistage Cancer, 2-Degree*



Della Porta et al., 1987: Table 4, B6C3 mice, male, hepatocellular carcinoma

F.2.27. Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular adenoma

F.2.27.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**1**	**0.468**	**99.355**	**3.695E+01**	**2.245E+01**	
Multistage Cancer, 2-Degree	0	NA	100.803	1.345E+02	2.353E+01	

[a] Best-fitting model, BMDS output presented in this appendix

F.2.27.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular adenoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\95_DPorta_1987_Female_Hep_Aden_MultiCanc1_1.(d)
         Gnuplot Plotting File:  C:\1\95_DPorta_1987_Female_Hep_Aden_MultiCanc1_1.plt
                                                 Fri Apr 02 13:58:32 2010
 ======================================================================

 Table 4, B6C3 mice, Female, Hepatocellular adenoma
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0244051
                        Beta(1) =  0.000306055


            Asymptotic Correlation Matrix of Parameter Estimates

              Background      Beta(1)
```

```
Background            1        -0.72

   Beta(1)        -0.72            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0369416            *               *                   *
        Beta(1)      0.000272012            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -47.4015         3
    Fitted model       -47.6775         2      0.552146      1          0.4574
   Reduced model       -51.6367         1       8.47042      2         0.01448

           AIC:         99.3551


                                Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0369         1.810     2.000          49        0.144
  357.1429     0.1261         5.296     4.000          42       -0.602
  714.2857     0.2070         9.936    11.000          48        0.379

 Chi^2 = 0.53      d.f. = 1        P-value = 0.4677


   Benchmark Dose Computation

Specified effect =           0.01

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        36.9482

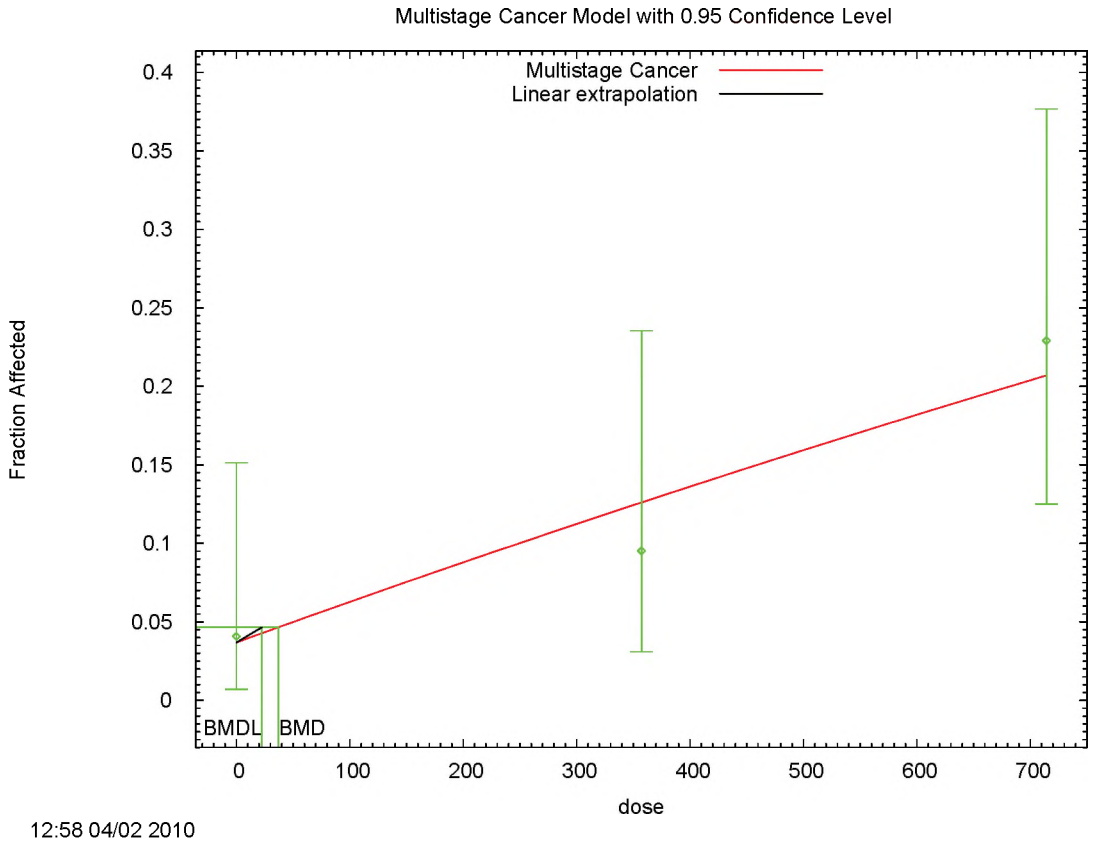
             BMDL =        22.4477

             BMDU =        86.1826

Taken together, (22.4477, 86.1826) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =   0.000445481
```

F.2.27.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular adenoma

F.2.28. Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular carcinoma

F.2.28.1. *Summary Table of BMDS Modeling Results*

Model	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
Multistage Cancer, 1-Degree [a]	**1**	**0.010**	**116.588**	**2.425E+01**	**1.605E+01**	
Multistage Cancer, 2-Degree	1	0.010	116.588	2.425E+01	1.605E+01	final ß=0

[a] Best-fitting model, BMDS output presented in this appendix

F.2.28.2. *Output for Selected Model: Multistage Cancer, 1-Degree*

Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular carcinoma

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\1\96_DPorta_1987_Female_Hep_Carc_MultiCanc1_1.(d)
         Gnuplot Plotting File:  C:\1\96_DPorta_1987_Female_Hep_Carc_MultiCanc1_1.plt
                                                 Fri Apr 02 13:59:01 2010
 ======================================================================

 Table 4, B6C3 mice, Female, Hepatocellular carcinoma
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = DichEff
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0903848
                        Beta(1) =  0.000261828


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)
```

Background	1	-0.8
Beta(1)	-0.8	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0300271	*	*	*
Beta(1)	0.000414523	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-53.1726	3			
Fitted model	-56.2941	2	6.24292	1	0.01247
Reduced model	-60.7146	1	15.084	2	0.0005303

AIC: 116.588

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0300	1.471	1.000	49	-0.395
357.1429	0.1635	6.867	12.000	42	2.142
714.2857	0.2786	13.373	9.000	48	-1.408

Chi^2 = 6.72 d.f. = 1 P-value = 0.0095

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 24.2455

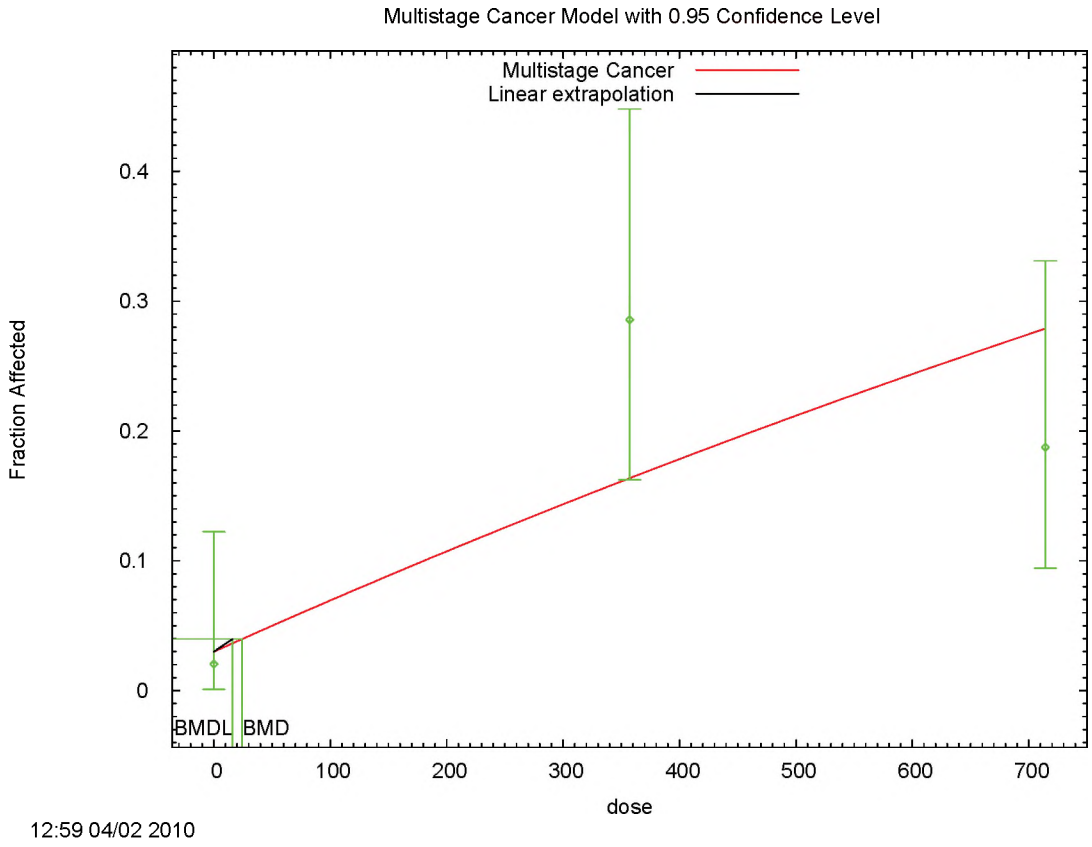
BMDL = 16.0512

BMDU = 49.7176

Taken together, (16.0512, 49.7176) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000623007

F.2.28.3. *Figure for Selected Model: Multistage Cancer, 1-Degree*



Della Porta et al., 1987: Table 4, B6C3 mice, female, hepatocellular carcinoma

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APPENDIX G

Endpoints Excluded From Reference Dose Derivation Based on Toxicological Relevance

NOTICE

THIS DOCUMENT IS AN EXTERNAL REVIEW DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

APPENDIX G. ENDPOINTS EXCLUDED FROM REFERENCE DOSE DERIVATION BASED ON TOXICOLOGICAL RELEVANCE

The National Academy of Sciences (NAS) committee commented on the low dose model predictions and the need to discuss the biological significance of the noncancer health effects modeled in the 2003 Reassessment. In selecting point of departure (POD) candidates from the animal bioassays for derivation of the reference dose (RfD), U.S. Environmental Protection Agency (EPA) had to consider the toxicological relevance of the identified endpoint(s) from any given study. Often endpoints/effects may be sensitive, but lack general toxicological significance due to not being clearly adverse (defined in the Integrated Risk Information System (IRIS) glossary as a biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge), being an adaptive response, or not being clearly linked to downstream functional or pathological alterations. It is standard EPA RfD derivation policy not to base a reference value on endpoints that are not adverse or not obvious precursors to an adverse effect. For select studies, a rationale for lack of toxicological relevance of particular endpoints reported is listed here. These endpoints were not considered for derivation of the RfD.

Kitchin and Woods (1979) administered female Sprague-Dawley rats a single gavage dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and measured cytochrome P450 levels and benzo(a)pyrene hydroxylase (BPH) activity as a marker of hepatic microsomal cytochrome P448-mediated enzyme activity. They found a statistically significant increase in BPH at doses $\geq$2 ng/kg and a significant increase in cytochrome P450 levels at doses $\geq$600 ng/kg. Aryl hydrocarbon hydrolase and EROD were both significantly increased 3 months after exposure; however the elevation did not maintain statistical significance at 6 months. No other indicators of hepatic effects were analyzed. CYP induction alone is not considered a significant toxicologically adverse effect given that CYPs are induced as a means of hepatic processing of xenobiotic agents. Additionally, the role of CYP induction in hepatotoxicity and carcinogenicity of TCDD is unknown, and CYP induction is not considered a relevant POD without obvious pathological significance.

In multiple studies by Hassoun et al. (1998, 2000, 2002, 2003), various indicators of oxidative stress were measured in hepatic and brain tissue of female B6C3F1 mice and Sprague-

Dawley rats following 13 or 30 weeks of TCDD gavage dosing (5 days a week). Biomarkers for oxidative stress included production superoxide anion, lipid peroxidation, and DNA single-strand breaks. The authors report a statistically significant effect on several oxidative stress markers as a result of TCDD exposure, the lowest dose producing an effect being 0.32 ng/kg-day (Hassoun et al., 1998). In this study, all oxidative stress markers were significantly effected, but no other indicators of brain pathology were assessed. Thus, it is impracticable to link the markers of oxidative stress to a toxicological outcome in the brain, and this study and its endpoints are not considered relevant POD candidates.

Burleson et al. (1996) analyzed the effect of a TCDD on viral host resistance following a single gavage dose of TCDD by measuring mortality mediated by influenza virus challenge in B6C3F1 female mice. The study authors found that TCDD at ≥10 ng/kg-day increased influenza-induced mortality. The experimental design calls for a 30% mortality in untreated animals (15% was achieved); mortality, itself, is not a direct result of TCDD exposure. None of the other immunologically-relevant measures were affected by TCDD treatment in this study, and no other effects were reported. The interpretation of these results with respect to humans is problematic. Furthermore, the findings were not reproduced by Nohara et al. (2002) using the same experimental design (see Section 2.4.2). Therefore, this endpoint is not considered relevant as a POD candidate.

To examine the central nervous system response to TCDD, Kuchiiwa et al (2002) analyzed the effect of in utero and lactational TCDD exposure on the serotonergic system in the brainstem of male ddY mice. Female mice were administered TCDD by oral gavage once a week for 8 weeks prior to pregnancy and, using an immunocytochemical detection method, the raphe nuclei in the brainstem of male offspring was monitored for serotogergic neurons. TCDD at 0.7 ng/kg-day caused a 25−50% reduction in the immunostaining of serotonin, however there were no differences in external morphology, birth or postnatal body weights between TCDD-exposed and control offspring. The authors suggest that these findings may indicate that TCDD acts as a neuroteratogen by mediating long-term alterations in neuronal serotonin synthesis and serotonergic function. However, no other relevant neurotoxicity endpoints were examined or reported. Thus, reduced serotonin is not an adverse endpoint of toxicological significance in and of itself, and this study is deemed unsuitable as a POD candidate.

Mally and Chipman (2002) evaluated the effect of TCDD on gap junctions, hypothesizing that as a nongenotoxic carcinogen, TCDD may induce tumor formation by disturbing tissue homeostasis. Female F344 rats were dosed with TCDD by oral gavage for either 3 consecutive days or 2 days a week for 28 days. Gap junction connexin (Cx) plaque expression and hepatocyte proliferation was measured. The study authors report a decrease in Cx32 plaque number and area in the liver of rats exposed to 0.7 ng/kg-day and higher, however they did not find an associated increase in hepatocyte proliferation. No clinical signs of toxicity were observed, and histological examination of the liver revealed no abnormalities. In the absence of additional indicators of hepatotoxicity, a decrease in Cx32 plaque formation is not clearly linked to TCDD-mediated hepatotoxicity or hepatocarcinogenicty, nor is it considered an adverse effect. This endpoint is not considered a toxicologically relevant POD.

Vanden Heuvel et al. (1994) analyzed changes in hepatic mRNA following a single administration of TCDD to female Sprague-Dawley rats by oral gavage. Four days after treatment, animals were sacrificed and livers were excised. Using reverse transcriptase-polymerase chain reaction (RT-PCR) on hepatic RNA, they compared levels of "dioxin responsive" mRNA's (CYP1A1, UDP-glucuronosyltransferase I, plasminogen activator inhibitor 2, and transforming growth factor α) at various doses of TCDD and at control (baseline) levels. They determined that CYP1A1 elicited the most sensitive response to TCDD, with a statistically significant increase (3-fold) in mRNA from rat livers exposed to 1 ng/kg-day TCDD. Induction of CYP1A1 expression is not considered an adverse effect, as the role of CYP1A1 in TCDD-mediated carcinogenicity is unsettled. Therefore, in the absence of other indicators of hepatoxicity, increases in liver CYP1A1 cannot be considered toxicologically relevant for a POD candidate.

Devito et al. (1994) assessed the activity of CYP1A1 and CYP1A2, the amount of phosphorylation of phosphotyrosyl proteins (pp32, pp34, and pp38), and the levels of estrogen receptor in the liver, uterus, lung and skin tissue of female B6C3F1 mice administered TCDD for 5 days a week for 13 weeks. The authors hypothesized that these measurements may be sensitive biomarkers for exposure to TCDD. Body weights were also recorded weekly. Induction of CY1A1 and CYP1A2, as well as increased phosphorylated forms of pp32, pp34, and pp38 were sensitive indicators of TCDD exposure, with statistically significant changes seen at 1.07 ng/kg-day. EROD activity in the ling, skin, and liver was also observed with significant

increases at this dose. However, the authors did not find a change in rat body or terminal organ weights, nor did they note any pathology in the animals at this dose level. The role of CYPs and phosphorylated pp32, pp34, and pp38 in TCDD-mediated toxicity is unknown, and changes in the activity or function of these proteins are not considered adverse. Therefore, these endpoints are not considered suitable as PODs.

Because TCDD had been detected in the soil of contaminated locations, determining the bioavailability of TCDD from ingested soil may be important to the calculation of safe exposure levels. Lucier et al. (1986) fed adult female Sprague-Dawley rats TCDD contaminated soil or gave them TCDD in corn oil at various doses and compared the effects of TCDD on biochemical parameters from liver tissue. They found that equivalent doses of TCDD in corn oil and soil produced similar increases in hepatic aryl hydrocarbon hydroxylase activity (AHH) and UDP glucuronyltransferase activity. They determined that AHH was statistically induced 1.8-fold at 15 ng/kg in corn oil and 40 ng/kg in soil. Cytochrome P450 was significantly increased at higher doses. No clinical signs of acute toxicity or changes in body weight were observed. The association between AHH activity and TCDD-mediated hepatotoxicity is unknown and no adverse endpoints were measured. Thus, this endpoint is not suitable as a POD candidate.

Sugita-Konishi et al. (2003) investigated the change in host resistance of mice offspring lactationally exposed to TCDD. Pregnant C57BL/6NCji mice were administered TCDD via drinking water from parturition to weaning of the offspring (17 days). One group of offspring was then infected with *Listeria monocytogenes* and blood and spleen samples were collected various time points post infection. Uninfected, TCDD exposed offspring were weighed and their spleens and thymuses removed for assay of cellular content and protein expression. TCDD exposure caused a statistically-significant decrease in relative spleen weight and a statistically-significant increase in thymic CD4+ cells in the high-dose group (11.3 ng/kg-day). Offspring infected with *Listeria* following TCDD exposure exhibited a statistically significant increase in serum tumor necrosis factor alpha (TNF-α) 2 days after infection in both sexes in the low- (1.14 ng/kg-day) and high-dose groups. The authors conclude that exposure to TCDD disrupted the host resistance of the offspring at the lowest dose tested, despite the primary immune parameters being unaffected. Without an obvious association between TCDD and immune function, however, this endpoint is not suitable for identification of a LOAEL. Thus, the LOAEL for this study is 11.3 ng/kg-day, and the NOAEL is 1.14 ng/kg-day.

Sewall et al. (1993) investigated alterations in the epidermal growth factor receptor (EGFR) pathway in a two-stage initiation promotion model of TCDD hepatic cancer. EGFR signaling has been implicated in the altered cell growth induction by tumor promoters. Female Sprague-Dawley rats were administered TCDD biweekly by oral gavage for 30 weeks following initiation by a single dose of diethylnitrosamine (DEN). A group also received TCDD without prior DEN initiation. Livers were harvested and fixed from sacrificed animals and sections tested for EGFR binding, autophosphorylation, immunolocalization, and hepatic cell proliferation. The authors report a significant dose-dependent decrease in plasma membrane EGFR maximum binding capacity in TCDD-exposed rats beginning at 3.5 ng/kg-day. However, at this same dose, the authors note a statistically significant decrease in cell proliferation (as measured by DNA replication labeling), with increases in proliferation only occurring at higher doses (125 ng/kg-day). No other indicators of hepatic toxicity or tumorigenicity were assessed. The role of EGFR in TCDD-mediated hepatotoxicity and hepatocarcinogenicity is unknown, and as such, this endpoint cannot be unequivocally linked to TCDD-induced hepatic effects nor labeled as adverse. Thus, it is not suitable as a POD candidate.

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APPENDIX H

Cancer Precursor Benchmark Dose Modeling

NOTICE

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National Center for Environmental Assessment
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Cincinnati, OH

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APPENDIX H. CANCER PRECURSOR BENCHMARK DOSE MODELING

H.1. BMDS INPUT TABLES

H.1.1. Hassoun et al. (2000)

Endpoint	Administered Dose (ng/kg-day)					
	0	3	10	22	46	100
	Internal Dose (ng/kg blood) [a]					
	0	1.94	4.61	8.15	14.01	25.34
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
Cytochrome C reductase [d]	0.15 ± 0.07	0.18 ± 0.05 [b]	0.19 ± 0.06	0.27 ± 0.06 [c]	0.39 ± 0.06 [c]	0.44 ± 0.11 [c]
DNA single-strand breaks [f]	7.41 ± 1.54	10.78 ± 1.25 [b,c]	13.6 ± 1.69 [c]	15.3 ± 1.71 [c]	20.4 ± 2.25 [c]	23.5 ± 1.37 [c]
TBARs [e]	1.47 ± 0.29	1.55 ± 0.54 [b]	2.15 ± 0.36 [c]	2.28 ± 0.25 [c]	2.62 ± 0.52 [c]	2.29 ± 0.49 [c]

[a]From the Emond PBPK model described in 3.3.
[b]LOEL for selected endpoint.
[c]Statistically significant as compared to control ($p < 0.05$).
[d]Values are the mean ± SD. Data obtained from Table 1 in Hassoun et al. 2000.
[e]Values are the mean ± SD. Data obtained from Table 2 in Hassoun et al. 2000.
[f]Values are the mean ± SD. Data obtained from Table 3 in Hassoun et al. 2000.

H.1.2. Kitchin and Woods (1979)

Endpoint	Administered Dose (ng/kg-day)					
	0	0.6	2	4	20	60
	Internal Dose (ng/kg blood) [a]					
	0	0.06	0.20	0.38	1.61	4.15
	n = 9	n = 4	n = 4	n = 4	n = 4	n = 4
BaP hydroxylase activity [f] (continued on next line)	4.9 ± 0.37	4.9 ± 0.59 [b]	6.7 ± 0.70 [c,d]	7.2 ± 0.90 [d]	8.3 ± 0.13 [e]	14 ± 2.5 [e]
	Administered Dose (ng/kg-day)					
	200	600	2000	5000	20,000	
	Internal Dose (ng/kg blood) [a]					
	11.59	30.26	90.90	218.02	863.18	
	n = 4	n = 4	n = 4	n = 4	n = 4	
BaP hydroxylase activity [f] (continued)	59 ± 3.4 [e]	96 ± 23 [e]	155 ± 8.2 [e]	182 ± 13 [e]	189 ± 13 [e]	

[a]From the Emond PBPK model described in 3.3.
[b]NOEL for selected endpoint.
[c]LOEL for selected endpoint.
[d]Statistically significant as compared to control ($p < 0.05$).
[e]Statistically significant as compared to control ($p < 0.001$).
[f]Values are the mean ± SE. Data obtained from Table 3 in Kitchin and Woods 1979.

H.1.3. National Toxicology Program (2006), 31 Week Exposure

Endpoint	Administered Dose (ng/kg-day)					
	0	2.14	7.14	15.7	32.9	71.4
	Internal Dose (ng/kg blood) [a]					
	0	2.33	5.32	9.21	15.66	28.13
	n = 9	n = 10	n = 10	n = 10	n = 10	n = 10
Labeling Index ,week 31 [c]	0.33 ± 0.0.06	0.85 ± 0.21 [b]	0.96 ± 0.23 [b]	0.79 ± 0.15 [b]	1.33 ± 0.36 [b]	3.85 ± 0.97 [b]

[a]From the Emond PBPK model described in 3.3.
[b]Statistically significant as compared to control ($p < 0.05$).
[c]Values are the mean ± SE. Data obtained from Table 11 in NTP 2006.

H.1.4. National Toxicology Program (2006), 53 Week Exposure

Endpoint	Administered Dose (ng/kg-day)					
	0	2.14	7.14	15.7	32.9	71.4
	Internal Dose (ng/kg blood) [a]					
	0.00	2.46	5.53	9.54	16.18	29.04
	n = 8	n = 8	n = 8	n = 8	n = 8	n = 8
Liver EROD, week 53 [c]	30.22 ± 1.59	569.38 ± 24.62 [b]	1280.00 ± 95.30 [b]	1551.16 ± 112.36 [b]	1726.81 ± 107.58 [b]	1871.47 ± 109.14 [b]
Lung EROD, week 53 [c]	3.01 ± 0.56	27.15 ± 1.87 [b]	42.85 ± 3.94 [b]	36.57 ± 4.59 [b]	43.75 ± 6.56 [b]	43.71 ± 2.24 [b]

[a]From the Emond PBPK model described in 3.3.
[b]Statistically significant as compared to control ($p < 0.01$).
[c]Values are the mean ± SE. Data obtained from Table 12 in NTP 2006.

H.1.5. Vanden Heuvel et al. (1994)

Endpoint	Administered Dose (ng/kg-day)						
	0	0.1	1	10	100	1,000	10,000
	Internal Dose (ng/kg blood) [a]						
	0.00	0.01	0.11	0.88	6.45	48.32	434.50
	n = 13	n = 5	n = 12	n = 7	n = 7	n = 11	n = 5
Hepatic CYP1A1 mRNA Expression [c]	5.4 ± 1.0	7.2 ± 2.5	14.8 ± 4.3 [b]	12.8 ± 1.7 [b]	536 ± 121 [b]	18000 ± 4590 [b]	36700 ± 9900 [b]

[a]From the Emond PBPK model described in 3.3.
[b]Statistically significant as compared to control ($p < 0.05$).
[c]Values are the mean ± SE. Data obtained from Table 2 in vanden Heuvel 1994.

H.2. ALTERNATE DOSE: WHOLE BLOOD BMDS RESULTS

H.2.1. Hassoun et al., 2000: Cytochrome C Reductase

H.2.1.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	4	0.016	-143.333	9.274E+00	7.737E+00	
exponential (M3)	4	0.016	-143.333	9.274E+00	7.737E+00	power hit bound (d = 1)
exponential (M4)	3	0.339	-150.139	3.364E+00	2.170E+00	
exponential (M5) [b]	2	0.788	-151.027	5.913E+00	3.102E+00	
Hill	2	0.743	-150.910	6.208E+00	3.190E+00	
linear	4	0.170	-149.086	5.613E+00	4.429E+00	
polynomial, 5-degree	4	0.170	-149.086	5.613E+00	4.429E+00	
power	4	0.170	-149.086	5.613E+00	4.429E+00	power bound hit (power = 1)

[a] Constant variance model selected ($p = 0.3871$)

[b] Best-fitting model, BMDS output presented in this appendix

H.2.1.2. *Output for Selected Model: Exponential (M5)*

Hassoun et al., 2000: Cytochrome C reductase

```
 ======================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\5\Blood\17_Has_2000_CytCLiv_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                Fri Apr 30 14:14:34 2010
 ======================================================================

 TBARs, liver only (Table 2)
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   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
```

```
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


               Initial Parameter Values

               Variable          Model 5
               --------          --------
                 lnalpha            -5.48625
                     rho(S)                0
                       a              0.1387
                       b           0.0225296
                       c             6.40231
                       d                   1

  (S) = Specified


                  Parameter Estimates

                Variable          Model 5
                --------          -------
                 lnalpha          -5.47298
                     rho                0
                       a          0.156024
                       b         0.0891513
                       c           2.85355
                       d           2.14235


         Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0      6         0.146       0.06614
  1.938      6         0.177       0.05389
  4.614      6         0.191       0.05634
  8.147      6         0.271       0.05634
  14.01      6         0.388       0.06369
  25.34      6         0.444        0.1102


               Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0         0.156       0.0648          -0.3789
  1.938        0.1627       0.0648           0.5416
  4.614        0.1961       0.0648          -0.1919
  8.147        0.2705       0.0648          0.01769
  14.01        0.3874       0.0648          0.02224
  25.34        0.4443       0.0648          -0.0107


Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma^2
```

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                            Likelihoods of Interest

                 Model      Log(likelihood)      DF         AIC
                -------    -----------------    ----   ------------
                    A1        80.75258           7     -147.5052
                    A2        83.37355          12     -142.7471
                    A3        80.75258           7     -147.5052
                     R        55.82002           2       -107.64
                     5        80.51364           5     -151.0273


Additive constant for all log-likelihoods =     -33.08.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                              Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 7a: Does Model 5 fit the data? (A3 vs 5)


                          Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                      55.11            10           < 0.0001
  Test 2                      5.242             5             0.3871
  Test 3                      5.242             5             0.3871
 Test 7a                     0.4779             2             0.7875


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 7a is greater than .1.  Model 5 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

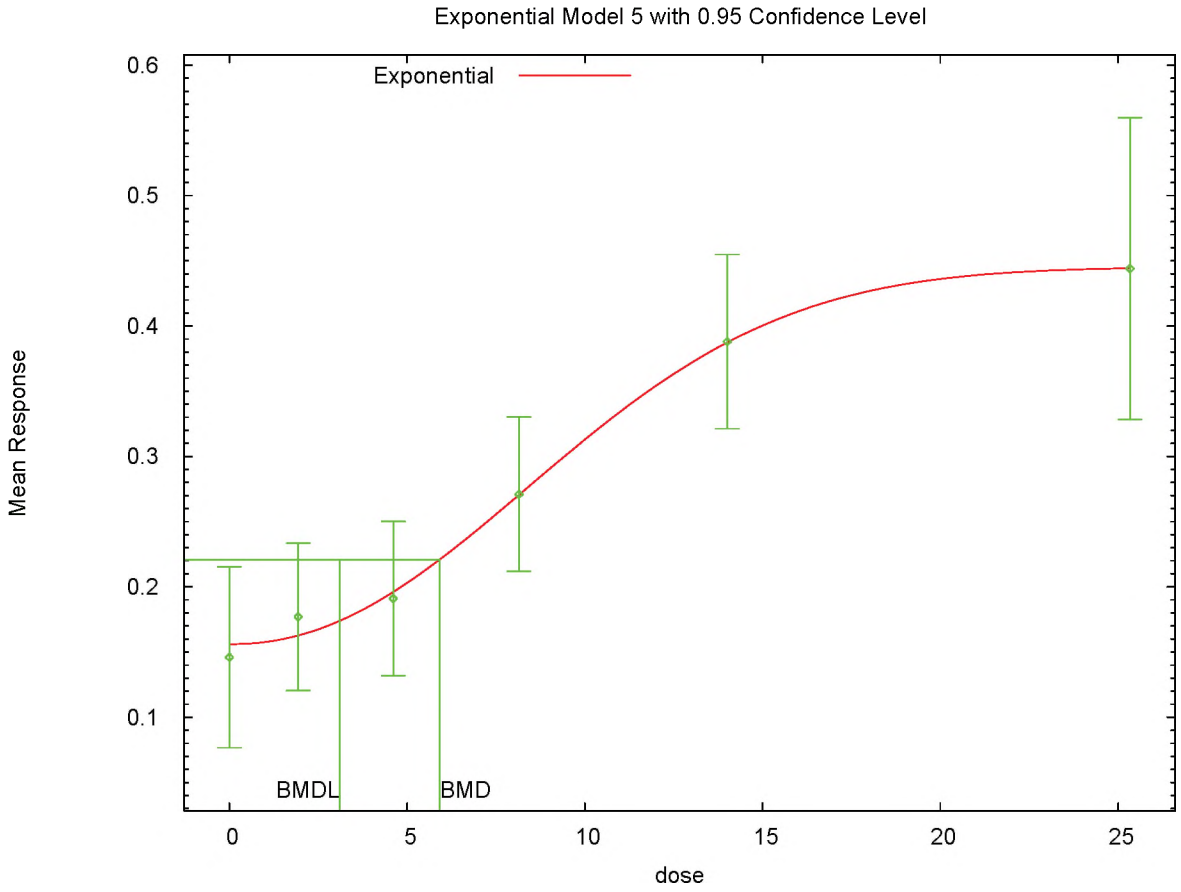
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

              BMD =      5.91298

             BMDL =      3.10234
```

H.2.1.3. *Figure for Selected Model: Exponential (M5)*



H.2.2. Hassoun et al., 2000: DNA Single-Strand Breaks

H.2.2.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	4	<0.0001	111.134	6.551E+00	5.472E+00	
exponential (M3)	4	<0.0001	111.134	6.551E+00	5.472E+00	power hit bound (d = 1)
exponential (M4) [b]	3	0.231	78.588	1.207E+00	9.165E-01	
exponential (M5)	3	0.231	78.588	1.207E+00	9.165E-01	power hit bound (d = 1)
Hill	3	0.230	78.590	1.097E+00	7.966E-01	n lower bound hit (n = 1)
linear	4	<.0001	97.616	3.552E+00	2.890E+00	
polynomial, 5-degree	4	<.0001	97.616	3.552E+00	2.890E+00	
power	4	<.0001	97.616	3.552E+00	2.890E+00	power bound hit (power = 1)
power, unrestricted [c]	3	0.132	79.893	4.522E-01	2.027E-01	unrestricted (power = 0.576)

[a] Constant variance model selected ($p = 0.7521$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

H.2.2.2. *Output for Selected Model: Exponential (M4)*

Hassoun et al., 2000: DNA single-strand breaks

```
 ================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\5\Blood\18_Has_2000_SSB_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                Fri Apr 30 14:15:16 2010
 ================================================================

 DNA single-strand breaks, liver only (Table 3)
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   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	0.841244
rho(S)	0
a	7.0395
b	0.103521
c	3.50522
d	1

(S) = Specified

Parameter Estimates

Variable	Model 4
lnalpha	0.960789
rho	0
a	7.7528
b	0.075429
c	3.39665
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	6	7.41	1.543
1.938	6	10.78	1.249
4.614	6	13.6	1.69
8.147	6	15.3	1.715
14.01	6	20.4	2.254
25.34	6	23.5	1.372

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	7.753	1.617	-0.5194
1.938	10.28	1.617	0.7575
4.614	13.21	1.617	0.5853
8.147	16.28	1.617	-1.49
14.01	19.87	1.617	0.7958
25.34	23.59	1.617	-0.1293

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)

```
            Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
            Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                   Model      Log(likelihood)      DF         AIC
                  -------    -----------------    ----   ------------
                      A1       -33.14239           7      80.28478
                      A2       -31.81197          12      87.62394
                      A3       -33.14239           7      80.28478
                       R       -80.44209           2      164.8842
                       4       -35.29421           4      78.58842


Additive constant for all log-likelihoods =     -33.08.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.

                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                        97.26          10            < 0.0001
  Test 2                        2.661           5              0.7521
  Test 3                        2.661           5              0.7521
 Test 6a                        4.304           3              0.2305


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

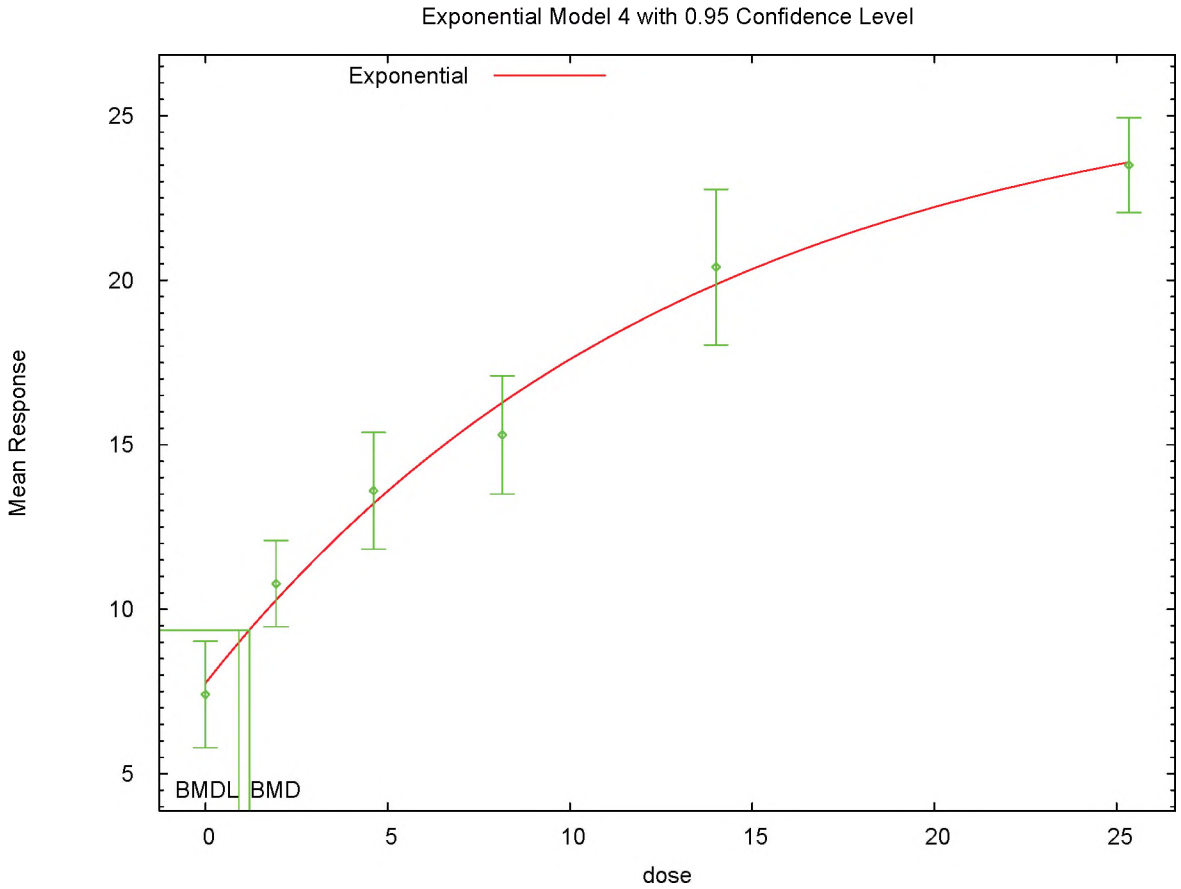
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      1.20684

              BMDL =     0.916526
```

H.2.2.3. *Figure for Selected Model: Exponential (M4)*



H.2.2.4. *Output for Additional Model Presented: Power, Unrestricted*

Hassoun et al., 2000: DNA single-strand breaks

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\5\Blood\18_Has_2000_SSB_PwrCV_U_1.(d)
         Gnuplot Plotting File:  C:\5\Blood\18_Has_2000_SSB_PwrCV_U_1.plt
                                              Fri Apr 30 14:15:20 2010
 ================================================================

 DNA single-strand breaks, liver only (Table 3)
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   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The power is not restricted
   A constant variance model is fit

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                         alpha =       2.7831
                           rho =            0   Specified
                       control =         7.41
                         slope =      2.16848
                         power =     0.620048


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -rho
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                  alpha      control        slope        power

    alpha             1     2.5e-009    -4.6e-009     5.7e-009

  control     2.5e-009            1        -0.79         0.66

    slope    -4.6e-009        -0.79            1        -0.97

    power     5.7e-009         0.66        -0.97            1


                               Parameter Estimates

                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         alpha          2.71022         0.638804             1.45818             3.96225
       control          7.26415         0.644159             6.00163             8.52668
         slope          2.60017         0.530762             1.55989             3.64044
         power         0.575946        0.0589669            0.460373            0.691519


      Table of Data and Estimated Values of Interest
```

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	6	7.41	7.26	1.54	1.65	0.217
1.938	6	10.8	11.1	1.25	1.65	-0.432
4.614	6	13.6	13.5	1.69	1.65	0.094
8.147	6	15.3	16	1.71	1.65	-0.993
14.01	6	20.4	19.2	2.25	1.65	1.85
25.34	6	23.5	24	1.37	1.65	-0.735

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-33.142389	7	80.284779
A2	-31.811970	12	87.623940
A3	-33.142389	7	80.284779
fitted	-35.946504	4	79.893008
R	-80.442086	2	164.884172

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	97.2602	10	<.0001
Test 2	2.66084	5	0.7521
Test 3	2.66084	5	0.7521
Test 4	5.60823	3	0.1323

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems

```
to adequately describe the data


                Benchmark Dose Computation

Specified effect =             1

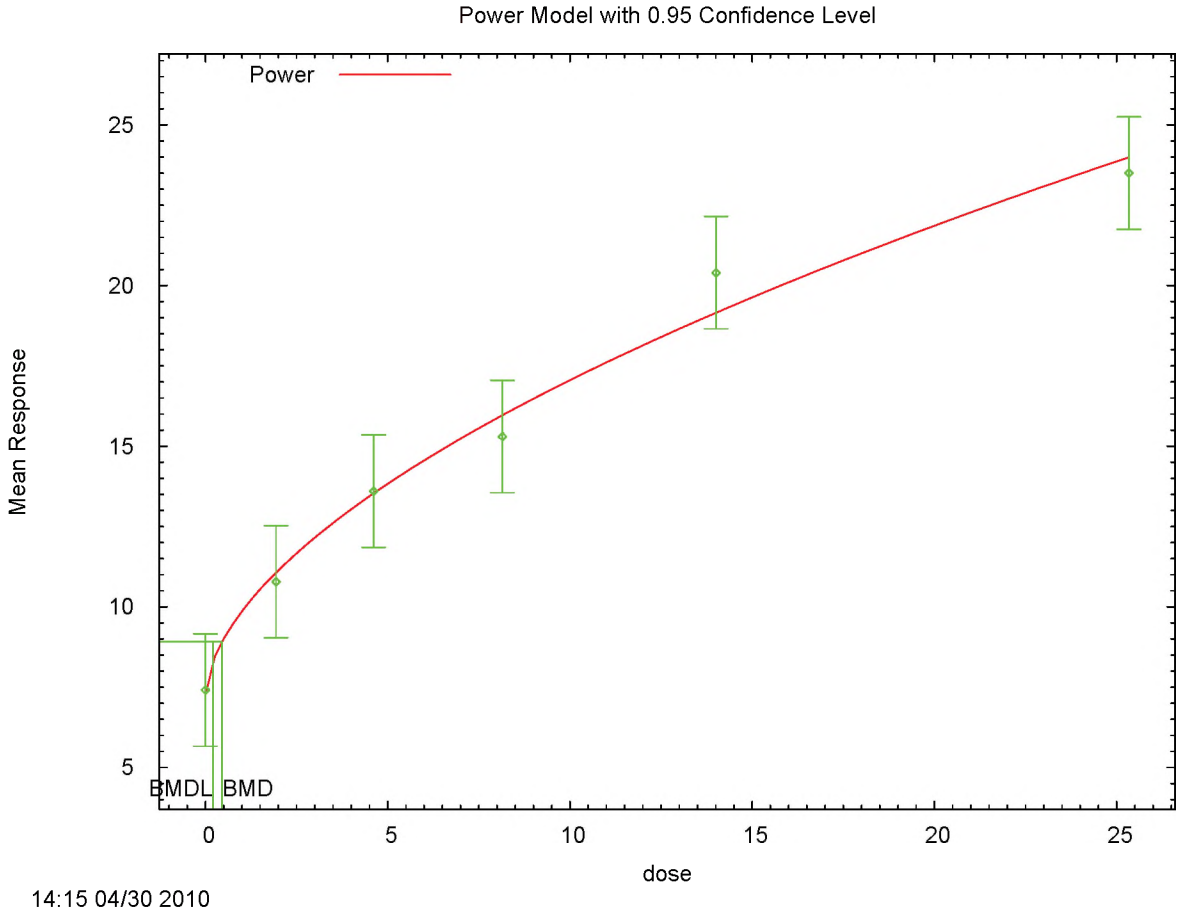
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD = 0.452221


             BMDL = 0.202688
```

H.2.2.5. *Figure for Additional Model Presented: Power, Unrestricted*



H.2.3. Hassoun et al., 2000: TBARS

H.2.3.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	4	0.001	-8.517	1.736E+01	1.223E+01	
exponential (M3)	4	0.001	-8.517	1.736E+01	1.223E+01	power hit bound (d = 1)
exponential (M4)	3	0.188	-19.755	2.189E+00	1.151E+00	
exponential (M5)	2	0.240	-19.681	3.470E+00	1.525E+00	
Hill [b]	2	0.272	-19.935	3.292E+00	1.737E+00	
linear	4	0.002	-9.793	1.444E+01	9.622E+00	
polynomial, 5-degree	4	0.002	-9.793	1.444E+01	9.622E+00	
power	4	0.002	-9.793	1.444E+01	9.622E+00	power bound hit (power = 1)

[a] Constant variance model selected ($p = 0.3348$)

[b] Best-fitting model, BMDS output presented in this appendix

H.2.3.2. *Output for Selected Model: Hill*

Hassoun et al., 2000: TBARS

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\5\Blood\19_Has_2000_TBARsLiv_HillCV_1.(d)
         Gnuplot Plotting File:  C:\5\Blood\19_Has_2000_TBARsLiv_HillCV_1.plt
                                          Fri Apr 30 14:16:02 2010
 ================================================================

 TBARs, liver only (Table 2)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	0.178788	
rho =	0	Specified
intercept =	1.469	
v =	1.15	
n =	1.2785	
k =	5.08547	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	n	k
alpha	1	2.8e-008	-4.4e-008	4.9e-008	-1.5e-008
intercept	2.8e-008	1	-0.82	0.48	0.52
v	-4.4e-008	-0.82	1	-0.61	-0.22
n	4.9e-008	0.48	-0.61	1	0.29
k	-1.5e-008	0.52	-0.22	0.29	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.16017	0.0377523	0.0861764	0.234163
intercept	1.46138	0.152797	1.1619	1.76086
v	0.963033	0.20228	0.566571	1.3595
n	3.44642	2.43468	-1.32547	8.21832
k	3.63417	1.02019	1.63464	5.6337

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	6	1.47	1.46	0.291	0.4	0.0466
1.938	6	1.55	1.56	0.539	0.4	-0.0696
4.614	6	2.15	2.13	0.363	0.4	0.12
8.147	6	2.28	2.37	0.247	0.4	-0.54
14.01	6	2.62	2.42	0.517	0.4	1.25
25.34	6	2.29	2.42	0.487	0.4	-0.803

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	16.269770	7	-18.539539
A2	19.127827	12	-14.255654
A3	16.269770	7	-18.539539
fitted	14.967391	5	-19.934782
R	2.442940	2	-0.885880

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	33.3698	10	0.000236
Test 2	5.71611	5	0.3348
Test 3	5.71611	5	0.3348
Test 4	2.60476	2	0.2719

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

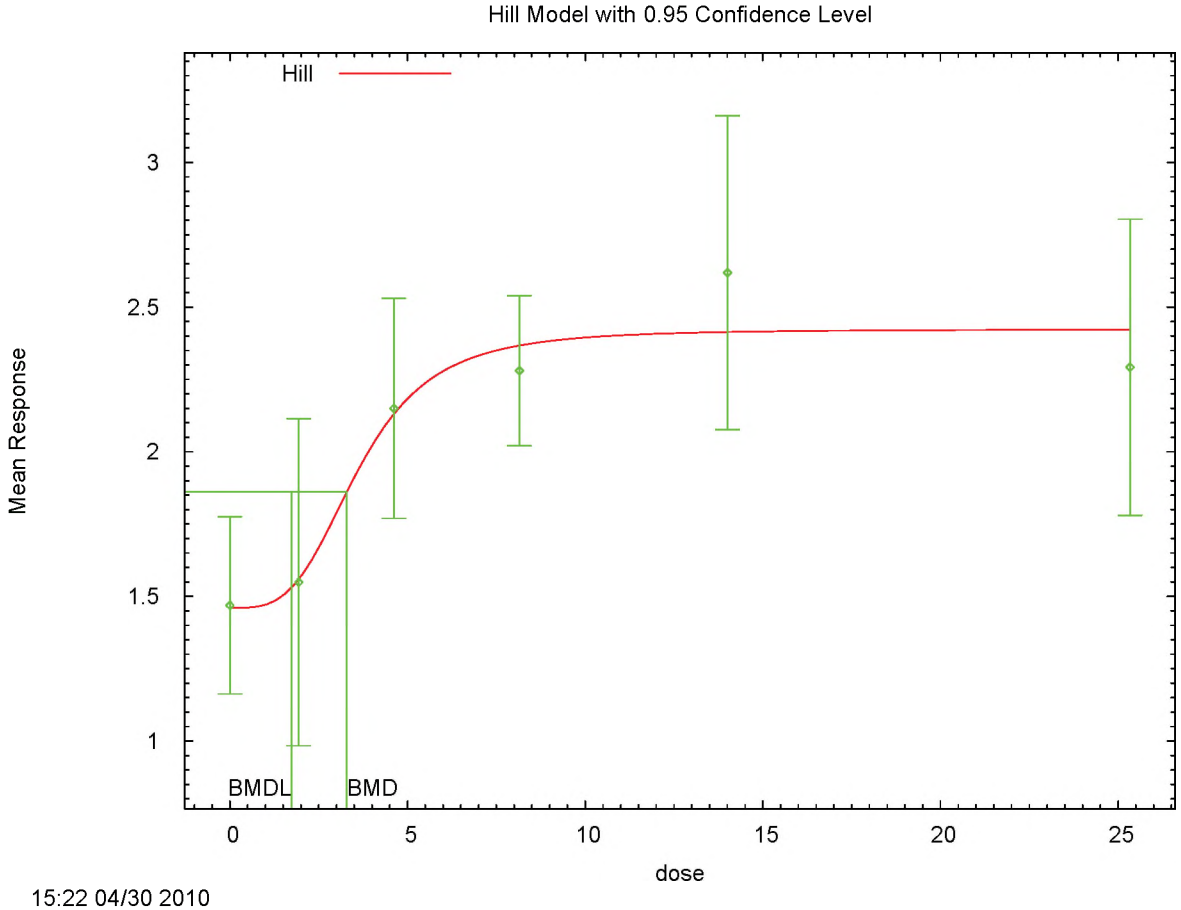
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 3.29185

BMDL = 1.73738

H.2.3.3. *Figure for Selected Model: Hill*



15:22 04/30 2010

H.2.4. Kitchin and Woods, 1979: Bap Hydroxylase Activity

H.2.4.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	9	<0.0001	452.100	2.960E+02	1.446E+02	
exponential (M3)	9	<0.0001	452.100	2.960E+02	1.446E+02	power hit bound (d = 1)
exponential (M4)	8	0.002	232.110	3.182E-01	2.373E-01	
exponential (M5) [b]	7	0.015	227.004	9.321E-01	4.900E-01	
Hill	8	<.0001	479.250	5.340E+00	4.528E+00	
linear	9	<.0001	291.380	4.552E-01	3.303E-01	
polynomial, 8-degree	6	<.0001	468.198	1.012E+03	7.899E-01	
power	9	<.0001	291.380	4.552E-01	3.303E-01	power bound hit (power = 1)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

H.2.4.2. *Output for Selected Model: Exponential (M5)*

Kitchin and Woods, 1979: BaP Hydroxylase Activity

```
 ======================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\5\Blood\27_Kitchin_1979_Hydrolase_Exp_1.(d)
         Gnuplot Plotting File:
                                                  Fri Apr 30 14:17:28 2010
 ======================================================================

 Kitchin 1979, Tbl3, BaP hydrolase activity
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
```

The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 11
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 5
lnalpha	-3.27793
rho	1.92227
a	4.655
b	0.0041206
c	42.6316
d	1

Parameter Estimates

Variable	Model 5
lnalpha	-2.64071
rho	1.94046
a	5.46248
b	0.0382278
c	30.9208
d	1.42906

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	9	4.9	1.11
0.0645	4	4.9	1.18
0.2023	4	6.7	1.4
0.3839	4	7.2	1.8
1.613	4	8.3	0.26
4.146	4	14	5
11.59	4	59	6.8
30.26	4	96	46
90.9	4	155	16.4
218	4	182	26
863.2	4	189	26

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	5.462	1.387	-1.217
0.0645	5.493	1.394	-0.8507
0.2023	5.619	1.425	1.516
0.3839	5.854	1.483	1.815
1.613	8.483	2.126	-0.1723
4.146	16.8	4.125	-1.358
11.59	49.32	11.73	1.65
30.26	121.2	28.06	-1.796
90.9	168.5	38.62	-0.6975
218	168.9	38.72	0.6765
863.2	168.9	38.72	1.038

Other models for which likelihoods are calculated:

```
  Model A1:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
             Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-158.1306	12	340.2613
A2	-84.80028	22	213.6006
A3	-98.82189	13	223.6438
R	-234.6252	2	473.2504
5	-107.5022	6	227.0044

Additive constant for all log-likelihoods = -45.03. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 7a: Does Model 5 fit the data? (A3 vs 5)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	299.6	20	< 0.0001
Test 2	146.7	10	< 0.0001
Test 3	28.04	9	0.0009381
Test 7a	17.36	7	0.01521

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 7a is less than .1. Model 5 may not adequately describe the data; you may want to consider another model.

Benchmark Dose Computations:

Specified Effect = 1.000000

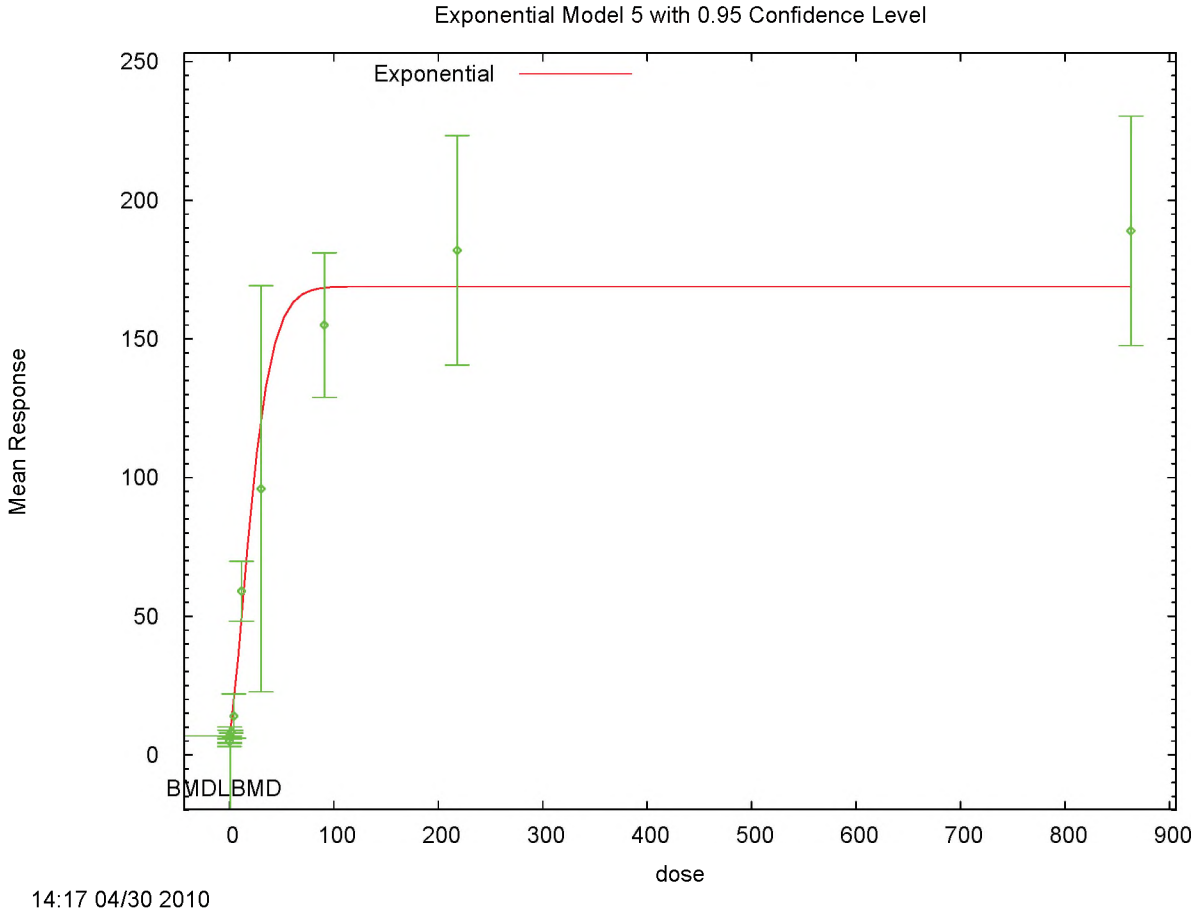
Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD = 0.9321

BMDL = 0.490004

H.2.4.3. *Figure for Selected Model: Exponential (M5)*



14:17 04/30 2010

H.2.5. National Toxicology Program, 2006: Liver EROD 53 Weeks

H.2.5.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	4	<0.0001	648.094	2.011E+01	1.464E+01	
exponential (M3)	4	<0.0001	648.094	2.011E+01	1.464E+01	power hit bound (d = 1)
exponential (M4)	3	0.015	521.251	1.430E-02	9.808E-03	
exponential (M5)	2	0.354	514.812	7.656E-02	3.202E-02	
Hill [b]	2	0.760	513.286	1.853E-01	9.351E-02	
linear	4	<.0001	639.841	1.034E+01	6.557E-03	
polynomial, 5-degree	1	<.0001	14.000	error	error	
power	4	<.0001	592.889	2.254E-02	1.527E-02	power bound hit (power = 1)

[a] Non-constant variance model selected (*p* = <.0001)

[b] Best-fitting model, BMDS output presented in this appendix

H.2.5.2. *Output for Selected Model: Hill*

National Toxicology Program, 2006: Liver EROD 53 Weeks

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\5\Blood\46_NTP_2006_ERODliv53_Hill_1.(d)
         Gnuplot Plotting File:  C:\5\Blood\46_NTP_2006_ERODliv53_Hill_1.plt
                                              Sun May 02 15:34:21 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
```

lalpha =	11.0197
rho =	0
intercept =	30.215
v =	1841.26
n =	7.0105
k =	6.95814

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	intercept	v	n	k
lalpha	1	-0.97	-0.18	0.065	-0.025	0.046
rho	-0.97	1	0.17	-0.093	0.025	-0.048
intercept	-0.18	0.17	1	-0.022	0.011	0.00084
v	0.065	-0.093	-0.022	1	-0.73	0.87
n	-0.025	0.025	0.011	-0.73	1	-0.83
k	0.046	-0.048	0.00084	0.87	-0.83	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
lalpha	-4.47504	0.923978	-6.286	-2.66407
rho	2.12799	0.137849	1.85781	2.39817
intercept	30.2685	1.41935	27.4866	33.0504
v	1813.88	100.554	1616.8	2010.96
n	2.02516	0.29717	1.44272	2.6076
k	3.78554	0.349266	3.101	4.47009

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	30.2	30.3	4.5	4.02	-0.0377
2.458	8	569	564	69.6	90.3	0.17
5.533	8	1.28e+003	1.27e+003	270	214	0.137
9.543	8	1.55e+003	1.6e+003	318	274	-0.529
16.18	8	1.73e+003	1.75e+003	304	302	-0.248
29.04	8	1.87e+003	1.82e+003	309	313	0.507

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-285.269096	7	584.538193
A2	-249.237836	12	522.475671
A3	-250.368300	8	516.736600
fitted	-250.643212	6	513.286424
R	-338.451300	2	680.902600

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	178.427	10	<.0001
Test 2	72.0625	5	<.0001
Test 3	2.26093	4	0.6879
Test 4	0.549824	2	0.7596

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

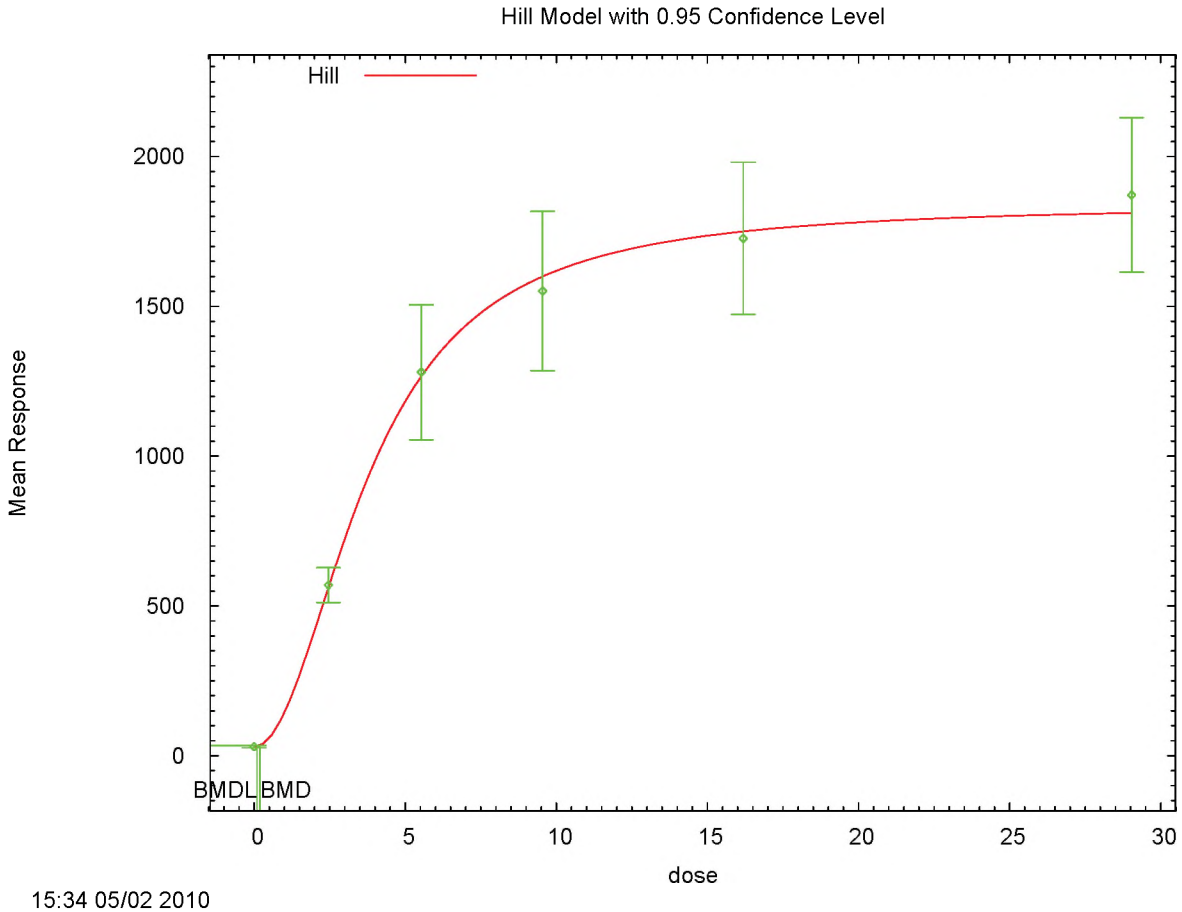
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 0.185269

BMDL = 0.0935065

H.2.5.3. *Figure for Selected Model: Hill*



H.2.6. National Toxicology Program, 2006: Lung Erod 53 Weeks

H.2.6.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	4	<0.0001	314.332	3.281E+01	2.047E+01	
exponential (M3)	4	<0.0001	555.061	5.210E+00	8.194E-01	power hit bound (d = 1)
exponential (M4) [b]	3	0.302	255.955	9.586E-02	5.907E-02	
exponential (M5)	2	0.276	256.882	1.044E+00	6.588E-02	
Hill	2	0.275	256.882	1.903E+00	3.469E-01	
linear	4	<.0001	313.237	2.662E+01	1.251E+01	
polynomial, 5-degree	5	<.0001	330.180	error	2.718E+01	
power	4	<.0001	313.237	2.662E+01	1.251E+01	power bound hit (power = 1)
power, unrestricted [c]	3	0.032	261.083	1.875E-07	1.875E-07	unrestricted (power = 0.18)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

H.2.6.2. *Output for Selected Model: Exponential (M4)*

National Toxicology Program, 2006: Lung EROD 53 Weeks

```
 ========================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\5\Blood\52_NTP_2006_LungEROD53_Exp_1.(d)
         Gnuplot Plotting File:
                                                 Fri Apr 30 14:20:27 2010
 ========================================================================

 Tbl 12, Week 53, Lung Microsomes EROD
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

     Note: Y[dose] is the median response for exposure = dose;
           sign = +1 for increasing trend in data;
           sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	-0.80064
rho	1.47683
a	2.86045
b	0.134268
c	16.0581
d	1

Parameter Estimates

Variable	Model 4
lnalpha	-1.14455
rho	1.63458
a	3.06102
b	0.371249
c	14.1551
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	8	3.011	1.584
2.458	8	27.15	5.269
5.533	8	42.85	11.15
9.543	8	36.57	12.99
16.18	8	43.75	18.55
29.04	8	43.71	6.322

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	3.061	1.408	-0.1005
2.458	27.16	8.383	-0.003073
5.533	38.17	11.07	1.196
9.543	42.16	12.01	-1.318
16.18	43.23	12.26	0.1191
29.04	43.33	12.28	0.08864

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                    Model      Log(likelihood)      DF         AIC
                    -------    -----------------    ----   ------------
                       A1       -135.2677           7      284.5353
                       A2       -115.6885          12      255.3771
                       A3       -121.1517           8      258.3034
                        R       -162.0902           2      328.1805
                        4       -122.9773           5      255.9546


Additive constant for all log-likelihoods =     -44.11.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                                Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                         92.8           10           < 0.0001
  Test 2                        39.16            5           < 0.0001
  Test 3                        10.93            4             0.0274
 Test 6a                        3.651            3             0.3017


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is less than .1.  You may want to
  consider a different variance model.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

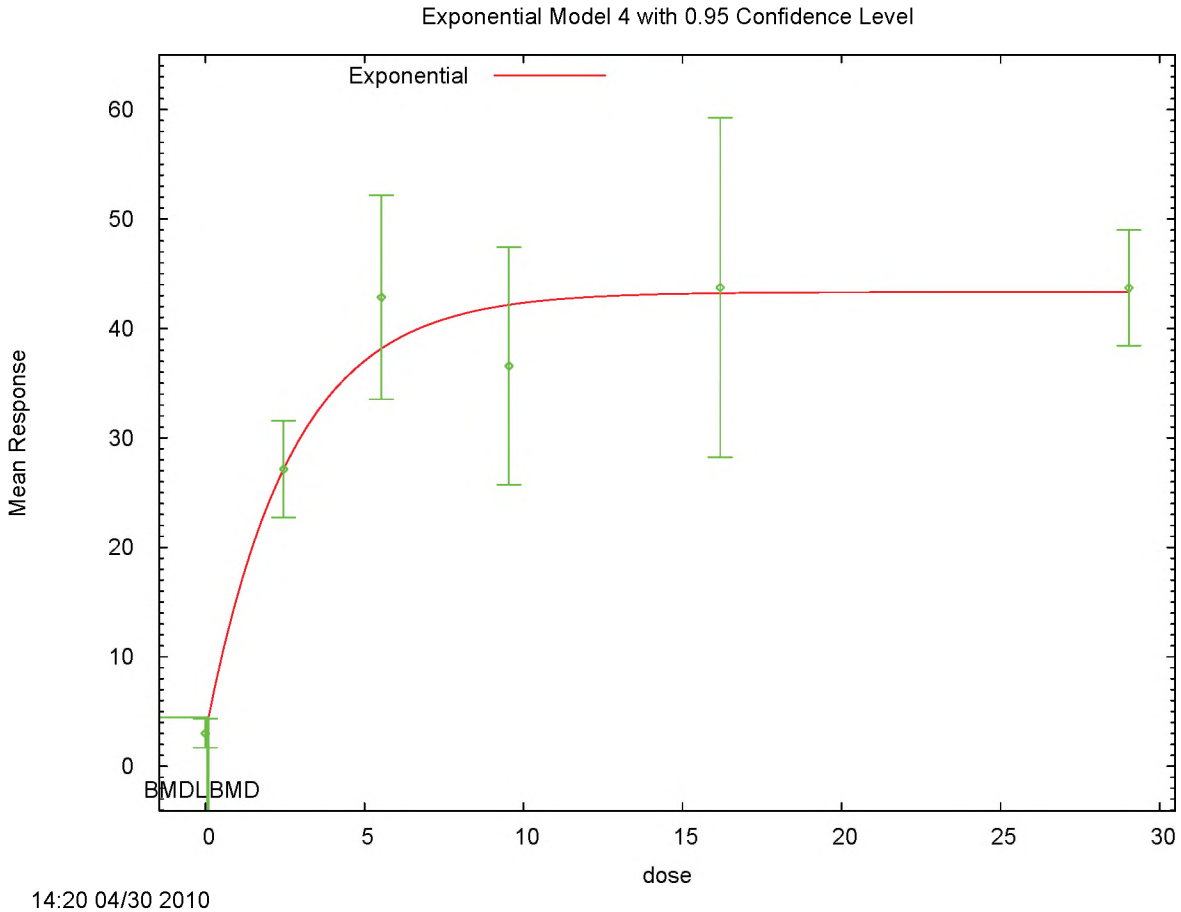
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      0.09586

              BMDL =    0.0590734
```

H.2.6.3. *Figure for Selected Model: Exponential (M4)*



14:20 04/30 2010

H.2.6.4. *Output for Additional Model Presented: Power, Unrestricted*

National Toxicology Program, 2006: Lung EROD 53 Weeks

```
 ================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\5\Blood\52_NTP_2006_LungEROD53_Pwr_U_1.(d)
         Gnuplot Plotting File:  C:\5\Blood\52_NTP_2006_LungEROD53_Pwr_U_1.plt
                                              Fri Apr 30 14:20:33 2010
 ================================================================

 Tbl 12, Week 53, Lung Microsomes EROD
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   The power is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                Default Initial Parameter Values
                       lalpha =      4.76968
                          rho =            0
                      control =        3.011
                        slope =      23.2411
                        power =     0.187468


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho      control        slope        power

    lalpha            1        -0.96        -0.49          0.1       -0.045

       rho        -0.96            1         0.45        -0.13         0.05

   control        -0.49         0.45            1        -0.14        0.048

     slope          0.1        -0.13        -0.14            1        -0.94

     power       -0.045         0.05        0.048        -0.94            1


                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha         -1.02668         0.818488           -2.63088            0.577531
            rho          1.63033          0.24056            1.15884             2.10182
        control          3.01543         0.519355            1.99751             4.03335
          slope          23.8167          3.70401            16.5569             31.0764
          power         0.179731        0.0639681           0.054356            0.305106


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
```

```
------     ---   --------     --------   -----------  -----------   ----------

     0     8       3.01         3.02         1.58         1.47       -0.00851
 2.458     8       27.1           31         5.27         9.84          -1.11
 5.533     8       42.8         35.4         11.2           11           1.92
 9.543     8       36.6         38.7           13         11.8          -0.52
 16.18     8       43.7         42.3         18.5         12.7          0.323
 29.04     8       43.7         46.6         6.32         13.7         -0.605


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -135.267662          7     284.535325
              A2         -115.688533         12     255.377067
              A3         -121.151707          8     258.303413
          fitted         -125.541690          5     261.083380
               R         -162.090242          2     328.180484


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              92.8034         10          <.0001
   Test 2              39.1583          5          <.0001
   Test 3              10.9263          4          0.0274
   Test 4              8.77997          3         0.03236

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is less than .1.  You may want to try a different
model
```

```
                Benchmark Dose Computation

Specified effect =             1

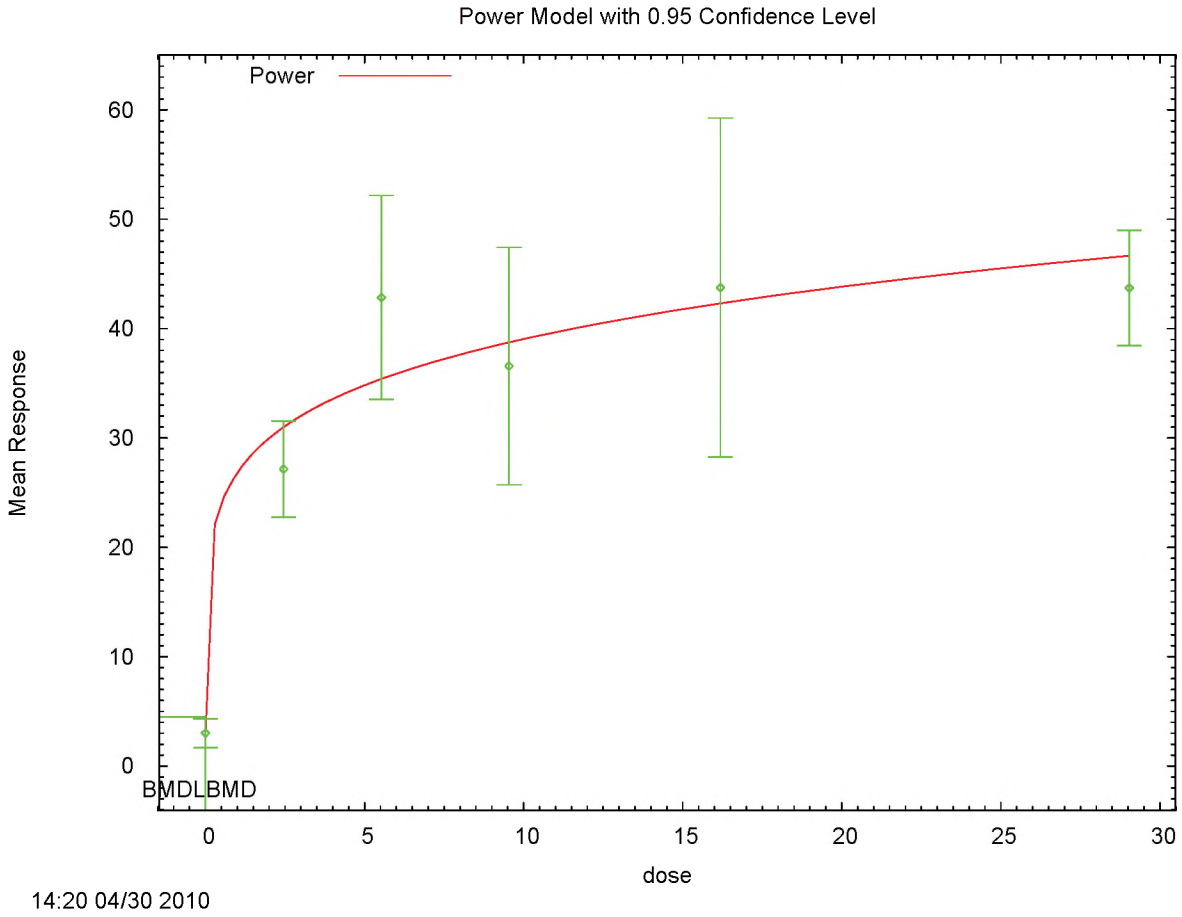
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD = 1.8745e-007


             BMDL = 1.8745e-007
```

H.2.6.5. *Figure for Additional Model Presented: Power, Unrestricted*



H.2.7. National Toxicology Program, 2006: Labeling Index 31 Weeks

H.2.7.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	4	0.000	46.547	8.660E+00	6.926E+00	
exponential (M3)	4	0.000	46.547	8.660E+00	6.926E+00	power hit bound (d = 1)
exponential (M4)	3	<0.0001	50.958	3.151E+00	1.865E+00	
exponential (M5)	3	<0.0001	50.958	3.151E+00	1.864E+00	power hit bound (d = 1)
Hill	3	<.0001	50.963	3.145E+00	error	n lower bound hit (n = 1)
linear	4	0.000	48.958	3.151E+00	1.865E+00	
polynomial, 5-degree [b]	3	0.000	46.230	7.607E+00	3.125E+00	
power	4	0.000	48.958	3.151E+00	1.865E+00	power bound hit (power = 1)

[a] Non-constant variance model selected (*p* = <.0001)

[b] Best-fitting model, BMDS output presented in this appendix

H.2.7.2. *Output for Selected Model: Polynomial, 5-degree*

National Toxicology Program, 2006: Labeling Index 31 Weeks

```
 ======================================================================
          Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
          Input Data File: C:\5\Blood\38_NTP_2006_HepIndex_Poly5_1.(d)
          Gnuplot Plotting File:  C:\5\Blood\38_NTP_2006_HepIndex_Poly5_1.plt
                                                 Fri Apr 30 14:21:16 2010
 ======================================================================

 Tbl 11, 31wk, Hep Cell Proliferation Labeling Index
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   The polynomial coefficients are restricted to be positive
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
```

```
                          lalpha =     0.708431
                             rho =            0
                          beta_0 =        0.327
                          beta_1 =            0
                          beta_2 =            0
                          beta_3 =            0
                          beta_4 =            0
                          beta_5 =            0


            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -beta_2    -beta_3    -beta_4
                  have been estimated at a boundary point, or have been specified by the user,
                  and do not appear in the correlation matrix )

                  lalpha          rho       beta_0       beta_1       beta_5

    lalpha             1       -0.086        0.012       -0.032        0.043

       rho       -0.086            1      -0.0027       -0.011        0.076

    beta_0        0.012      -0.0027            1         -0.6         0.23

    beta_1       -0.032       -0.011         -0.6            1        -0.53

    beta_5        0.043        0.076         0.23        -0.53            1


                                 Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha        -0.501559         0.185039          -0.864229           -0.138889
            rho          1.90452         0.272948            1.36955             2.43948
         beta_0         0.500197         0.102837           0.298641            0.701753
         beta_1        0.0525247        0.0192967          0.0147038           0.0903456
         beta_2     8.00068e-025               NA
         beta_3                0               NA
         beta_4                0               NA
         beta_5     1.08658e-007     6.10451e-008       -1.09879e-008        2.28305e-007

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.



     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     9      0.327          0.5        0.189        0.402          -1.29
2.331    10      0.852        0.623        0.651        0.496           1.46
5.315    10      0.956         0.78        0.737        0.614          0.907
9.207    10      0.792        0.991        0.462        0.772         -0.816
15.66    10       1.33         1.42         1.12         1.09         -0.266
28.13    10       3.85         3.89         3.08         2.84        -0.0523


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2
```

```
Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          -47.234977            7     108.469953
             A2           -8.679256           12      41.358512
             A3           -8.980651            8      33.961301
         fitted          -18.115050            5      46.230101
              R          -63.448285            2     130.896571


                   Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

  Test    -2*log(Likelihood Ratio)  Test df        p-value

  Test 1              109.538         10          <.0001
  Test 2              77.1114          5          <.0001
  Test 3              0.60279          4          0.9628
  Test 4              18.2688          3       0.0003871

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


             Benchmark Dose Computation

Specified effect =             1

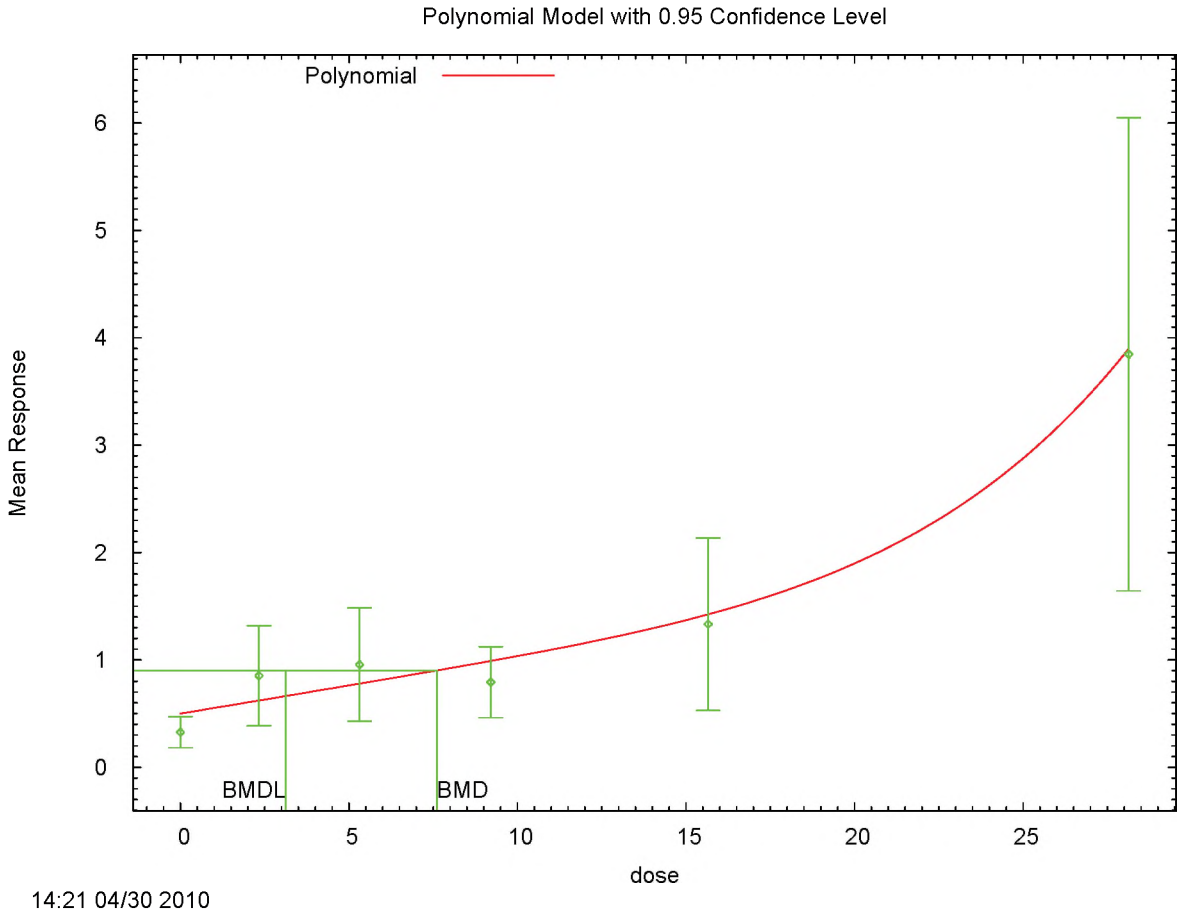
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =         7.6073


             BMDL =        3.12526
```

H.2.7.3. *Figure for Selected Model: Polynomial, 5-degree*



H.2.8. Vanden Heuvel et al., 1994: Hepatic CYP1A1 Mrna Expression

H.2.8.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
exponential (M2)	5	<0.0001	1147.626	1.769E+01	1.257E+01	
exponential (M3)	4	<0.0001	1149.626	1.769E+01	1.257E+01	power hit bound (d = 1)
exponential (M4)	4	<0.0001	666.337	6.104E-02	2.871E-02	
exponential (M5)	3	<0.0001	635.591	1.252E+00	9.089E-01	
Hill [b]	3	<.0001	664.418	2.429E-01	1.679E-01	
linear	5	<.0001	673.777	4.546E-02	2.487E-02	
polynomial, 6-degree	6	<.0001	1213.329	error	1.301E+03	
power	4	<.0001	673.418	6.269E-02	3.196E-02	

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

H.2.8.2. *Output for Selected Model: Hill*

Vanden Heuvel et al., 1994: Hepatic CYP1A1 mRNA Expression

```
================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\Usepa\BMDS21\Data\hil_Vanden_mRNA_Setting.(d)
          Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\hil_Vanden_mRNA_Setting.plt
                                                Tue May 18 05:24:48 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = mRNA_mean
   Independent variable = blood_conc
   Power parameter is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 7
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  User Inputs Initial Parameter Values
```

```
                          lalpha =           1
                             rho =         1.9
                       intercept =           6
                               v =       36000
                               n =           1
                               k =        1000


            Asymptotic Correlation Matrix of Parameter Estimates

                  lalpha          rho    intercept            v            n            k

     lalpha            1        -0.89        -0.43         0.27         0.68        -0.18

        rho        -0.89            1         0.31        -0.42        -0.72         0.22

  intercept        -0.43         0.31            1       -0.093         0.14        -0.04

          v         0.27        -0.42       -0.093            1        0.075          0.7

          n         0.68        -0.72         0.14        0.075            1        -0.52

          k        -0.18         0.22        -0.04          0.7        -0.52            1


                                  Parameter Estimates

                                                                95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          lalpha        -0.191631         0.711681            -1.5865             1.20324
             rho           2.0275         0.132551            1.76771             2.28729
       intercept            5.416          1.16292            3.13672             7.69529
               v          41657.2          16561.5            9197.25             74117.2
               n          1.29154         0.100513            1.09454             1.48854
               k          97.8648          41.0376            17.4325             178.297


       Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    13         5.4         5.42         3.61         5.04        -0.0115
0.0113     5         7.2         5.76         5.59         5.36          0.602
0.106     12        14.8         11.6         14.9         10.9           1.03
0.8828     7        12.8          100          4.5         97.2          -2.38
 6.46      7         536    1.21e+003          320    1.22e+003          -1.48
48.32     11    1.8e+004    1.19e+004    1.52e+004    1.24e+004           1.62
434.5      5   3.67e+004    3.64e+004    2.21e+004    3.82e+004         0.0199


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-572.470944	8	1160.941889
A2	-290.799287	14	609.598575
A3	-293.809342	9	605.618684
fitted	-326.209186	6	664.418372
R	-603.663396	2	1211.326792

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	625.728	12	<.0001
Test 2	563.343	6	<.0001
Test 3	6.02011	5	0.3043
Test 4	64.7997	3	<.0001

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation

Specified effect = 24

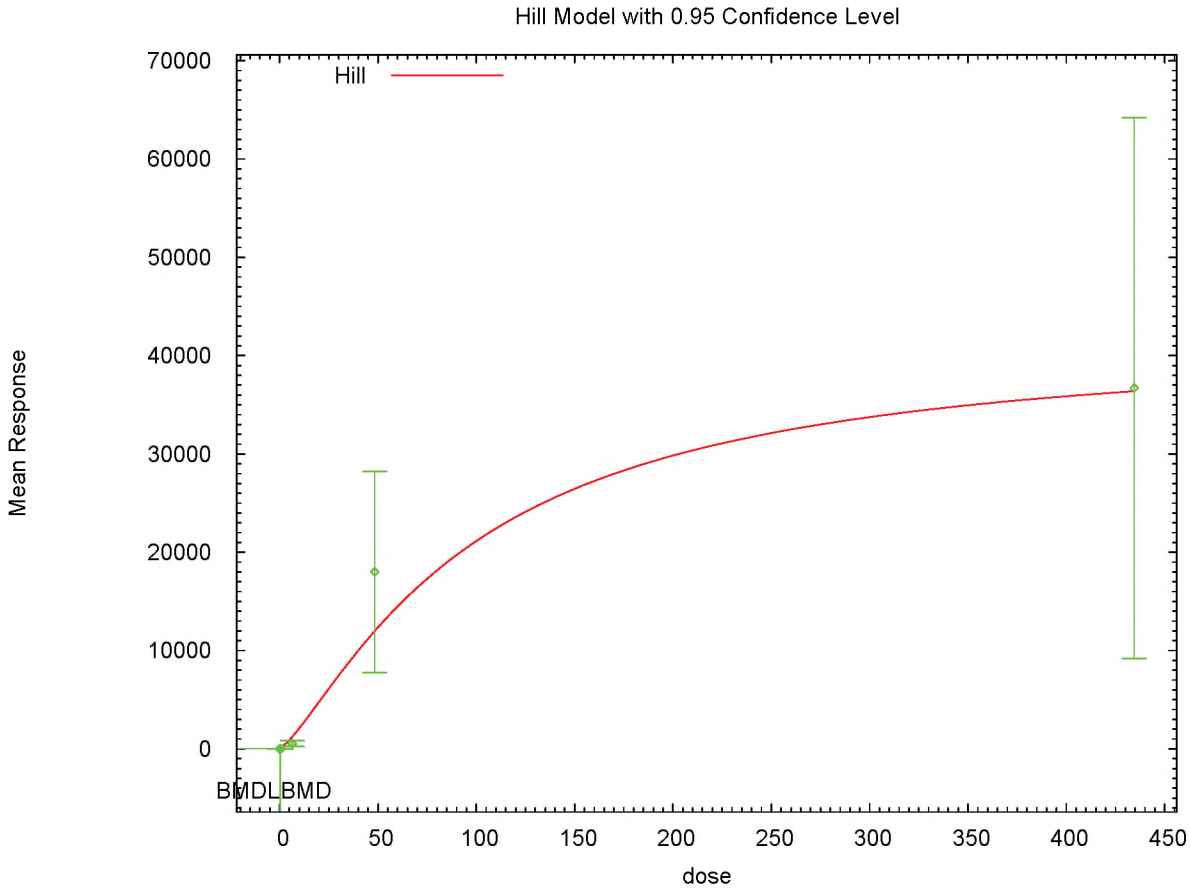
Risk Type = Point risk

Confidence level = 0.95

BMD = 0.249203

BMDL = 0.167897

H.2.8.3. *Figure for Selected Model: Hill*



05:24 05/18 2010

H.3. ADMINISTERED DOSE BMDS RESULTS

H.3.1. Hassoun et al., 2000: Cytochrome C Reductase

H.3.1.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	4	0.002	-139.075	3.939E+01	3.254E+01	
exponential (M3)	4	0.002	-139.075	3.939E+01	3.254E+01	power hit bound (d = 1)
exponential (M4) [b]	3	0.637	-151.807	9.085E+00	5.886E+00	
exponential (M5)	2	0.786	-151.023	1.420E+01	6.537E+00	
Hill	2	0.741	-150.905	1.513E+01	6.277E+00	
linear	4	0.032	-144.946	2.470E+01	1.933E+01	
polynomial, 5-degree	4	0.032	-144.946	2.470E+01	1.933E+01	
power	4	0.032	-144.946	2.470E+01	1.933E+01	power bound hit (power = 1)
power, unrestricted [c]	3	0.211	-148.989	6.573E+00	1.966E+00	unrestricted (power = 0.574)

[a] Constant variance model selected ($p = 0.3871$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

H.3.1.2. *Output for Selected Model: Exponential (M4)*

Hassoun et al., 2000: Cytochrome C reductase

```
 ========================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\5\17_Has_2000_CytCLiv_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                Fri Apr 30 21:15:20 2010
 ========================================================================

 TBARs, liver only (Table 2)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
```

Model 4 is nested within Model 5.

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	-5.48625
rho(S)	0
a	0.1387
b	0.027423
c	3.36121
d	1

(S) = Specified

Parameter Estimates

Variable	Model 4
lnalpha	-5.43908
rho	0
a	0.141259
b	0.0235562
c	3.42165
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	6	0.146	0.06614
3	6	0.177	0.05389
10	6	0.191	0.05634
22	6	0.271	0.05634
46	6	0.388	0.06369
100	6	0.444	0.1102

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	0.1413	0.06591	0.1762
3	0.1646	0.06591	0.4609
10	0.2131	0.06591	-0.8196
22	0.2796	0.06591	-0.3199
46	0.3676	0.06591	0.7587
100	0.4509	0.06591	-0.2564

Other models for which likelihoods are calculated:

```
  Model A1:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
             Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	80.75258	7	-147.5052
A2	83.37355	12	-142.7471
A3	80.75258	7	-147.5052
R	55.82002	2	-107.64
4	79.90337	4	-151.8067

Additive constant for all log-likelihoods = -33.08. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	55.11	10	< 0.0001
Test 2	5.242	5	0.3871
Test 3	5.242	5	0.3871
Test 6a	1.698	3	0.6373

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Benchmark Dose Computations:

```
  Specified Effect = 1.000000

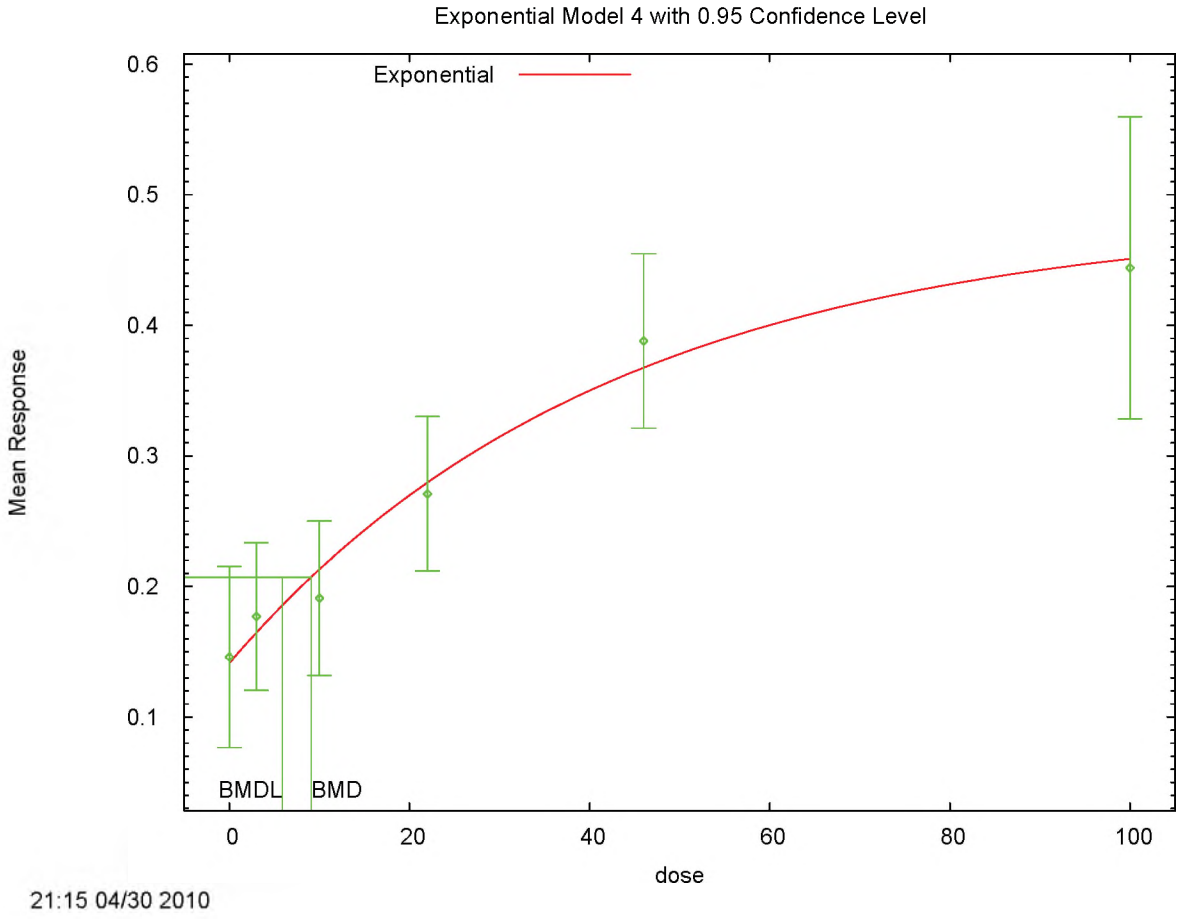
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000
```

```
BMD =        9.0851

BMDL =       5.88612
```

H.3.1.3. *Figure for Selected Model: Exponential (M4)*



H.3.1.4. *Output for Additional Model Presented: Power, Unrestricted*

Hassoun et al., 2000: Cytochrome C reductase

```
================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\5\17_Has_2000_CytCLiv_PwrCV_U_1.(d)
        Gnuplot Plotting File:  C:\5\17_Has_2000_CytCLiv_PwrCV_U_1.plt
                                          Fri Apr 30 21:15:26 2010
================================================================

TBARs, liver only (Table 2)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = control + slope * dose^power


  Dependent variable = Mean
  Independent variable = Dose
  rho is set to 0
  The power is not restricted
  A constant variance model is fit
```

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	0.004972	
rho =	0	Specified
control =	0.146	
slope =	0.0109242	
power =	0.717914	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	control	slope	power
alpha	1	-8.8e-010	-3.8e-009	4.5e-009
control	-8.8e-010	1	-0.77	0.68
slope	-3.8e-009	-0.77	1	-0.98
power	4.5e-009	0.68	-0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.00469717	0.00110713	0.00252723	0.00686711
control	0.135495	0.0246289	0.0872229	0.183766
slope	0.0232652	0.013381	-0.00296103	0.0494915
power	0.573772	0.119032	0.340474	0.80707

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	6	0.146	0.135	0.0661	0.0685	0.375
3	6	0.177	0.179	0.0539	0.0685	-0.0784
10	6	0.191	0.223	0.0563	0.0685	-1.13
22	6	0.271	0.273	0.0563	0.0685	-0.056
46	6	0.388	0.345	0.0637	0.0685	1.54
100	6	0.444	0.462	0.11	0.0685	-0.653

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          80.752584            7    -147.505168
              A2          83.373547           12    -142.747094
              A3          80.752584            7    -147.505168
          fitted          78.494318            4    -148.988637
               R          55.820023            2    -107.640047


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1               55.107         10          <.0001
   Test 2              5.24193          5          0.3871
   Test 3              5.24193          5          0.3871
   Test 4              4.51653          3          0.2108

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


               Benchmark Dose Computation

Specified effect =             1

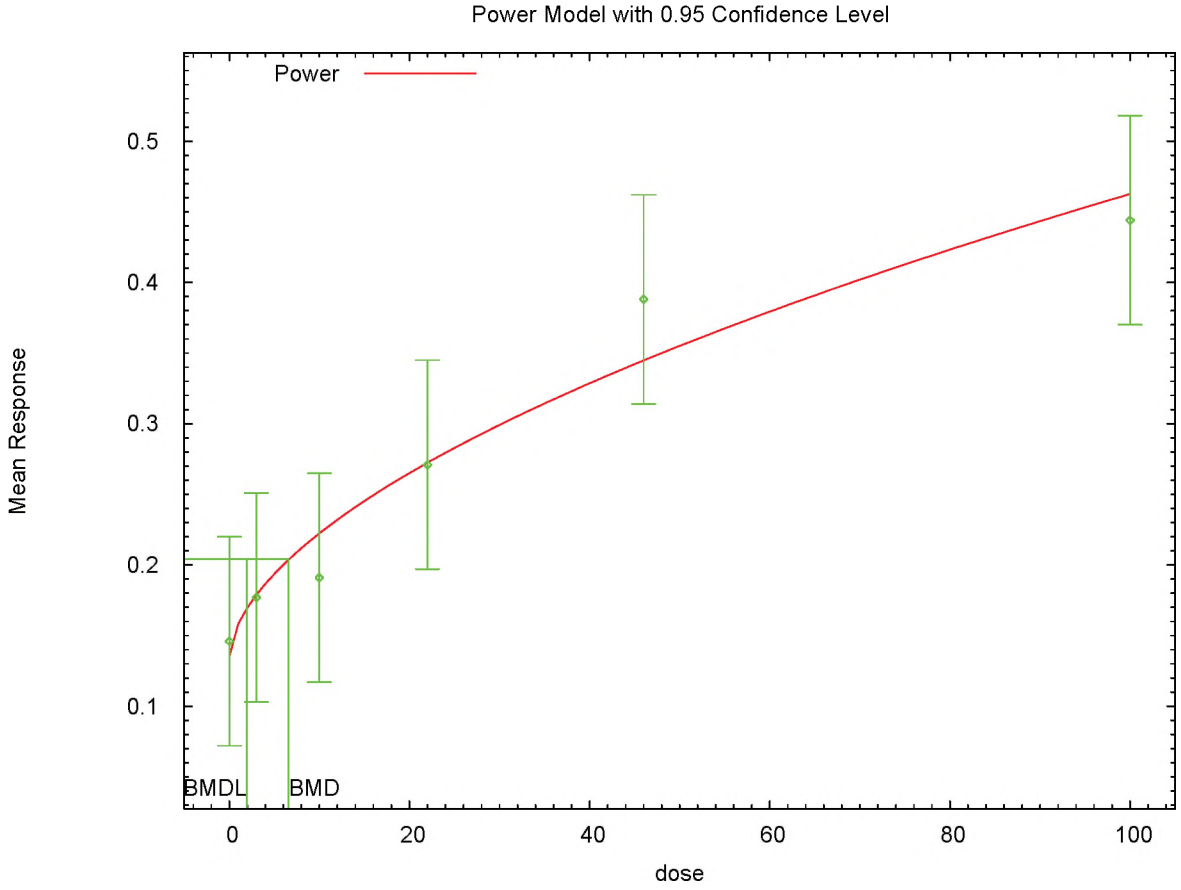
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 6.57302


            BMDL = 1.96558
```

H.3.1.5. *Figure for Additional Model Presented: Power, Unrestricted*



H.3.2. Hassoun et al., 2000: DNA Single-Strand Breaks

H.3.2.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	4	<0.0001	120.828	3.006E+01	2.491E+01	
exponential (M3)	4	<0.0001	120.828	3.006E+01	2.491E+01	power hit bound (d = 1)
exponential (M4)	3	0.036	82.814	3.734E+00	2.783E+00	
exponential (M5)	3	0.036	82.814	3.734E+00	2.783E+00	power hit bound (d = 1)
Hill [b]	3	0.068	81.407	2.890E+00	2.007E+00	n lower bound hit (n = 1)
linear	4	<.0001	111.165	1.807E+01	1.452E+01	
polynomial, 5-degree	4	<.0001	111.165	1.807E+01	1.452E+01	
power	4	<.0001	111.165	1.807E+01	1.452E+01	power bound hit (power = 1)
Hill, unrestricted [c]	2	0.133	80.318	9.618E-01	2.114E-01	unrestricted (n = 0.613)

[a] Constant variance model selected ($p = 0.7521$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

H.3.2.2. *Output for Selected Model: Hill*

Hassoun et al., 2000: DNA single-strand breaks

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\5\18_Has_2000_SSB_HillCV_1.(d)
         Gnuplot Plotting File:  C:\5\18_Has_2000_SSB_HillCV_1.plt
                                          Fri Apr 30 21:16:28 2010
 ================================================================

 DNA single-strand breaks, liver only (Table 3)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
```

Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	2.7831	
rho =	0	Specified
intercept =	7.41	
v =	16.09	
n =	0.174831	
k =	69.2706	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	k
alpha	1	1.1e-007	1.9e-007	1.9e-007
intercept	1.1e-007	1	0.099	0.61
v	1.9e-007	0.099	1	0.79
k	1.9e-007	0.61	0.79	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	2.82659	0.666233	1.5208	4.13238
intercept	8.16404	0.581043	7.02522	9.30286
v	20.1253	1.69013	16.8127	23.4379
n	1	NA		
k	31.702	8.35815	15.3203	48.0836

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	6	7.41	8.16	1.54	1.68	-1.1
3	6	10.8	9.9	1.25	1.68	1.28
10	6	13.6	13	1.69	1.68	0.889
22	6	15.3	16.4	1.71	1.68	-1.62
46	6	20.4	20.1	2.25	1.68	0.469
100	6	23.5	23.4	1.37	1.68	0.0802

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

```
Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          -33.142389           7      80.284779
              A2          -31.811970          12      87.623940
              A3          -33.142389           7      80.284779
          fitted          -36.703273           4      81.406545
               R          -80.442086           2     164.884172


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              97.2602         10          <.0001
   Test 2              2.66084          5          0.7521
   Test 3              2.66084          5          0.7521
   Test 4              7.12177          3         0.06812

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


        Benchmark Dose Computation

Specified effect =             1

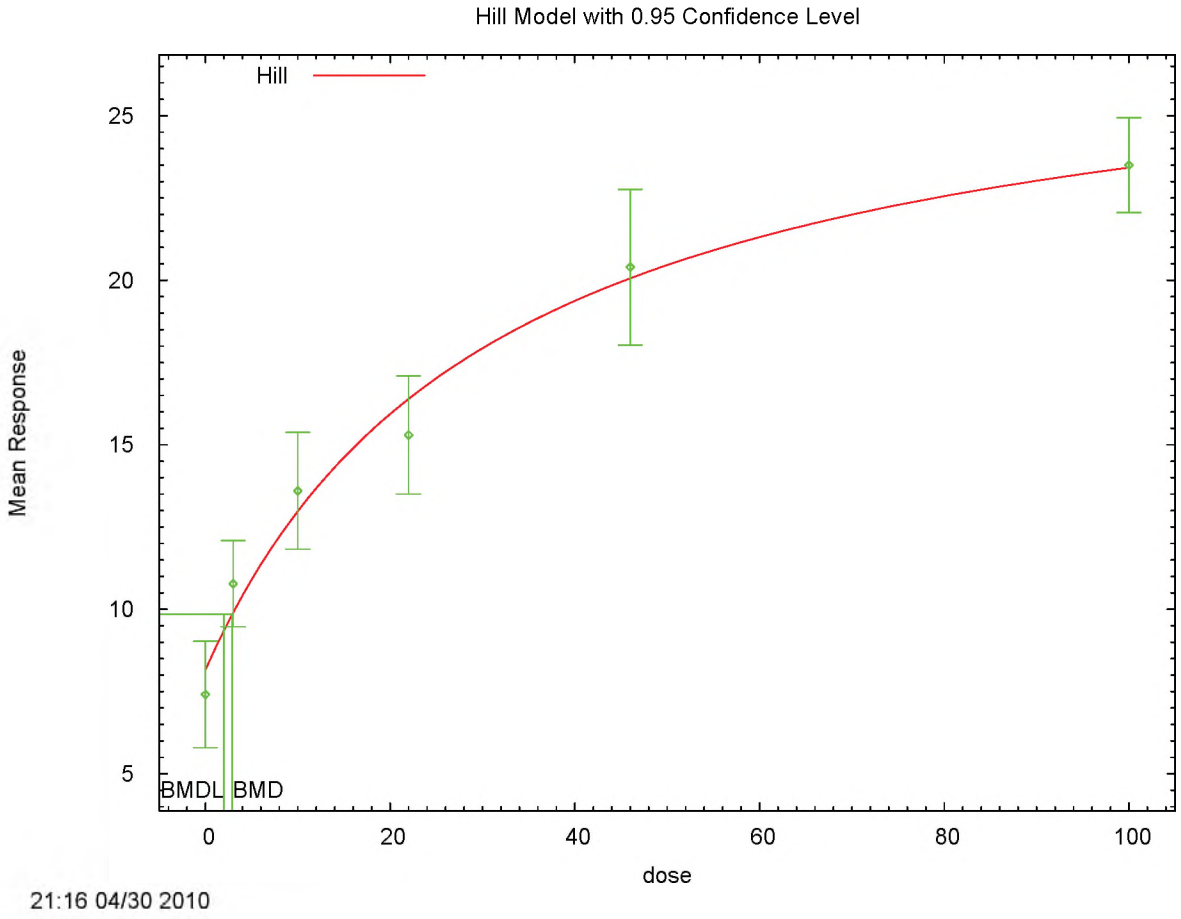
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        2.88976

            BMDL =       2.00669
```

H.3.2.3. *Figure for Selected Model: Hill*



H.3.2.4. *Output for Additional Model Presented: Hill, Unrestricted*

Hassoun et al., 2000: DNA single-strand breaks

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\5\18_Has_2000_SSB_HillCV_U_1.(d)
         Gnuplot Plotting File:  C:\5\18_Has_2000_SSB_HillCV_U_1.plt
                                              Fri Apr 30 21:16:30 2010
 ================================================================

 DNA single-strand breaks, liver only (Table 3)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter is not restricted
   A constant variance model is fit

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
```

Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	2.7831	
rho =	0	Specified
intercept =	7.41	
v =	16.09	
n =	0.174831	
k =	69.2706	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	n	k
alpha	1	-2.2e-008	-4.6e-008	8.4e-009	-4.3e-008
intercept	-2.2e-008	1	-0.33	0.47	-0.29
v	-4.6e-008	-0.33	1	-0.95	1
n	8.4e-009	0.47	-0.95	1	-0.96
k	-4.3e-008	-0.29	1	-0.96	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	2.5942	0.611459	1.39576	3.79264
intercept	7.47627	0.665055	6.17278	8.77975
v	36.9014	25.5466	-13.1689	86.9718
n	0.612877	0.190055	0.240376	0.985377
k	148.104	303.532	-446.809	743.016

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	6	7.41	7.48	1.54	1.61	-0.101
3	6	10.8	10.6	1.25	1.61	0.313
10	6	13.6	13.4	1.69	1.61	0.286
22	6	15.3	16.2	1.71	1.61	-1.41
46	6	20.4	19.6	2.25	1.61	1.24
100	6	23.5	23.7	1.37	1.61	-0.33

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          -33.142389           7      80.284779
              A2          -31.811970          12      87.623940
              A3          -33.142389           7      80.284779
          fitted          -35.159023           5      80.318046
               R          -80.442086           2     164.884172


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              97.2602         10          <.0001
   Test 2              2.66084          5          0.7521
   Test 3              2.66084          5          0.7521
   Test 4              4.03327          2          0.1331

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =             1

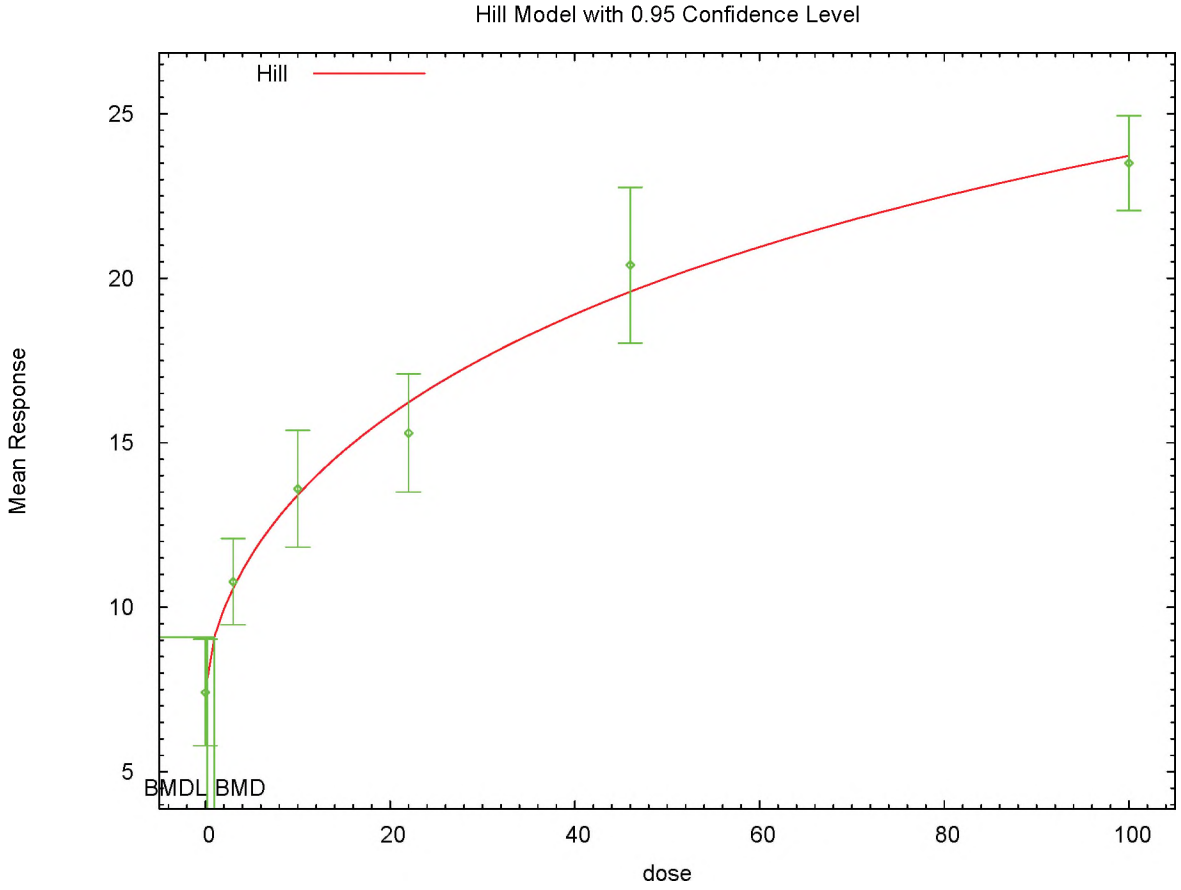
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       0.961789

            BMDL =      0.211403
```

H.3.2.5. *Figure for Additional Model Presented: Hill, Unrestricted*



H.3.3. Hassoun et al., 2000: TBARS

H.3.3.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	4	0.000	-6.143	7.977E+01	5.344E+01	
exponential (M3)	4	0.000	-6.143	7.977E+01	5.344E+01	power hit bound (d = 1)
exponential (M4) [b]	3	0.340	-21.181	4.916E+00	2.300E+00	
exponential (M5)	2	0.240	-19.681	6.732E+00	2.470E+00	
Hill	2	0.272	-19.932	6.261E+00	2.575E+00	
linear	4	0.001	-7.019	6.904E+01	4.373E+01	
polynomial, 5-degree	4	0.001	-7.019	6.904E+01	4.373E+01	
power	4	0.001	-7.019	6.904E+01	4.373E+01	power bound hit (power = 1)
power, unrestricted [c]	3	0.023	-14.993	2.902E+00	6.150E-02	unrestricted (power = 0.263)

[a] Constant variance model selected ($p = 0.3348$)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

H.3.3.2. *Output for Selected Model: Exponential (M4)*

Hassoun et al., 2000: TBARS

```
 ======================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\5\19_Has_2000_TBARsLiv_ExpCV_1.(d)
         Gnuplot Plotting File:
                                                Fri Apr 30 21:17:17 2010
 ======================================================================

 TBARs, liver only (Table 2)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	-1.90388
rho(S)	0
a	1.39555
b	0.0194898
c	1.97051
d	1

(S) = Specified

Parameter Estimates

Variable	Model 4
lnalpha	-1.81059
rho	0
a	1.40436
b	0.0996859
c	1.74329
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	6	1.469	0.2915
3	6	1.549	0.5389
10	6	2.15	0.3625
22	6	2.28	0.2474
46	6	2.619	0.5168
100	6	2.292	0.4874

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	1.404	0.4044	0.3915
3	1.674	0.4044	-0.7582
10	2.063	0.4044	0.527
22	2.332	0.4044	-0.3134
46	2.438	0.4044	1.099
100	2.448	0.4044	-0.9458

Other models for which likelihoods are calculated:

```
  Model A1:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                    Model      Log(likelihood)      DF         AIC
                    -------    -----------------    ----   ------------
                       A1        16.26977            7     -18.53954
                       A2        19.12783           12     -14.25565
                       A3        16.26977            7     -18.53954
                        R         2.44294            2    -0.8858799
                        4          14.5907           4     -21.18141


Additive constant for all log-likelihoods =     -33.08.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                         33.37          10            0.000236
  Test 2                         5.716           5              0.3348
  Test 3                         5.716           5              0.3348
 Test 6a                         3.358           3              0.3396


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1.  A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

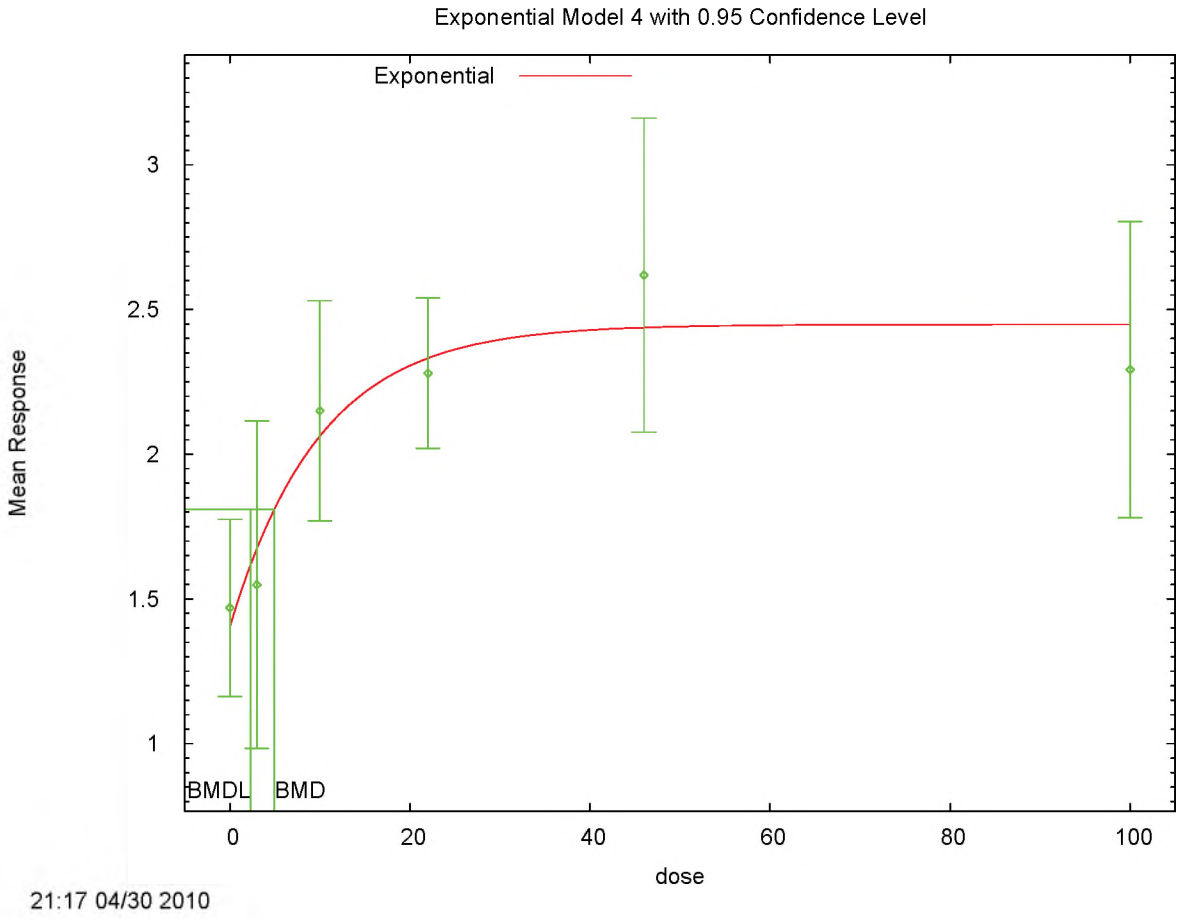
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

              BMD =      4.91639
```

BMDL = 2.29952

H.3.3.3. *Figure for Selected Model: Exponential (M4)*



H.3.3.4. *Output for Additional Model Presented: Power, Unrestricted*

Hassoun et al., 2000: TBARS

```
================================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\5\19_Has_2000_TBARsLiv_PwrCV_U_1.(d)
        Gnuplot Plotting File:  C:\5\19_Has_2000_TBARsLiv_PwrCV_U_1.plt
                                                Fri Apr 30 21:17:21 2010
================================================================================

TBARs, liver only (Table 2)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = control + slope * dose^power


  Dependent variable = Mean
  Independent variable = Dose
  rho is set to 0
  The power is not restricted
  A constant variance model is fit

  Total number of dose groups = 6
```

```
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                          alpha =     0.178788
                            rho =            0   Specified
                        control =        1.469
                          slope =    0.0756538
                          power =     0.652114


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                    alpha      control        slope        power

      alpha             1     1.1e-008    -1.1e-009    -1.5e-008

    control     1.1e-008            1        -0.75         0.47

      slope    -1.1e-009        -0.75            1        -0.91

      power    -1.5e-008         0.47        -0.91            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha         0.194232        0.0457809           0.104503            0.283961
         control          1.42104         0.171077            1.08573             1.75634
           slope         0.333105         0.166768         0.00624603            0.659963
           power         0.262735        0.0983956          0.0698836            0.455587


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0       6       1.47         1.42        0.291        0.441          0.267
    3       6       1.55         1.87        0.539        0.441          -1.76
   10       6       2.15         2.03        0.363        0.441          0.661
   22       6       2.28         2.17        0.247        0.441          0.603
   46       6       2.62         2.33        0.517        0.441            1.6
  100       6       2.29         2.54        0.487        0.441          -1.37


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
      Model A3 uses any fixed variance parameters that
      were specified by the user
```

```
Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                       Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1           16.269770           7     -18.539539
            A2           19.127827          12     -14.255654
            A3           16.269770           7     -18.539539
         fitted          11.496634           4     -14.993268
             R            2.442940           2      -0.885880


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              33.3698         10        0.000236
   Test 2              5.71611          5          0.3348
   Test 3              5.71611          5          0.3348
   Test 4              9.54627          3         0.02284

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1.  You may want to try a different
model


               Benchmark Dose Computation

Specified effect =             1

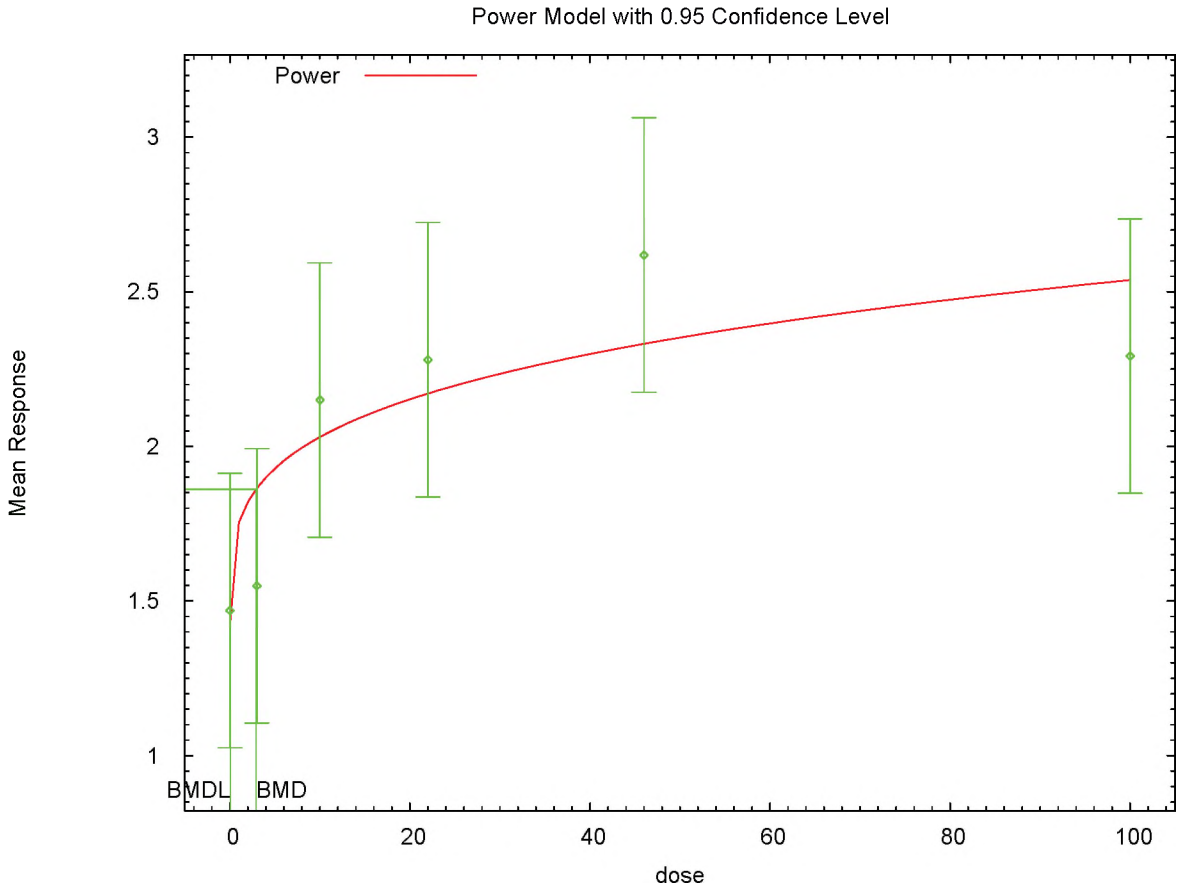
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD = 2.90232


            BMDL = 0.0614971
```

H.3.3.5. *Figure for Additional Model Presented: Power, Unrestricted*



21:17 04/30 2010

H.3.4. Kitchin and Woods, 1979: Bap Hydroxylase Activity

H.3.4.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	9	<0.0001	452.693	7.939E+03	3.663E+03	
exponential (M3)	9	<0.0001	452.693	7.939E+03	3.663E+03	power hit bound (d = 1)
exponential (M4)	8	0.015	226.600	5.458E+00	4.099E+00	
exponential (M5) [b]	7	0.019	226.401	1.022E+01	4.807E+00	
Hill	8	<.0001	504.527	error	error	n upper bound hit (n = 18)
linear	9	<.0001	299.732	8.276E+00	5.945E+00	
polynomial, 8-degree	3	<.0001	20.000	error	error	
power	9	<.0001	299.732	8.276E+00	5.945E+00	power bound hit (power = 1)

[a] Non-constant variance model selected (*p* = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

H.3.4.2. *Output for Selected Model: Exponential (M5)*

Kitchin and Woods, 1979: BaP Hydroxylase Activity

```
 ======================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\5\27_Kitchin_1979_Hydrolase_Exp_1.(d)
         Gnuplot Plotting File:
                                                 Fri Apr 30 21:18:04 2010
 ======================================================================

 Kitchin 1979, Tbl3, BaP hydrolase activity
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
```

The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 11
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 5
lnalpha	-3.27793
rho	1.92227
a	4.655
b	0.000177432
c	42.6316
d	1

Parameter Estimates

Variable	Model 5
lnalpha	-2.64304
rho	1.93753
a	5.43423
b	0.00191658
c	31.2033
d	1.21503

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	9	4.9	1.11
0.6	4	4.9	1.18
2	4	6.7	1.4
4	4	7.2	1.8
20	4	8.3	0.26
60	4	14	5
200	4	59	6.8
600	4	96	46
2000	4	155	16.4
5000	4	182	26
2e+004	4	189	26

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	5.434	1.375	-1.166
0.6	5.478	1.386	-0.8347
2	5.624	1.421	1.514
4	5.875	1.483	1.787
20	8.525	2.127	-0.2115
60	16.87	4.12	-1.394
200	49.41	11.67	1.643
600	119.4	27.43	-1.705
2000	168.6	38.31	-0.7091
5000	169.6	38.53	0.6454
2e+004	169.6	38.53	1.009

Other models for which likelihoods are calculated:

```
  Model A1:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
             Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-158.1306	12	340.2613
A2	-84.80028	22	213.6006
A3	-98.82189	13	223.6438
R	-234.6252	2	473.2504
5	-107.2005	6	226.4011

Additive constant for all log-likelihoods = -45.03. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 7a: Does Model 5 fit the data? (A3 vs 5)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	299.6	20	< 0.0001
Test 2	146.7	10	< 0.0001
Test 3	28.04	9	0.0009381
Test 7a	16.76	7	0.01903

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 7a is less than .1. Model 5 may not adequately describe the data; you may want to consider another model.

Benchmark Dose Computations:

Specified Effect = 1.000000

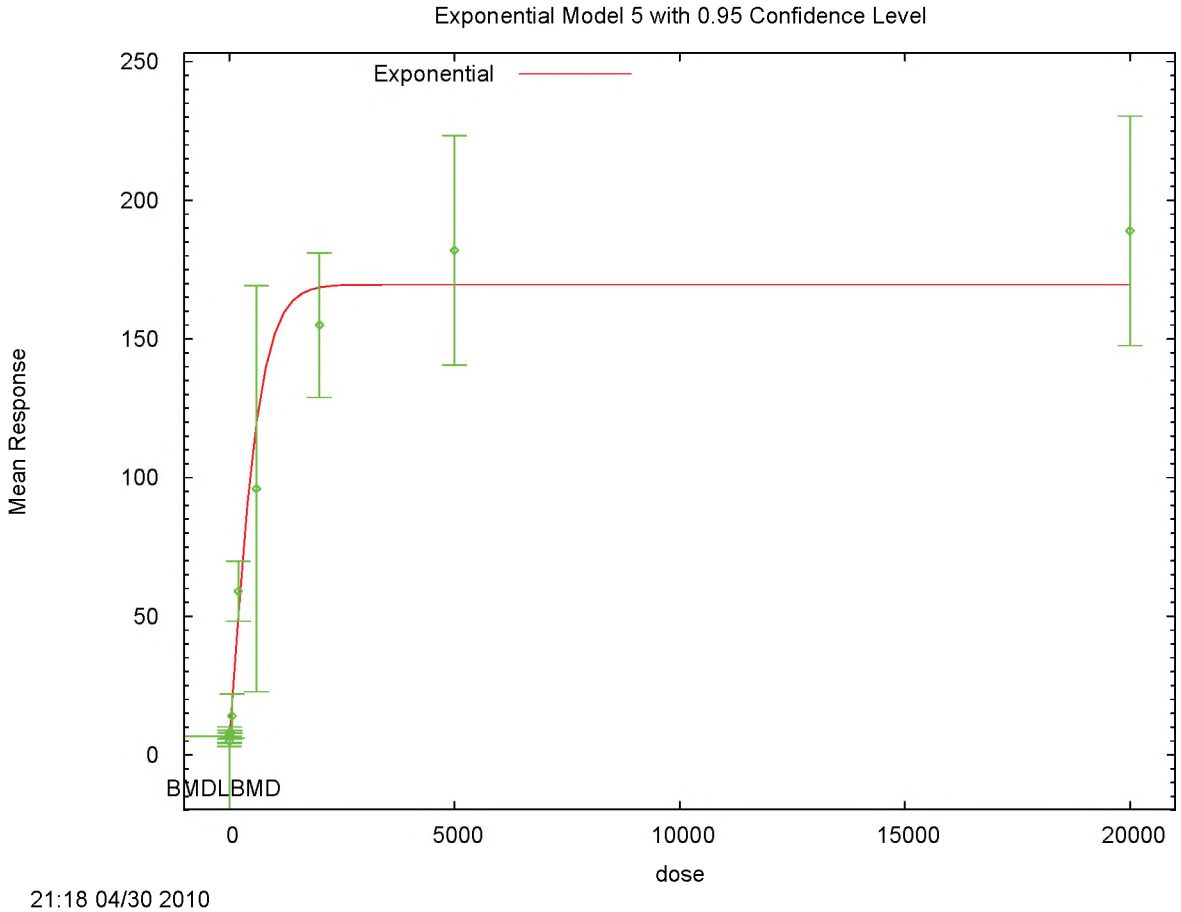
Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD = 10.2235

BMDL = 4.80673

H.3.4.3. *Figure for Selected Model: Exponential (M5)*



H.3.5. National Toxicology Program, 2006: Liver EROD 53 Weeks

H.3.5.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	4	<0.0001	210.749	4.068E+01	2.856E+01	
exponential (M3)	4	<0.0001	210.749	4.068E+01	2.856E+01	power hit bound (d = 1)
exponential (M4)	3	0.071	98.835	1.912E-01	1.384E-01	
exponential (M5)	2	0.040	100.232	2.394E-01	1.433E-01	
Hill [b]	2	0.219	96.847	3.823E-01	2.336E-01	
linear	4	<.0001	203.577	2.076E+01	8.128E+00	
polynomial, 5-degree	4	<.0001	203.577	2.076E+01	8.128E+00	
power	4	<.0001	203.577	2.076E+01	8.128E+00	power bound hit (power = 1)

[a] Non-constant variance model selected (*p* = <.0001)

[b] Best-fitting model, BMDS output presented in this appendix

H.3.5.2. *Output for Selected Model: Hill*

National Toxicology Program, 2006: Liver EROD 53 Weeks

```
 ================================================================
         Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\5\46_NTP_2006_ERODliv53_Hill_1.(d)
         Gnuplot Plotting File:  C:\5\46_NTP_2006_ERODliv53_Hill_1.plt
                                          Sun May 02 15:05:02 2010
 ================================================================

 0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
```

```
                          lalpha =      1.59547
                             rho =            0
                       intercept =        3.614
                               v =       17.599
                               n =      1.38542
                               k =      8.70663


            Asymptotic Correlation Matrix of Parameter Estimates

                  lalpha          rho    intercept            v            n            k

     lalpha            1        -0.96        -0.16        0.086       -0.057        0.041

        rho        -0.96            1         0.14        -0.11        0.059       -0.045

  intercept        -0.16         0.14            1        -0.18         0.13        0.069

          v        0.086        -0.11        -0.18            1        -0.72         0.84

          n       -0.057        0.059         0.13        -0.72            1        -0.79

          k        0.041       -0.045        0.069         0.84        -0.79            1


                                  Parameter Estimates

                                                                 95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha         -4.86522         0.741624           -6.31878            -3.41167
            rho          2.26949         0.287245             1.7065             2.83248
      intercept          3.62909         0.133823             3.3668             3.89138
              v          17.9802         0.989132            16.0416             19.9189
              n           1.4314         0.162447            1.11301             1.74979
              k          5.58259         0.717084            4.17713             6.98805


    Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0       8       3.61         3.63        0.486        0.379         -0.113
 2.14       8       7.27         7.27        0.557        0.833         0.0203
 7.14       8       14.8         14.2         1.61         1.78          0.911
 15.7       8       17.3         18.3         1.59         2.37          -1.19
 32.9       8       20.6         20.3         3.05         2.67          0.304
 71.4       8       21.2         21.2         3.82          2.8         0.0606


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
             Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-59.086537	7	132.173073
A2	-37.515858	12	99.031716
A3	-40.906180	8	97.812359
fitted	-42.423278	6	96.846556
R	-116.710291	2	237.420582

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	158.389	10	<.0001
Test 2	43.1414	5	<.0001
Test 3	6.78064	4	0.1479
Test 4	3.0342	2	0.2193

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

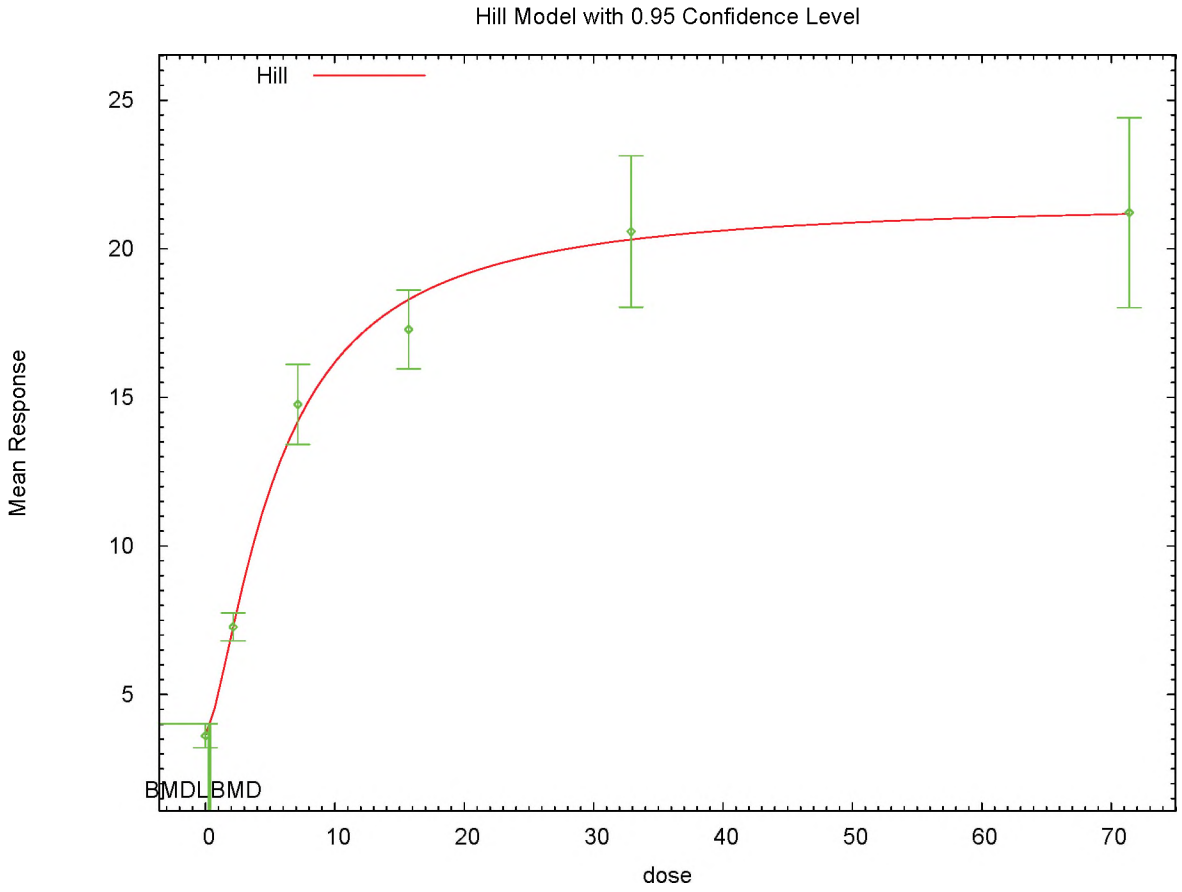
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 0.382287

BMDL = 0.233611

H.3.5.3. *Figure for Selected Model: Hill*



15:05 05/02 2010

H.3.6. National Toxicology Program, 2006: Lung Erod 53 Weeks

H.3.6.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	4	<0.0001	316.324	8.979E+01	5.757E+01	
exponential (M3)	4	<0.0001	316.324	8.979E+01	5.757E+01	power hit bound (d = 1)
exponential (M4) [b]	3	0.421	255.120	8.746E-02	5.370E-02	
exponential (M5)	2	0.276	256.882	6.769E-01	5.491E-02	
Hill	2	0.275	256.882	1.454E+00	1.138E-01	
linear	4	<.0001	315.961	8.550E+01	4.502E+01	
polynomial, 5-degree	4	<.0001	315.961	8.550E+01	4.502E+01	
power	4	<.0001	315.961	8.550E+01	4.502E+01	power bound hit (power = 1)
power, unrestricted [c]	3	0.037	260.794	2.688E-10	2.688E-10	unrestricted (power = 0.129)

[a] Non-constant variance model selected (p = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

[c] Alternate model, BMDS output also presented in this appendix

H.3.6.2. *Output for Selected Model: Exponential (M4)*

National Toxicology Program, 2006: Lung EROD 53 Weeks

```
 ========================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\5\52_NTP_2006_LungEROD53_Exp_1.(d)
         Gnuplot Plotting File:
                                                Fri Apr 30 21:22:36 2010
 ========================================================================

 Tbl 12, Week 53, Lung Microsomes EROD
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

     Note: Y[dose] is the median response for exposure = dose;
           sign = +1 for increasing trend in data;
           sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	-0.80064
rho	1.47683
a	2.86045
b	0.054659
c	16.0581
d	1

Parameter Estimates

Variable	Model 4
lnalpha	-1.15021
rho	1.63127
a	3.06838
b	0.414677
c	13.847
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	8	3.011	1.584
2.14	8	27.15	5.269
7.14	8	42.85	11.15
15.7	8	36.57	12.99
32.9	8	43.75	18.55
71.4	8	43.71	6.322

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	3.068	1.404	-0.1156
2.14	26.26	8.088	0.3116
7.14	40.45	11.5	0.5901
15.7	42.43	11.96	-1.386
32.9	42.49	11.98	0.2972
71.4	42.49	11.98	0.2894

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
  Model A2:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
              Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                    Model      Log(likelihood)      DF         AIC
                    -------    -----------------    ----   ------------
                       A1       -135.2677           7      284.5353
                       A2       -115.6885          12      255.3771
                       A3       -121.1517           8      258.3034
                        R       -162.0902           2      328.1805
                        4       -122.5601           5      255.1202


Additive constant for all log-likelihoods =     -44.11.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                         92.8           10           < 0.0001
  Test 2                        39.16            5           < 0.0001
  Test 3                        10.93            4             0.0274
 Test 6a                        2.817            3             0.4207


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is less than .1.  You may want to
  consider a different variance model.

  The p-value for Test 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000

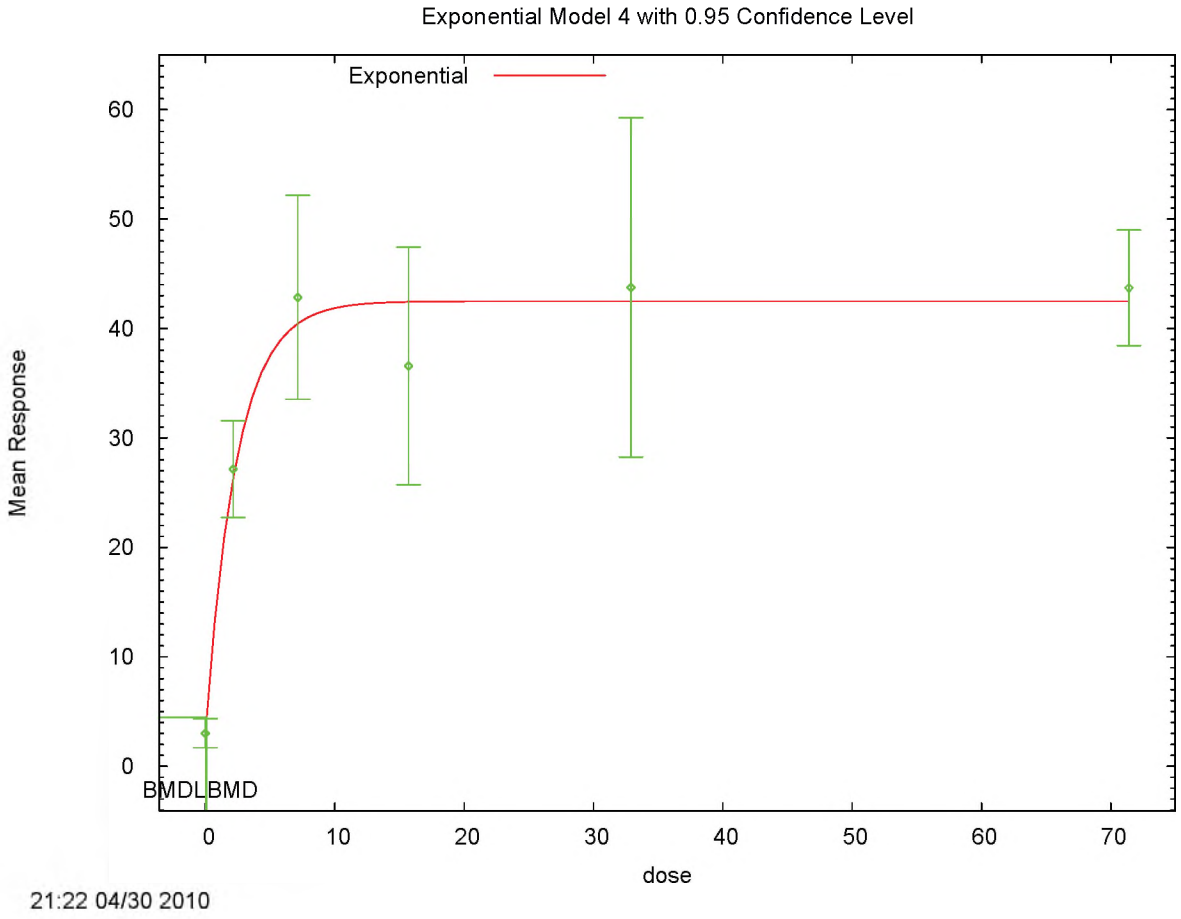
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =    0.0874595

              BMDL =    0.0537035
```

H.3.6.3. *Figure for Selected Model: Exponential (M4)*



H.3.6.4. *Output for Additional Model Presented: Power, Unrestricted*

National Toxicology Program, 2006: Lung EROD 53 Weeks

```
 ========================================================================
         Power Model. (Version: 2.15;  Date: 04/07/2008)
         Input Data File: C:\5\52_NTP_2006_LungEROD53_Pwr_U_1.(d)
         Gnuplot Plotting File:  C:\5\52_NTP_2006_LungEROD53_Pwr_U_1.plt
                                                Fri Apr 30 21:22:40 2010
 ========================================================================

 Tbl 12, Week 53, Lung Microsomes EROD
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Mean
   Independent variable = Dose
   The power is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	4.76968
rho =	0
control =	3.011
slope =	24.7003
power =	0.132996

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.96	-0.48	0.11	-0.048
rho	-0.96	1	0.45	-0.15	0.053
control	-0.48	0.45	1	-0.15	0.05
slope	0.11	-0.15	-0.15	1	-0.92
power	-0.048	0.053	0.05	-0.92	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-1.03242	0.815871	-2.6315	0.566654
rho	1.63031	0.239764	1.16038	2.10024
control	3.01793	0.518146	2.00238	4.03348
slope	25.144	3.39289	18.494	31.7939
power	0.128894	0.0448391	0.041011	0.216777

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	3.01	3.02	1.58	1.47	-0.0133
2.14	8	27.1	30.8	5.27	9.74	-1.05
7.14	8	42.8	35.4	11.2	10.9	1.92
15.7	8	36.6	38.9	13	11.8	-0.553
32.9	8	43.7	42.5	18.5	12.7	0.286
71.4	8	43.7	46.6	6.32	13.7	-0.598

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-135.267662	7	284.535325
A2	-115.688533	12	255.377067
A3	-121.151707	8	258.303413
fitted	-125.397022	5	260.794043
R	-162.090242	2	328.180484

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	92.8034	10	<.0001
Test 2	39.1583	5	<.0001
Test 3	10.9263	4	0.0274
Test 4	8.49063	3	0.03689

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .1. You may want to consider a different variance model

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation

Specified effect = 1

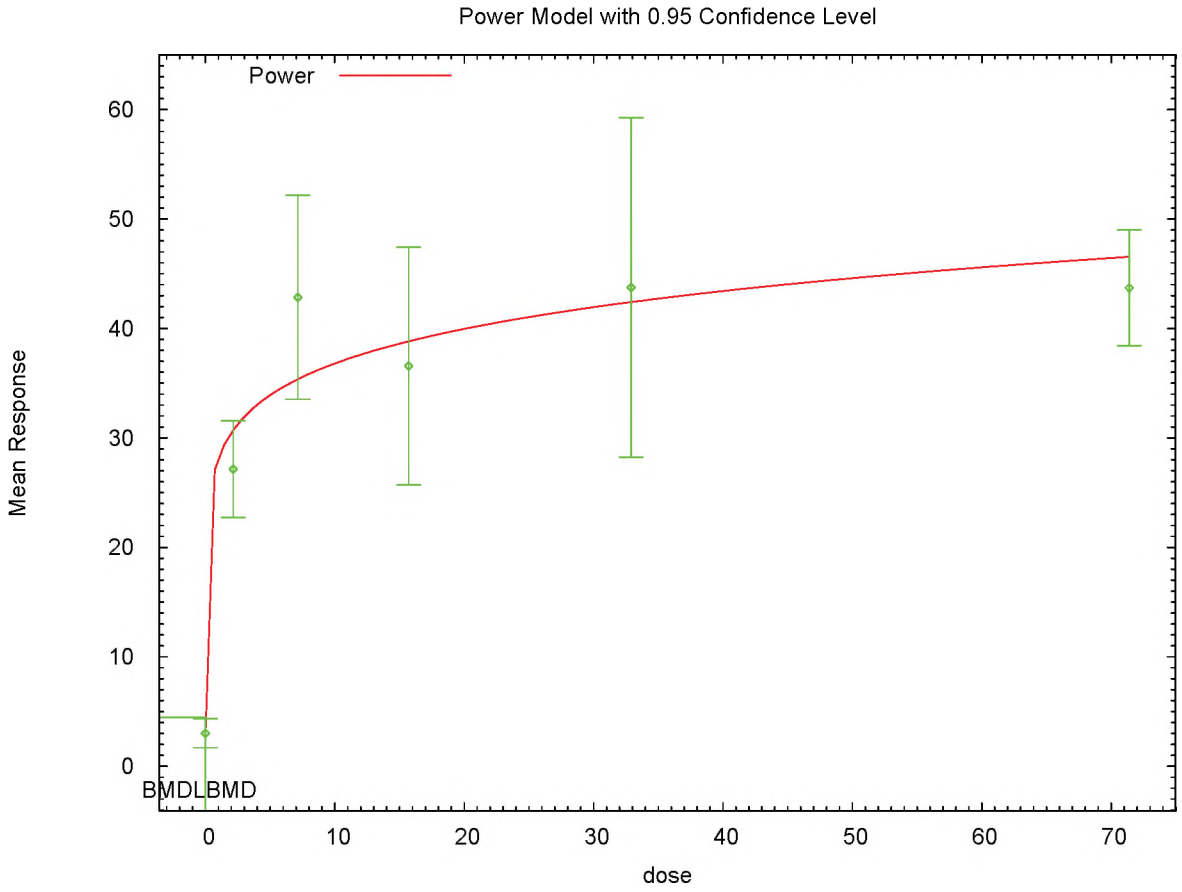
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 2.68823e-010

BMDL = 2.68823e-010

H.3.6.5. *Figure for Additional Model Presented: Power, Unrestricted*



H.3.7. National Toxicology Program, 2006: Labeling Index 31 Weeks

H.3.7.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2) [b]	4	0.000	47.304	2.336E+01	1.867E+01	
exponential (M3)	4	0.000	47.304	2.336E+01	1.867E+01	power hit bound (d = 1)
exponential (M4)	3	<0.0001	53.331	1.233E+01	7.562E+00	
exponential (M5)	2	<0.0001	51.057	3.279E+01	2.055E+01	
Hill	3	0.000	49.057	3.277E+01	error	n upper bound hit (n = 18)
linear	4	<.0001	51.331	1.233E+01	7.563E+00	
polynomial, 5-degree	3	0.000	48.698	2.510E+01	1.192E+01	
power	3	<.0001	49.826	3.238E+01	1.723E+01	

[a] Non-constant variance model selected (*p* = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

H.3.7.2. *Output for Selected Model: Exponential (M2)*

National Toxicology Program, 2006: Labeling Index 31 Weeks

```
 ======================================================================
         Exponential Model. (Version: 1.61;  Date: 7/24/2009)
         Input Data File: C:\5\38_NTP_2006_HepIndex_Exp_1.(d)
         Gnuplot Plotting File:
                                                  Fri Apr 30 21:23:28 2010
 ======================================================================

 Tbl 11, 31wk, Hep Cell Proliferation Labeling Index
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
```

The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2
lnalpha	-0.674004
rho	2.29189
a	0.576363
b	0.0266174
c	0
d	1

Parameter Estimates

Variable	Model 2
lnalpha	-0.471424
rho	1.90298
a	0.616539
b	0.0253715
c	0
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	9	0.327	0.189
2.14	10	0.852	0.6514
7.14	10	0.956	0.7368
15.7	10	0.792	0.4617
32.9	10	1.333	1.123
71.4	10	3.846	3.08

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	0.6165	0.4986	-1.742
2.14	0.6509	0.5251	1.211
7.14	0.739	0.5924	1.158
15.7	0.9182	0.7284	-0.548
32.9	1.421	1.103	-0.2511
71.4	3.773	2.795	0.08251

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

```
  Model  R:        Yij = Mu + e(i)
            Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                    Model      Log(likelihood)      DF         AIC
                    -------    -----------------    ----   ------------
                       A1       -47.23498           7        108.47
                       A2       -8.679256          12       41.35851
                       A3       -8.980651           8        33.9613
                        R       -63.44829           2       130.8966
                        2       -19.65195           4       47.30389


Additive constant for all log-likelihoods =     -54.22.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                               Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A2 vs. A1)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does Model 2 fit the data? (A3 vs. 2)


                           Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                         109.5          10           < 0.0001
  Test 2                         77.11           5           < 0.0001
  Test 3                        0.6028           4             0.9628
  Test 4                         21.34           4          0.0002708


  The p-value for Test 1 is less than .05.  There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is less than .1.  A non-homogeneous
  variance model appears to be appropriate.

  The p-value for Test 3 is greater than .1.  The modeled
  variance appears to be appropriate here.

  The p-value for Test 4 is less than .1.  Model 2 may not adequately
  describe the data; you may want to consider another model.


Benchmark Dose Computations:

  Specified Effect = 1.000000

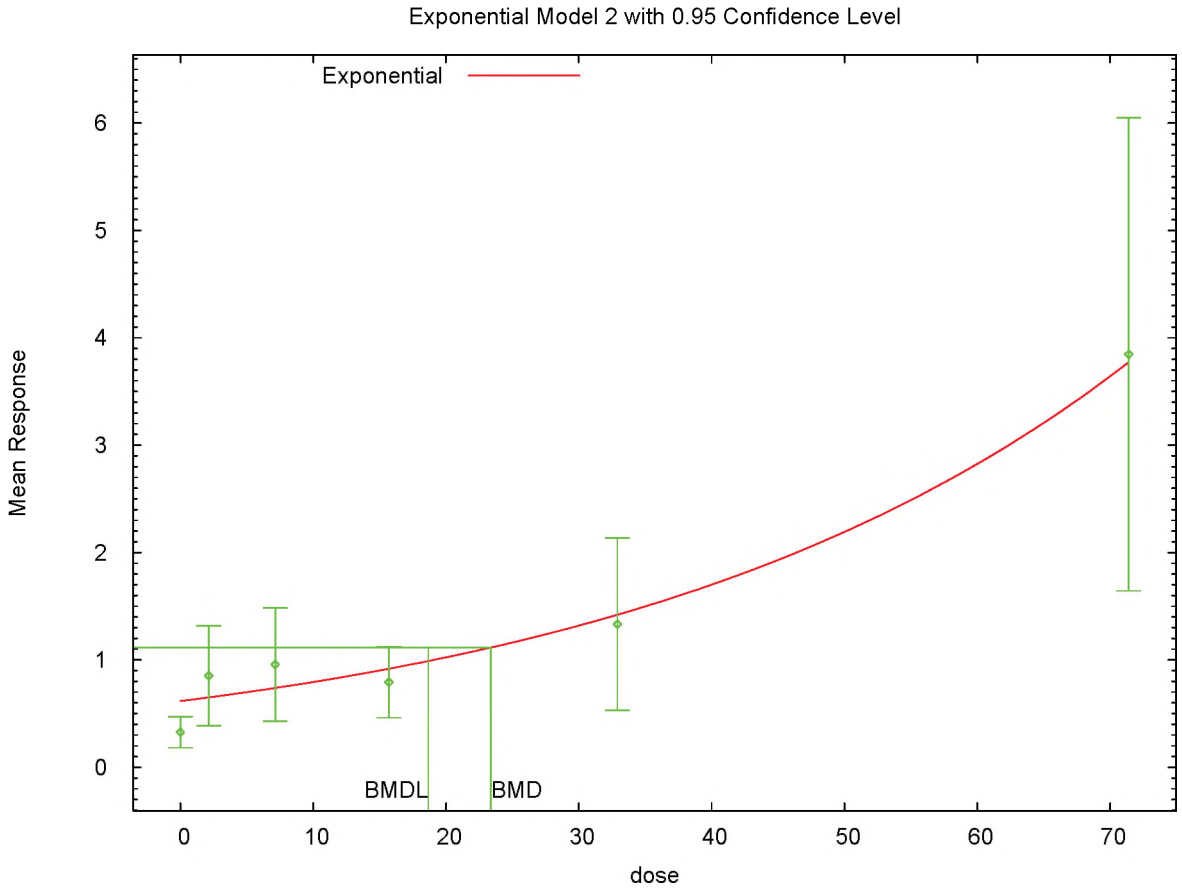
         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      23.3586

              BMDL =      18.6683
```

H.3.7.3. *Figure for Selected Model: Exponential (M2)*



21:23 04/30 2010

H.3.8. Vanden Heuvel et al., 1994: Hepatic CYP1A1 Mrna Expression

H.3.8.1. *Summary Table of BMDS Modeling Results*

Model [a]	Degrees of Freedom	χ^2 *p*-Value	AIC	BMD (ng/kg-d)	BMDL (ng/kg-d)	Notes
exponential (M2)	5	<0.0001	1164.377	4.699E+03	1.729E+03	
exponential (M3)	5	<0.0001	1164.377	4.699E+03	1.729E+03	power hit bound (d = 1)
exponential (M4)	4	<0.0001	661.006	4.550E-01	2.643E-01	
exponential (M5)	3	<0.0001	635.327	1.516E+01	1.046E+01	
Hill [b]	3	<.0001	662.251	8.091E-01	4.844E-01	
linear	5	<.0001	667.554	4.953E-01	3.093E-01	
polynomial, 6-degree	1	<.0001	715.412	5.774E+03	1.204E+01	
power	4	<.0001	669.441	5.571E-01	3.204E-01	

[a] Non-constant variance model selected (*p* = <0.0001)

[b] Best-fitting model, BMDS output presented in this appendix

H.3.8.2. *Output for Selected Model: Hill*

Vanden Heuvel et al., 1994: Hepatic CYP1A1 mRNA Expression

```
================================================================
          Hill Model. (Version: 2.14;  Date: 06/26/2008)
          Input Data File: C:\Usepa\BMDS21\Data\hil_Vanden_mRNA_Setting.(d)
          Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\hil_Vanden_mRNA_Setting.plt
                                                Wed May 19 14:25:06 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = mRNA_mean
   Independent variable = d
   Power parameter is not restricted
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 7
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
```

```
                          lalpha =      18.2064
                             rho =            0
                       intercept =          5.4
                               v =      36694.6
                               n =     0.720907
                               k =      18830.3


            Asymptotic Correlation Matrix of Parameter Estimates

                  lalpha          rho    intercept            v            n            k

     lalpha            1        -0.89        -0.41         0.37          0.7         -0.2

        rho        -0.89            1         0.29        -0.54        -0.75         0.24

  intercept        -0.41         0.29            1        -0.11         0.13       -0.034

          v         0.37        -0.54        -0.11            1         0.21         0.57

          n          0.7        -0.75         0.13         0.21            1        -0.53

          k         -0.2         0.24       -0.034         0.57        -0.53            1


                                  Parameter Estimates

                                                                 95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha         -0.28219         0.733221           -1.71928              1.1549
            rho          2.05171         0.146654            1.76427             2.33915
      intercept           5.4299          1.14997            3.17599             7.68381
              v          36598.9          13930.2            9296.23             63901.7
              n          1.13992        0.0919476           0.959705             1.32013
              k          2012.71           881.73            284.554             3740.87


      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    13        5.4          5.43         3.61         4.93        -0.0219
  0.1     5        7.2          5.88         5.59         5.35           0.55
    1    12       14.8          11.7         14.9         10.8          0.991
   10     7       12.8          91.8          4.5         89.6          -2.33
  100     7        536     1.16e+003          320     1.21e+003          -1.37
 1000    11   1.8e+004     1.14e+004     1.52e+004     1.26e+004           1.75
1e+004     5  3.67e+004     3.15e+004     2.21e+004     3.58e+004          0.323


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
      Model A3 uses any fixed variance parameters that
      were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-572.470944	8	1160.941889
A2	-290.799287	14	609.598575
A3	-293.809342	9	605.618684
fitted	-325.125462	6	662.250924
R	-603.663396	2	1211.326792

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	625.728	12	<.0001
Test 2	563.343	6	<.0001
Test 3	6.02011	5	0.3043
Test 4	62.6322	3	<.0001

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different
model

Benchmark Dose Computation

Specified effect = 1

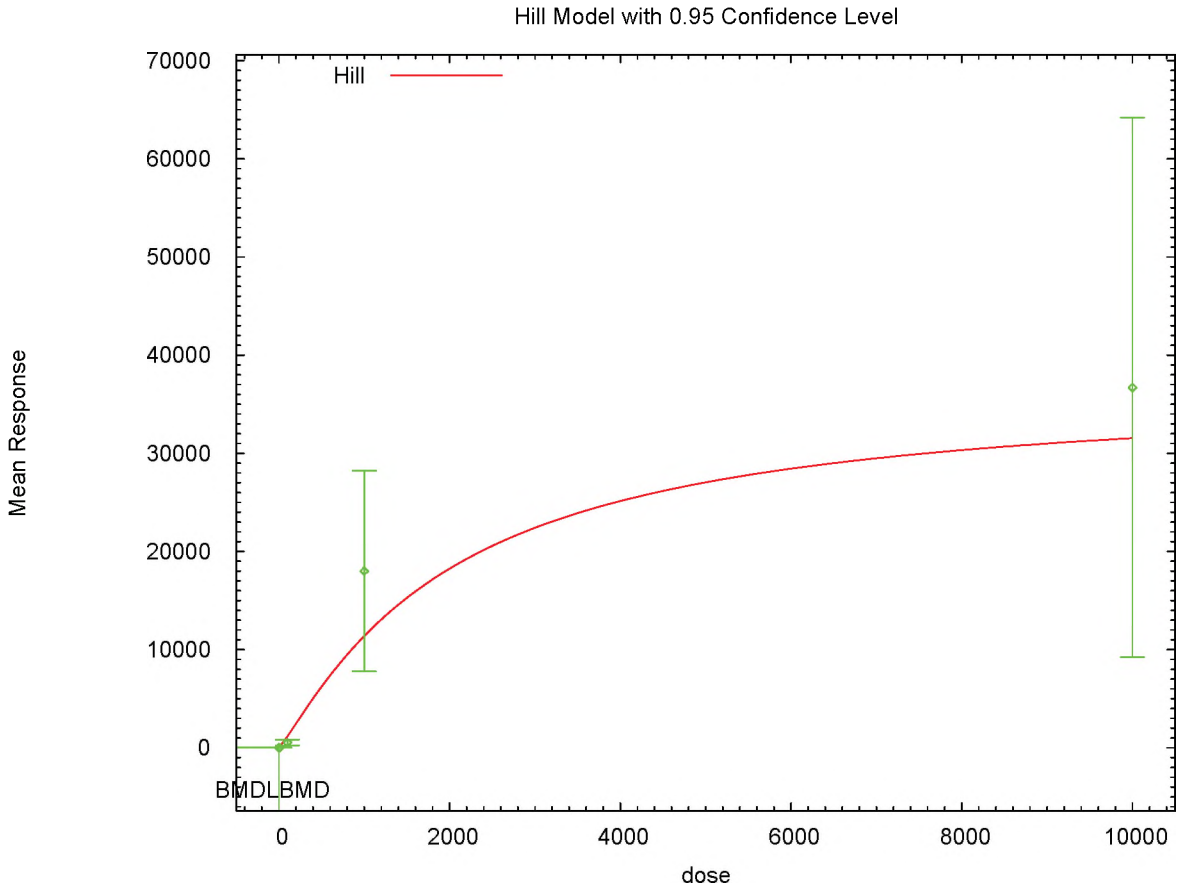
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 0.809125

BMDL = 0.484455

H.3.8.3. *Figure for Selected Model: Exponential (M5)*



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APPENDIX I

Effect of Background Exposure on Benchmark-Dose Modeling

NOTICE

THIS DOCUMENT IS AN EXTERNAL REVIEW DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

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APPENDIX I. EFFECT OF BACKGROUND EXPOSURE ON BENCHMARK-DOSE MODELING

I.1. NTP, 2006 (CHOLANGIOCARCINOMAS): UNADJUSTED BLOOD CONCENTRATIONS

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Usepa\BMDS21\Data\msc_NTP_2006_carcin_Setting.(d)
         Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\msc_NTP_2006_carcin_Setting.plt
                                              Wed Apr 14 12:59:57 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = cholang
   Independent variable = bl_nom

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 2.44609e-005


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(3)

   Beta(3)           1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	2.30992e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-55.408	6			
Fitted model	-55.7584	1	0.700706	5	0.9829
Reduced model	-96.9934	1	83.1708	5	<.0001

AIC: 113.517

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	49	0.000
2.5600	0.0004	0.019	0.000	48	-0.136
5.6900	0.0042	0.195	0.000	46	-0.443
9.7900	0.0214	1.072	1.000	50	-0.070
16.6000	0.1003	4.913	4.000	49	-0.434
29.7000	0.4540	24.063	25.000	53	0.259

Chi^2 = 0.48 d.f. = 5 P-value = 0.9930

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.57754

BMDL = 4.13907

BMDU = 8.42931

Taken together, (4.13907, 8.42931) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.002416

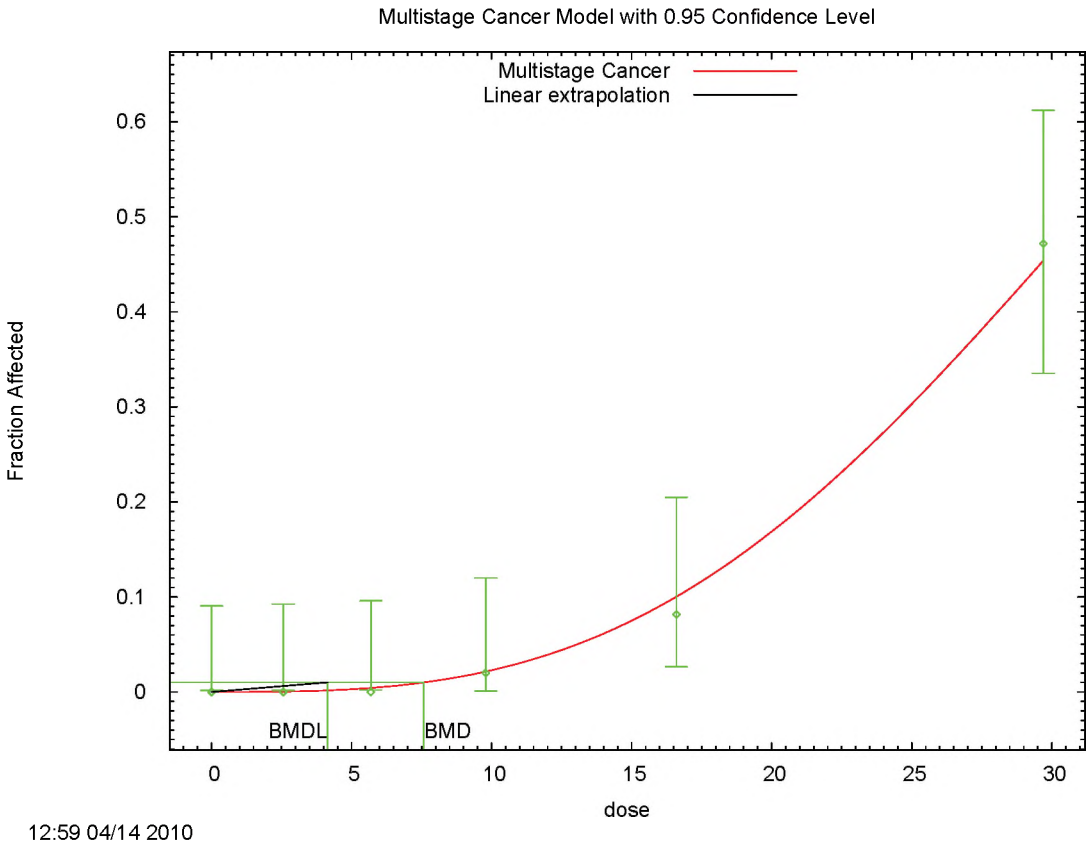


Figure I-1. NTP, 2006: Unadjusted blood concentrations (cholangiocarcinomas).

I.2. NTP, 2006 (CHOLANGIOCARCINOMAS): BACKGROUND DOSE = MEASURED TCDD CONCENTRATION ONLY

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Usepa\BMDS21\Data\msc_NTP_2006_carcin_Setting.(d)
         Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\msc_NTP_2006_carcin_Setting.plt
                                              Fri Apr 16 15:47:08 2010
 ================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = cholang
   Independent variable = bl_TCDDadj

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 2.43074e-005


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(3)

   Beta(3)            1



                                Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	2.29144e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-55.408	6			
Fitted model	-55.771	1	0.726	5	0.9815
Reduced model	-96.9934	1	83.1708	5	<.0001

AIC: 113.542

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0640	0.0000	0.000	0.000	49	-0.001
2.6240	0.0004	0.020	0.000	48	-0.141
5.7540	0.0044	0.200	0.000	46	-0.449
9.8540	0.0217	1.084	1.000	50	-0.082
16.6640	0.1006	4.930	4.000	49	-0.442
29.7640	0.4535	24.035	25.000	53	0.266

Chi^2 = 0.49 d.f. = 5 P-value = 0.9924

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.59785

BMDL = 4.19355

BMDU = 8.45188

Taken together, (4.19355, 8.45188) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00238461

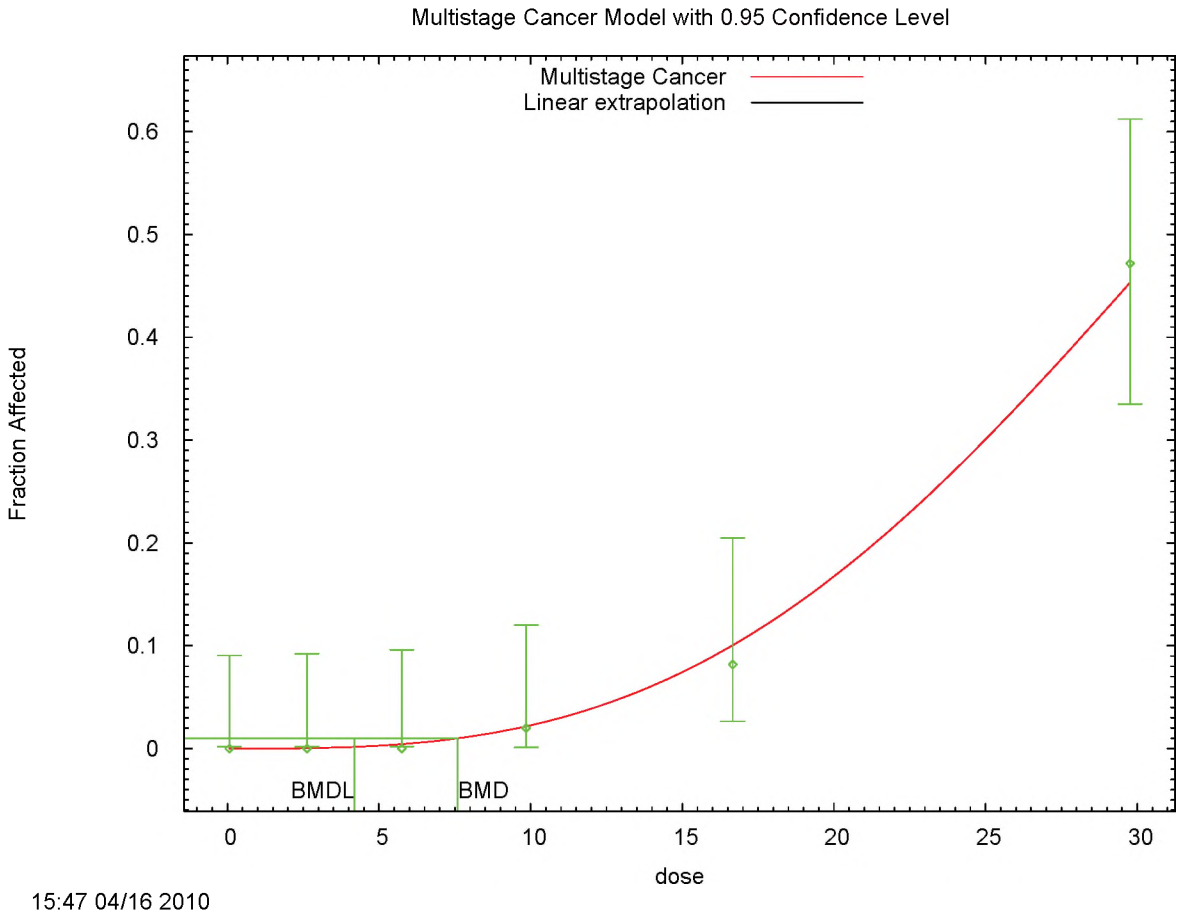


Figure I-2. NTP, 2006 (cholangiocarcinomas): Background dose = measured TCDD concentration only.

I.3. NTP, 2006 (CHOLANGIOCARCINOMAS): BACKGROUND DOSE = MEASURED TEQ CONCENTRATION (TCDD, PECDF, AND PCB-126)

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Usepa\BMDS21\Data\msc_NTP_2006_carcin_Setting.(d)
         Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\msc_NTP_2006_carcin_Setting.plt
                                              Fri Apr 16 15:50:00 2010
 ================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = cholang
   Independent variable = bl_TEQadj

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 2.40088e-005


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(3)

   Beta(3)            1



                                Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	2.25556e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-55.408	6			
Fitted model	-55.7969	1	0.777718	5	0.9784
Reduced model	-96.9934	1	83.1708	5	<.0001

AIC: 113.594

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.1900	0.0000	0.000	0.000	49	-0.003
2.7500	0.0005	0.023	0.000	48	-0.150
5.8800	0.0046	0.210	0.000	46	-0.460
9.9800	0.0222	1.109	1.000	50	-0.104
16.7900	0.1013	4.962	4.000	49	-0.455
29.8900	0.4525	23.981	25.000	53	0.281

Chi^2 = 0.53 d.f. = 5 P-value = 0.9909

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.63793

BMDL = 4.29872

BMDU = 8.4964

Taken together, (4.29872, 8.4964) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00232627

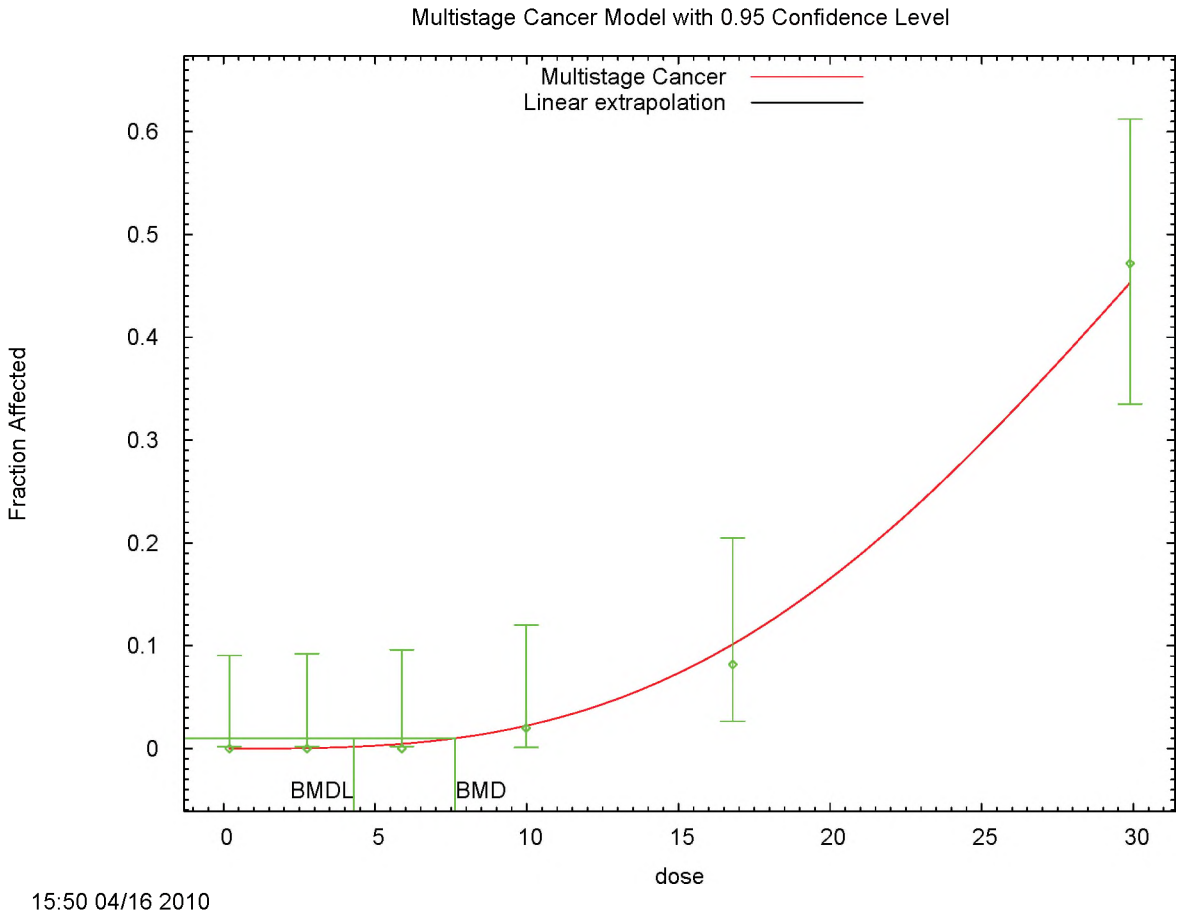


Figure I-3. NTP, 2006 (cholangiocarcinomas): Background dose = measured TEQ concentration (TCDD, PeCDF, and PCB-126).

I.4. NTP, 2006 (CHOLANGIOCARCINOMAS): BACKGROUND DOSE = 2× MEASURED TEQ CONCENTRATION (TCDD, PECDF, AND PCB-126)

```
 ================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Usepa\BMDS21\Data\msc_NTP_2006_carcin_Setting.(d)
         Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\msc_NTP_2006_carcin_Setting.plt
                                              Fri Apr 16 15:51:30 2010
 ================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = cholang
   Independent variable = bl_TEQ2x

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) =  2.3568e-005


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(3)

   Beta(3)            1



                                Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	2.20268e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-55.408	6			
Fitted model	-55.8382	1	0.860456	5	0.973
Reduced model	-96.9934	1	83.1708	5	<.0001

AIC: 113.676

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.3800	0.0000	0.000	0.000	49	-0.008
2.9400	0.0006	0.027	0.000	48	-0.164
6.0700	0.0049	0.226	0.000	46	-0.477
10.1700	0.0229	1.145	1.000	50	-0.137
16.9800	0.1022	5.009	4.000	49	-0.476
30.0800	0.4509	23.898	25.000	53	0.304

Chi^2 = 0.59 d.f. = 5 P-value = 0.9884

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.69856

BMDL = 4.45212

BMDU = 8.56376

Taken together, (4.45212, 8.56376) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00224612

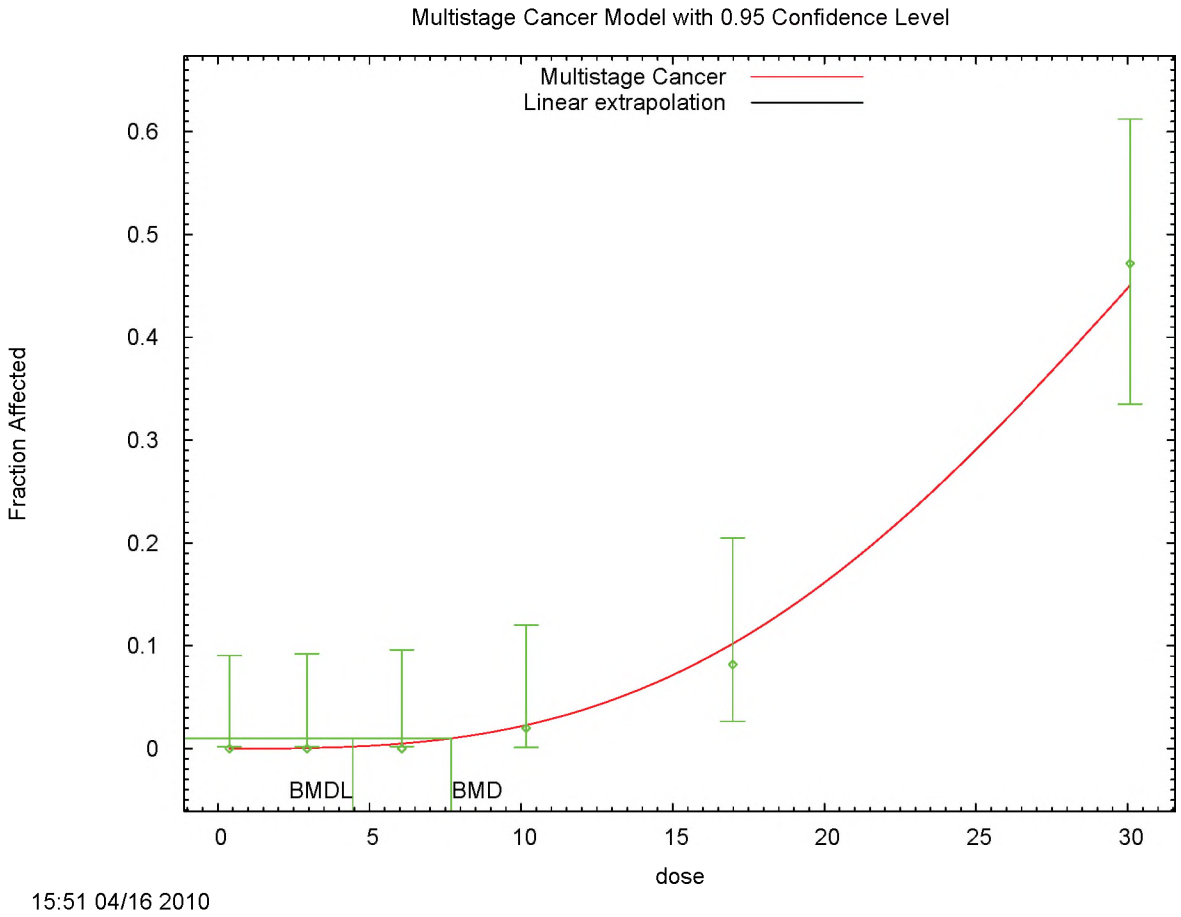


Figure I-4. NTP, 2006 (cholangiocarcinomas): Background dose = 2× measured TEQ concentration (TCDD, PeCDF, and PCB-126).

I.5. NTP, 2006 (CHOLANGIOCARCINOMAS): BACKGROUND DOSE = 10× MEASURED TCDD CONCENTRATION

```
 ================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\Usepa\BMDS21\Data\msc_NTP_2006_carcin_Setting.(d)
        Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\msc_NTP_2006_carcin_Setting.plt
                                            Fri Apr 16 15:55:37 2010
 ================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = cholang
   Independent variable = bl_TEQmax

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 2.29823e-005


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(3)

   Beta(3)            1



                                Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	2.13264e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-55.408	6			
Fitted model	-55.8994	1	0.982747	5	0.9639
Reduced model	-96.9934	1	83.1708	5	<.0001

AIC: 113.799

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.6400	0.0000	0.000	0.000	49	-0.017
3.2000	0.0007	0.034	0.000	48	-0.183
6.3300	0.0054	0.248	0.000	46	-0.499
10.4300	0.0239	1.195	1.000	50	-0.181
17.2400	0.1035	5.072	4.000	49	-0.503
30.3400	0.4488	23.785	25.000	53	0.336

Chi^2 = 0.68 d.f. = 5 P-value = 0.9840

Benchmark Dose Computation

Specified effect = 0.01

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.78193

BMDL = 4.65224

BMDU = 8.65638

Taken together, (4.65224, 8.65638) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0021495

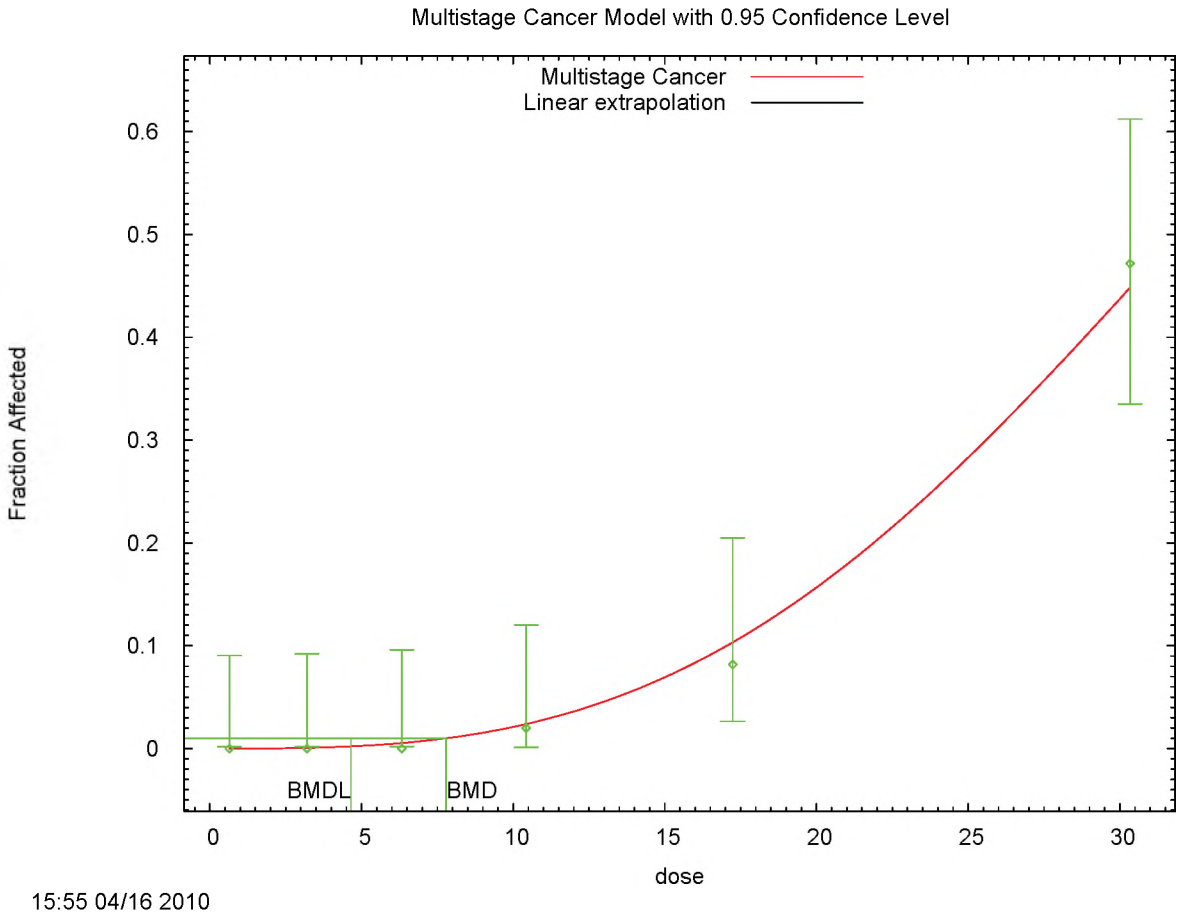


Figure I-5. NTP, 2006 (cholangiocarcinomas): Background dose = 10× measured TCDD concentration.

I.6. REFERENCE

NTP (National Toxicology Program). (2006a) NTP technical report on the toxicology and carcinogenesis studies of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (CAS No. 1746-01-6) in female Harlan Sprague-Dawley rats (Gavage Studies). Natl Toxicol ProgramTech Rep 521. Public Health Service, National Institute of Health, U.S. Department of Health and Human Services, Research Triangle Park, NC.