PROPOSAL FOR PHARMACOKINETICS STUDY OF 1,1,2-TRICHLOROETHANE

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1.0 INTRODUCTION

On June 26, 1996, the USEPA proposed a test rule under Section 4(a) of the Toxic Substances Control Act (TSCA) which would require manufacturers and processors of 21 hazardous air pollutants, including 1,1,2-trichloroethane, to test these compounds for specific health effects. The tests proposed to be conducted for 1,1,2-trichloroethane via inhalation exposures include the following:

- acute toxicity,
- subchronic toxicity,
- developmental toxicity,
- reproductive toxicity,
- neurotoxicity,
- carcinogenicity,
- *in vivo* cytogenicity, and
- immunotoxicity.

The cost for conducting these tests was estimated by the USEPA to be approximately $3.8 million. As an alternative to conducting these tests, the USEPA is soliciting proposals regarding the use of pharmacokinetic studies which would permit the extrapolation of toxicity information from other routes of exposure (*i.e.*, oral) to predict risks from inhalation exposures. This document serves as

a proposal to conduct pharmacokinetic studies for 1,1,2-trichloroethane as a means of filling specific data gaps and as a means of offering additional evidence for other data gaps.

Prior to extrapolating information from one route to another, an evaluation of available information must be made with respect to its adequacy for route-to-route extrapolation. A decision tree for conducting route-to-route extrapolations has been proposed (Gerrity and Henry, 1990), and is provided in Figure 1. Following an evaluation of the information available, there are four possible options that can be pursued:

Option 1: Collect data (all routes)

Option 2: Use data for a structurally analogous compound

Option 3: Collect data for relevant routes (inhalation)

Option 4: Route-to-route extrapolation

Route-to-route extrapolation may be conducted using various levels of complexity ranging from the use of default absorption values to physiologically based pharmacokinetic (PBPK) modeling. The approach proposed here centers on the use of validated PBPK models.

Three general requirements are necessary to conduct proper route to route extrapolations using PBPK models:

1. A scientifically plausible (defensible) mechanism of toxicological action is needed

2. Studies must be available that are considered adequate to assess relevant toxicological endpoints via a route(s) of exposure other than that of interest.

3. A validated PBPK model for the chemical and species of interest must be available which is capable of predicting the pertinent internal dose measures based on mechanism of action.

When these criteria exist for a compound that also has no toxicological effects on the portal of entry, a route-to-route extrapolation can be performed as follows:

1. The internal dose metric is determined under the experimental conditions for the studies to be extrapolated (*i.e.*, oral) for the NOAEL and/or LOAEL doses.

2. Equivalent NOAEL and/or LOAEL concentrations for route extrapolated to (*i.e.* inhalation) are determined by estimating an exposure that produces the same internal dose metric as determined in Step 1 above.

For 1,1,2-trichloroethane, an evaluation was made of primary and secondary toxicological literature to determine the adequacy of the dose-response data for the effects listed above following inhalation, oral, and other relevant exposures. Limited information from inhalation studies for 1,1,2-trichloroethane in animals suggest that effects remote from the respiratory tract (*i.e.*, the central nervous system, liver) may be of potential concern. However, adequate information for inhalation exposures were not located for any of the effect categories listed above. For oral exposures, potential

candidate studies were located for route-to-route extrapolation (Option 4) for subchronic toxicity, neurotoxicity, carcinogenicity, and immunotoxicity. No adequate inhalation or oral studies were identified for acute toxicity, developmental toxicity, reproductive toxicity, nor *in vivo* cytogenicity. However, as will be presented in later sections, it is proposed that only limited (if any) inhalation studies will be needed to evaluate acute toxicity (Sections 3.1 and 3.2), *in vivo* cytogenicity studies will not be required (Section 3.7), and weight of evidence arguments, which include a summary of data for structurally analogous compounds (Option 2), will be presented indicating that developmental and reproductive (Section 3.4) studies are also not necessary for 1,1,2-trichloroethane.

The remainder of this proposal will address the status of each of these requirements with regard to 1,1,2-trichloroethane, and will describe the specific approaches we propose to use to fill the data gaps identified in the test rule (both PBPK modeling and otherwise).

2.0 MECHANISM OF ACTION

1,1,2-Trichloroethane is metabolized to reactive intermediates via two potential pathways. Along one pathway free radicals and acyl chlorides (reactive metabolites) are generated from metabolism of 1,1,2-trichloroethane by cytochrome P-450. 1,1,2-Trichloroethane and its metabolites can also react with glutathione which may possibly give rise to a glutathione episulfonium ion, a reactive intermediate which has been implicated in the toxicity of chemicals like 1,2-dichloroethane (NTP, 1991). The reactive metabolites generated by both pathways are capable of binding to cellular macromolecules. Following exposure of rats and mice to a single dose of radiolabeled 1,1,2-trichloroethane, the radiolabel was covalently bound to DNA, RNA, and protein of the liver, kidney, lung, and stomach (Mazzulo *et al.* 1986). Binding to DNA was greatest in the liver, and was greater in mice than rats, correlating with the carcinogenic potency of 1,1,2-trichloroethane (NCI, 1978). *In vitro* studies demonstrate that binding of the radiolabel is decreased by the addition of glutathione, suggesting that cytochrome P-450-mediated metabolism is the primary activating pathway for metabolites to bind to these macromolecules (Mazzulo *et al.* 1986).

Binding to key cellular molecules following metabolic activation is offered as a potential mechanism of action for the subchronic toxicity and carcinogenic effects of 1,1,2-trichloroethane on the liver. The rapid onset of the anesthetic effects of chlorinated solvents in general precludes the involvement of a metabolite in the mechanism of action (Cassaret and Doull, 1996), and suggests that the neurological effects of 1,1,2-trichloroethane are associated with the parent compound. Information regarding the potential mechanism(s) of action for 1,1,2-trichloroethane-induced immunotoxicity

were not located, but we propose to use parent chemical present in the lymphoid tissue as an appropriate internal dose metric (see Section 3.9).

3.0 EVALUATION OF CANDIDATE STUDIES FOR ROUTE-TO-ROUTE EXTRAPOLATION

3.1 Potential for Direct Contact Effects

An important factor to consider prior to conducting a route-to-route extrapolation is the potential for direct contact (*i.e.* portal of entry) effects on the lung following inhalation exposures (see Figure 1). In general, the USEPA has classified inhaled chemicals into one of three categories based on water solubility and reactivity (USEPA, 1994):

Category 1: Do not penetrate to blood (*i.e.*, highly water soluble/very reactive)

Category 2: Water soluble/blood accumulation

Category 3: Water insoluble/perfusion limited

Portal of entry effects do not appear to be of primary concern for 1,1,2-trichloroethane based on the following rationale:

- Macroscopic evaluation of lungs revealed no effects in rats acutely exposed to concentrations exceeding 1,000 ppm (Bonnet *et al.* 1980).

- Preferential accumulation of 1,1,2-trichloroethane in the lungs following inhalation exposures does not appear to occur. Rather, 1,1,2-trichloroethane rapidly distributes to other tissues following exposure. For example, following inhalation exposure of

mice to 1,000 ppm 1,1,2-trichloroethane for 1 hour, the levels of 1,1,2-trichloroethane in the lungs (20-35 µg/g) were less than those observed in the blood and brain (45-60 µg/g), kidney and liver (80 µg/g), and adipose tissue (600 µg/g) in mice (Takahara 1986).

- Although evidence of lung damage (hemorrhagic, reddened areas) were observed following a single gavage dose above the LD50 (500 - 600 mg/kg-day) in mice (White *et al.* 1985), this effect was not observed in mice exposed to lower doses, or in mice exposed to up to 38 mg/kg for 14 days, or in mice exposed to up to 384 mg/kg-day 1,1,2-trichloroethane in the drinking water (White *et al.* 1985). Furthermore, histopathological changes of respiratory tract tissues were not observed in mice exposed to up to 390 mg/kg-day 1,1,2-trichloroethane via gavage for 78 weeks (NCI, 1978).

- One of the metabolically activating pathways for 1,1,2-trichloroethane in the liver (*i.e.*, reductive dechlorination to a free radical) is not applicable for the lungs. Reductive dechlorination of chlorinated compounds by cytochrome P-450 requires conditions of low oxygen content, a condition that is not likely to be found in the lung.

Based on the low reactivity of 1,1,2-trichloroethane in the lung and the fact that 1,1,2-trichloroethane has low water solubility (see saline:air partition coefficient, Gargas *et al.*, 1989b), this compound

should be considered a Category 3 compound (USEPA, 1994) in which direct effects on the portal of entry are not expected.

3.2 Acute Toxicity

As described in the introduction, no adequate inhalation or oral studies were identified that assessed acute toxicity. Since 1,1,2-trichloroethane is a Category 3 compound and based on the data presented in Section 3.1, it would seem unlikely that 1,1,2-trichloroethane will have direct toxicological effects on the lung (portal of entry). However, it is proposed that appropriate acute toxicity data (including for the lung) be collected during both the oral and inhalation studies needed to support PBPK model development and validation as described in Section 4.0. It is further proposed that evaluation of acute toxicity be conducted as per OPPTS Guidelines for acute studies, including the lung (*i.e.* gross necropsy and histopathology, [if deemed necessary]) as part of the studies described in Section 4.0.

3.3 Subchronic Toxicity

Three studies were identified as potential candidates for route-to-route extrapolation, one study by White *et al.* (1985) and two (rats and mice) by NCI (1978). These studies are summarized briefly below.

- *White et al. (1985)* - Groups of 32-48 male and 32-48 female CD-1 mice were exposed to doses of 0, 4.4, 46, or 305 mg/kg-day (males), or 0, 3.9, 44, or 384 (females) 1,1,2-trichloroethane in the drinking water for 90 days. Drinking water consumption and body weight gain were reduced in concentration-dependent manner in male mice, but not in female mice. Absolute liver and kidney weights were decreased in male mice receiving 46 or 305 mg/kg-day; however, this was not observed when organ weights were expressed relative to body weight. Liver weights were significantly increased (absolutely and relatively) in female mice exposed to the highest dose. Absolute spleen and kidney weights were also elevated in this group. Hematological changes in male mice did not appear to be significant. However, hemoglobin and hematocrit values were significantly decreased in female mice exposed to the highest dose. Fibrinogen was increased in all exposed females, however this was not in a dose-dependent manner. Prothrombin time was significantly decreased in a dose-dependent manner in female mice exposed to 44 or 384 mg/kg-day. In male mice, serum cholesterol and serum alkaline phosphatase activity was significantly increased at the highest dose. In female mice, serum cholesterol and SGPT activity was significantly increased at the highest dose. Liver glutathione was significantly decreased in a dose-dependent manner in males exposed to the two highest doses, but was increased in females exposed to the highest dose. The dose-response information from female mice were used to base a LOAEL and NOAEL since they present a more consistent pattern of toxicity. This study identifies a LOAEL of 384 and a NOAEL of 44 mg/kg-day for increased liver weight, serum

cholesterol, and SGPT activity, and decreased (10%) hemoglobin and hematocrit in female mice.

- *NCI (1978)* - Groups of 50 male and 50 female rats (Osborne-Mendel) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 30, and 70 mg/kg-day were raised to 0, 50, and 100 mg/kg-day after week 8 since animals seemed to tolerate exposure. Time-weighted average doses of 0, 46, and 92 mg/kg-day were calculated by the study authors. There was no appreciable difference in mortality or body weight gain from controls. However, high mortality was noted in the vehicle control group. During the first 6 months, the incidence of clinical signs (*i.e.*, appearance and behavior changes) were comparable between exposed and control animals. However, after 6 months, exposed animals exhibited a higher frequency of hunched appearance, rough fur, urine stains, wheezing, dyspnea, and squinted eye (sometimes with reddish exudate). Histopathological examinations revealed no significant differences between exposed and control animals for noncancer effects in any tissue site (including the respiratory tract). This study identifies a NOAEL of 92 mg/kg/day for histopathological changes.

- *NCI (1978)* - Groups of 50 male and 50 female mice (B6C3F1) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study.

Initial doses of 0, 150, and 300 mg/kg-day were raised to 0, 200, and 400 mg/kg-day after week 20 since the animals seemed to tolerate exposure. Time-weighted average doses of 0, 195, and 390 mg/kg-day were calculated by the study authors. Mortality was significantly increased in exposed female mice, although this may not have been dose-related (mortality was greater in the low dose group compared to the high dose group). In any event, the number of animals surviving the entire exposure exceeded the requirement of the OPPTS guideline for number of animals/dose group (10/sex/group). For males, there was no appreciable difference in mortality from controls. Body weight gain was not affected in either sex. Clinical signs (*i.e.*, appearance and behavior changes) were observed at comparable rates in exposed and control groups. Histopathological examinations revealed no significant differences between exposed and control animals for noncancer effects in any tissue site (including the respiratory tract). This study identifies a NOAEL of 390 mg/kg-day for noncancer histopathological effects in all tissues examined.

These two studies are compared with toxicity test guidelines from the USEPA (OPPTS) in Table 1. Based on this comparison, the study by White *et al.* (1985) appears to be adequate, however this study lacks a histopathological examination of tissues. For this reason, the liver histopathology results of the NCI (1978) study are used to supplement this study. As discussed in Section 3.1, direct contact (portal of entry) effects do not appear to be of concern for subchronic exposures to 1,1,2-trichloroethane.

Based upon the mechanism of action proposed in Section 2.0, it appears that cytochrome P-450 metabolites of 1,1,2-trichloroethane are likely to be involved in the hepatic effects described above for both studies. Therefore, it is proposed that some internal measure of these metabolites (*i.e.*, total amount metabolized in a 24 hour period) be modeled as the appropriate internal dose measure. Final choice of the internal dose measure will be decided after the internal dose/response relationship is evaluated.

3.4 Developmental and Reproductive Toxicity

The USEPA identified developmental and reproductive toxicity as a data gap for 1,1,2-trichloroethane, based on the availability of only a single study (Seidenberg et al. 1986). This study is summarized below.

- *Seidenberg et al. (1986)* - Groups of 30 ICR/SIM mice were exposed to 0 (vehicle control) or 350 mg/kg-day 1,1,2-trichloroethane on days 8 through 12 of gestation via corn oil gavage. Significant toxicity (3 deaths) were observed in exposed animals. However, no effects were observed on pup viability, pup weight, litter size, or terata. The dose level of 350 mg/kg-day serves as a LOAEL for maternal toxicity and a NOAEL for developmental effects.

This study is compared with USEPA (OPPTS) guidelines for developmental toxicity tests in Table 2. Although there are limitations to this study (single dose tested, short exposure duration), the study

demonstrates a lack of developmental effects for 1,1,2-trichloroethane at a dose approximate to the maximum tolerable dose. For this reason, little information would be obtained from additional study since the testing of higher doses would only result in greater maternal toxicity, while the testing of lower doses would only result in the identification of lower NOAELs for developmental effects.

A weight-of-evidence argument is presented below for the developmental and reproductive effects of 1,1,2-trichloroethane. This argument includes issues regarding the toxicity, potential for exposure, and current regulations for 1,1,2-trichloroethane.

Toxicity

Although there is very little information regarding the potential for developmental effects of 1,1,2-trichloroethane, data are available which suggest that developmental effects are not of primary concern for chlorinated ethanes in general. A summary of the available information regarding the developmental effects of chlorinated ethanes is provided in the table below (1,1,2-TCA = 1,1,2-trichloroethane; 1,1-DCA = 1,1-dichloroethane; 1,1,1-TCA = 1,1,1-trichloroethane; 1,2-DCA = 1,2-dichloroethane).

Summary of Developmental Toxicity Data for Chlorinated Ethanes

Study	Species	Route	Duration	Effect	LOAEL	NOAEL	Chemical
Seidenberg et al. 1986	Mouse	Oral	Gd 8-12	None	None	350 mg/kg-day	1,1,2-TCA

Study	Species	Route	Duration	Effect	LOAEL	NOAEL	Chemical
Schwetz et al. 1974	Rat	Inhalation	Gd 6-15	Skeletal anomalies	6000 ppm	3800 ppm	1,1-DCA
BRRC 1987	Rat, rabbit	Inhalation	Gd 6-15	Decreased fetal weight, delayed ossification, extra rib	6000 ppm	3000 ppm	1,1,1-TCA
Schwetz et al. 1975	Rat, mouse	Inhalation	Gd 6-15	None	None	875 ppm	1,1,1-TCA
York et al. 1982	Rat	Inhalation	2 wk premating; Gd 1-20	Delayed ossification, reduced clavicle	2100 ppm	None	1,1,1-TCA
NTP 1988	Rat	Oral	70 d	None	None	3.5 mg/kg-day	1,1,1-TCA
Lane et al. 1982	Mouse	Oral	Multigeneration	None	None	1000 mg/kg-day	1,1,1-TCA
Maurissen et al. 1994	Rat	Oral	Gd 6-10	None	None	750 mg/kg-day	1,1,1-TCA
George et al. 1989	Rat	Oral	2 wk premating through PND 21	None	None	30 ppm (drinking water)	1,1,1-TCA
Payan et al. 1995	Rat	Inhalation	Gd 6-21	None	None	300 ppm	1,2-DCA
Rao et al. 1980	Rat, rabbit	Inhalation	Gd 1-21	None	None	150 ppm	1,2-DCA
Lane et al. 1982	Mouse	Oral	Multigeneration	None	None	50 mg/kg-day	1,2-DCA

In general, most studies have reported either no developmental effects or effects only at very high concentrations of chlorinated ethanes. Therefore, when the negative results of the Seidenberg et al. (1986) study are considered along with the weight-of-negative evidence for structurally related compounds, additional study on the developmental effects of 1,1,2-trichloroethane do not appear to

be necessary, particularly if potential human exposures are minimal and are likely to be less than minimal potential risks estimated for carcinogenicity (see below).

Reproductive toxicity does not appear to be a sensitive endpoint for 1,1,2-trichloroethane based on the following rationale. In general, there are three mechanisms by which chemicals may affect reproduction:

(1) Direct toxic action on reproductive tissues;

(2) Indirect action on reproductive tissues through endocrine system modulation; and

(3) Indirect action on reproductive behavior through central nervous system effects.

Under the assumption that 1,1,2-trichloroethane is capable of producing reproductive effects, endocrine system modulation does not appear to be a likely mechanism for 1,1,2-trichloroethane since there is no evidence which suggests that 1,1,2-trichloroethane is capable of interacting with hormone receptors. In fact, the chemical structure for 1,1,2-trichloroethane suggests that such interactions are unlikely. Additionally, since CNS effects (anesthesia) are generally only observed following relatively high exposures to 1,1,2-trichloroethane (ATSDR, 1989), reproductive behavior effects are unlikely as well. Instead, the mechanism by which 1,1,2-trichloroethane may impact reproduction most likely involves the direct toxic action of 1,1,2-trichloroethane on reproductive tissues. However, studies in which reproductive tissues were histopathologically evaluated have generally reported negative

results. For example, no histopathological effects were observed in the testes, prostate, tunica vaginalis, uterus, mammary gland, and ovary in rats exposed orally to 46-92 mg/kg-day or in mice exposed orally to 195-390 mg/kg-day for 78 weeks (NCI, 1978).

Additionally, available data suggest that reproductive effects are not of primary concern for chlorinated ethanes in general. A summary of the available information regarding the reproductive effects of chlorinated ethanes is provided in the table below (1,1,2-TCA = 1,1,2-trichloroethane; 1,1-DCA = 1,1-dichloroethane; 1,1,1-TCA = 1,1,1-trichloroethane; 1,1,2,2-TCA = 1,1,2,2-tetrachloroethane; 1,2-DCA = 1,2-dichloroethane).

Summary of Reproductive Toxicity Data for Chlorinated Ethanes

Study	Species	Route	Duration	Effect	LOAEL	NOAEL	Chemical
NCI 1978	Rat, mouse	Oral	78 wk	No histopathological changes	None	92-390 mg/kg-day	1,1,2-TCA
NCI 1978	Rat, mouse	Oral	78 wk	No histopathological changes	None	284 mg/kg-day	1,1,2,2-TCA
Lane et al. 1982	Mouse	Oral	Multigeneration	None	None	50 mg/kg-day	1,2-DCA
Rao et al. 1980	Rat	Inhalation	Single generation	None	None	150 ppm	1,2-DCA
NTP 1988	Rat	Oral	70 d	None	None	3.5 mg/kg-day	1,1,1-TCA

Study	Species	Route	Duration	Effect	LOAEL	NOAEL	Chemical
NCI 1977	Rat, mouse	Oral	78 wk	No histopathological changes	None	1500-5615 mg/kg-day	1,1,1-TCA

Based on the lack of histopathological effects of 1,1,2-trichloroethane on reproductive tissues, along with the negative available data on structurally related compounds and very low human exposure potential (see below), additional study on the reproductive effects of 1,1,2-trichloroethane does not appear to be necessary.

Exposure

The extremely low human exposure to 1,1,2-trichloroethane is due to the fact that virtually the entire U.S. production of 1,1,2-trichloroethane is as a captive or "site-limited" intermediate in the production of vinylidene chloride (VDC) or as a byproduct of the production of ethylene dichloride (EDC). Thus, 1,1,2-trichloroethane is found primarily in the vicinity of VDC and EDC production facilities, where in many cases the 1,1,2-trichloroethane is not isolated. The one manufacturer that markets 1,1,2-trichloroethane sells less than 3 million pounds annually. The small group (fewer than 10) of its customers use 1,1,2-trichloroethane as a carrier solvent in the manufacture of other materials. Two of these customers have indicated that they intend to switch to alternative carriers. These users also recycle/recover as much 1,1,2-trichloroethane in their operations as possible.

Available data on the concentrations of 1,1,2-trichloroethane in the environment are consistent with the foregoing paragraph. The Agency for Toxic Substances and Disease Registry (ATSDR) has

stated that "where 1,1,2-trichloroethane is found, levels appear to be about 10-50 ppt [parts per trillion]." *Toxicological Profile for 1,1,2-Trichloroethane* (1989), 63. These concentrations would have been undetectable without advances in detection capability. The low environmental exposure is consistent with available information as to use. ATSDR found that "no use with significant consumer and general population exposures has been identified." *Id.*, 61. Moreover, there is some doubt as to whether 1,1,2-trichloroethane meets the criteria articulated in EPA's so-called "B policy" for "substantial release" and "substantial human exposure," as will be addressed in greater detail in the comments of the HAP Task Force on the proposed test rule. For present purposes, it may be noted that the preamble to the proposed test rule does not identify any exposure as meeting the threshold for "substantial human exposure" except for 1,036 workers -- the threshold being 1,000 workers. The HAP Task Force will be providing a better estimate of the number of workers actually exposed to 1,1,2-trichloroethane as part of its comments.

Regulations and Guidelines

On top of the fact that human exposure to this site-limited intermediate is extremely low, EPA's regulatory structure would appear to make superfluous any determination as to the reproductive or developmental toxicity of 1,1,2-trichloroethane. As discussed above, the most significant potential exposure would appear to be from production facilities for VDC or EDC. VDC, EDC, and 1,1,2-trichloroethane obviously are subject to regulation under the MACT standards for chemical production facilities, and any additional controls imposed as part of the residual risk determination for VDC and EDC will reduce exposure to 1,1,2-trichloroethane. The Agency's Integrated Risk

Information System (IRIS) lists a unit risk estimate or cancer potency for 1,1,2-trichloroethane of $1.6x10^{-5}$ $(\mu g/m^3)^{-1}$. The maximum 8-hour time-weighted-average (TWA) exposure allowed for 1,1,2-trichloroethane is 10 parts per million (1.8 mg/m^3), 29 C.F.R. § 1910.1000, Table Z, and the American Conference of Governmental Industrial Hygienists (ACGIH) lists it as an animal carcinogen (A3). In drinking water, the maximum contaminant level goal (MCLG) is 3 µg/l, which according to EPA corresponds to a theoretical cancer risk of 10^{-5}, and the maximum contaminant level (the enforceable limit) is 5 µg/l, the same as for organic contaminants which have an MCLG of zero. The HAP Task Force is aware of no instance where the allowable exposure level for a substance so regulated has ever been reduced on account of such substance being identified as a reproductive or developmental toxin.

A specific illustration of these points is provided by an evaluation of 1,1,2-trichloroethane outside of one of the fewer than 20 U.S. facilities where VDC or EDC is produced, specifically, the Dow Chemical Company facility in Lake Jackson, Texas. Actual monitoring data in the community near the facility showed that out of 306 samples, 97% were non-detects at 0.55 $\mu g/m^3$ (0.0001 ppm). Only three values exceeded the detection limit. Put another way, only three samples exceeded an upper bound cancer risk of $9x10^{-6}$ (using the EPA unit risk estimate of $1.6x10^{-5}$). It is therefore inconceivable that any additional reproductive/developmental studies would result in setting exposure levels below those established for carcinogenic effects.

3.5 Neurotoxicity

Three studies were identified as potential candidates for route-to-route extrapolation, one for acute effects, White *et al.* (1985), and two for longer-term effects, both of which were conducted by NCI (1978). These studies are summarized briefly below.

- *White et al. (1985)* - Seven groups of 8 male and female CD-1 mice were administered a single dose of 200 to 600 mg/kg 1,1,2-trichloroethane via gavage. Sedation and loss of righting reflex were noted in mice soon after receiving a single gavage dose of 450 mg/kg 1,1,2-trichloroethane or more. These animals recovered four hours after exposure. No gross changes to the central nervous system were noted upon necropsy. This study identifies an acute LOAEL of 450 mg/kg for the anesthetic effects of 1,1,2-trichloroethane.

- *NCI (1978)* - Groups of 50 male and 50 female rats (Osborne-Mendel) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 30, and 70 mg/kg-day were raised to 0, 50, and 100 mg/kg-day after week 8 since animals seemed to tolerate exposure. Time-weighted average doses of 0, 46, and 92 mg/kg-day were calculated by the study authors. During the first 6 months, the incidence clinical signs (*i.e.*, appearance and behavior changes) were comparable between exposed and control animals. However, after 6 months,

exposed animals exhibited a higher frequency of hunched appearance, rough fur, urine stains, dyspnea, and squinted eye (sometimes with reddish exudate). Histopathological examinations revealed no significant differences between exposed and control animals for noncancer effects in the brain or nerves. This study identifies a NOAEL of 92 mg/kg-day for histopathological effects on the central nervous system.

- *NCI (1978)* - Groups of 50 male and 50 female mice (B6C3F1) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 150, and 300 mg/kg-day were raised to 0, 200, and 400 mg/kg-day after week 20 since the animals seemed to tolerate exposure. Time-weighted average doses of 0, 195, and 390 mg/kg-day were calculated by the study authors. Clinical signs (*i.e.*, appearance and behavior changes) were observed at comparable rates in exposed and control groups. Histopathological examinations revealed no significant differences between exposed and control animals for noncancer effects in the brain and nerves. This study identifies a NOAEL of 390 mg/kg-day for histopathological changes.

These studies are compared with toxicity test guidelines from the USEPA (OPPTS) in Table 3. Based on this comparison, there seems to be a lack of evaluation of subtle neurological effects of

1,1,2-trichloroethane. However, we still believe that these studies should be considered adequate for evaluating neurological effects.

We propose that the parent chemical in the central nervous system is likely responsible for the effects described above and that the parent compound in the CNS (or proportionately in the blood) be modeled as the appropriate dose measure.

3.6 Carcinogenicity

Two studies were identified as potential candidates for route-to-route extrapolation, both of which were conducted by NCI (1978). These studies are summarized briefly below.

- *NCI (1978)* - Groups of 50 male and 50 female rats (Osborne-Mendel) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 30, and 70 mg/kg-day were raised to 0, 50, and 100 mg/kg-day after week 8 of exposure. Time-weighted average doses of 0, 46, and 92 mg/kg-day were calculated by the study authors. High mortality was noted in the vehicle control group. Animals were followed up to 34 weeks after exposure. No statistically significant increase in tumor incidence was observed in exposed rats. The authors concluded that there was no convincing evidence for the carcinogenicity of

1,1,2-trichloroethane in rats. However, the maximum tolerable dose may not have been achieved in this study.

- *NCI (1978)* - Groups of 50 male and 50 female mice (B6C3F1) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 150, and 300 mg/kg-day were raised to 0, 200, and 400 mg/kg-day after week 20 of exposure. Time-weighted average doses of 0, 195, and 390 mg/kg-day were calculated by the study authors. Animals were followed for up to 12 weeks following exposure. Mortality was significantly increased in exposed female mice, however this may not have been dose-related (mortality was greater in the low dose group compared to the high dose group). The incidence of hepatocellular carcinomas was significantly increased in exposed mice of both sexes. Additionally, adrenal pheochromocytomas were elevated in female mice. The authors concluded that 1,1,2-trichloroethane was carcinogenic to mice under the conditions of this bioassay.

These studies are compared with toxicity test guidelines from the USEPA (OPPTS) in Table 4 and are considered adequate. Although there are limitations in these studies (*i.e.*, less than lifetime exposure, mortality in vehicle control group in rats, and in low dose female mice), these limitations do not detract from the results of the mouse study, which are clearly positive for liver tumors. Furthermore, the USEPA has placed sufficient confidence in this study as to base both the oral and inhalation cancer potency factors on its results (IRIS, 1996) For this reason, a repetition of the

cancer bioassay for 1,1,2-trichloroethane does not appear to be a justified use of laboratory animals, particularly in light of the fact that a chronic bioassay (negative results reported following chronic subcutaneous injection) was also recently conducted for this chemical (Norpoth *et al.* 1988). Extrapolation of the NCI (1978) studies to inhalation exposures using PBPK modeling is offered as an appropriate alternative to repeating the cancer bioassay.

Based upon the mechanism of action proposed in Section 2.0, it appears likely that cytochrome P-450 metabolites of 1,1,2-trichloroethane are likely to be involved in the hepatic effects described above for both studies. Therefore, it is proposed that some internal measure of these metabolites (*i.e.*, total amount metabolized in a 24 hour period) in the liver be modeled as the appropriate internal dose measure.

3.7 *In Vivo* Cytogenicity

Two key studies were identified regarding the potential cytogenicity of 1,1,2-trichloroethane, an *in vivo* study (Mazzulo *et al.*, 1986), and an *in vitro* study (Doherty *et al.*, 1996). These studies are summarized below.

- *Mazzulo et al. (1986)* - A group of 4 Wistar rats and 12 BALB/c mice were administered a single dose of 6.35 umol/kg of radiolabeled 1,1,2-trichloroethane via intraperitoneal injection. Twenty-two hours after exposure, radiolabel was found covalently bound to DNA, RNA, and proteins of the liver, kidney, lung, and stomach.

The DNA binding in all tissues was greater in mice than rats, thus correlating with observations on the relative susceptibility of these species to 1,1,2-trichloroethane-induced carcinogenesis (NCI, 1978).

- *Doherty et al. (1996)* - In this *in vitro* study, metabolically competent human cell lines (AHH-1 MCL-5 and h2E1) were exposed to 1,1,2-trichloroethane. These cell lines differed with respect to the cytochrome P-450 isozyme expressed. Micronuclei induction (3.5-4 fold) was noted in 2 of the 3 cell lines.

These studies clearly demonstrate a genotoxic effect for 1,1,2-trichloroethane *in vivo*, as well as a cytogenetic effect for 1,1,2-trichloroethane *in vitro*. Therefore, it is reasonably safe to assume that both genotoxic and cytogenetic effects would be observed following inhalation exposures to 1,1,2-trichloroethane. In fact, we will assume a genotoxic mechanism when the positive oral NCI (1978) bioassay data are extrapolated to equivalent inhalation exposures (see Section 3.6). Since further study on the cytogenicity of 1,1,2-trichloroethane would provide little additional insight regarding the mechanism of action for tumor induction, additional study is not recommended.

3.8 Immunotoxicity

Two studies were identified as potential candidates for route-to-route extrapolation, Sanders *et al.* (1985) and NCI (1978). These two studies are summarized briefly below.

- *Sanders et al. (1985)* - Groups of 32-48 male and 32-48 female CD-1 mice were exposed to doses of 0, 4.4, 46, or 305 mg/kg-day (males), or 0, 3.9, 44, or 384 (females) 1,1,2-trichloroethane in the drinking water for 90 days. Drinking water consumption and body weight gain were reduced in concentration-dependent manner in male mice, but not in female mice. Spleen weights were decreased in a dose-dependent manner in male mice, but were significantly increased in females exposed to the highest dose. Bone marrow status as indicated by DNA synthesis was not affected by exposure. Cell-mediated immunity, as measured by delayed-type hypersensitivity and popliteal lymph node proliferation responses to sheep erythrocytes, was unaffected in both sexes. However, humoral immune status was significantly depressed in both sexes in a dose dependent manner. For example, hemagglutination titers were significantly depressed in both sexes at the two highest doses. In addition, lymphocyte responsiveness to Con A and LPS was significantly depressed in female mice exposed to the highest dose, but unaffected in exposed male mice. This study identifies a LOAEL of 44-46 mg/kg-day and a NOAEL of 3.9-4.4 mg/kg-day for depression of humoral immunity.

- *NCI (1978)* - Groups of 50 male and 50 female mice (B6C3F1) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 150, and 300 mg/kg-day were raised to 0, 200, and 400 mg/kg-day after week 20 since the animals seemed to tolerate exposure. Time-weighted average

doses of 0, 195, and 390 mg/kg-day were calculated by the study authors. Histopathological examinations revealed no significant differences between exposed and control animals for noncancer effects in immunological tissues (*i.e.*, spleen, bone marrow, lymph nodes). This study identifies a NOAEL of 390 mg/kg-day for histopathological effects.

These two studies are compared with guidelines for conducting immunotoxicity tests from the USEPA (OPPTS) in Table 5. Based on this comparison, the study by Sanders *et al.* (1985) was considered adequate for route-to-route extrapolation. The primary limitation with this study is the lack of histopathological examination of immune system organs (spleen, thymus, lymph nodes). For this reason, the histopathology results from the NCI (1978) could be used to supplement this study.

In the absence of specific information regarding the potential mechanisms of action for 1,1,2-trichloroethane-induced immunological effects, we propose that parent chemical present in the lymphoid tissue (*i.e.*, spleen) is the appropriate dose metric leading to these effects.

4.0 PBPK MODELS FOR 1,1,2-TRICHLOROETHANE

This section summarizes the toxicokinetics of 1,1,2-trichloroethane and describes an existing PBPK model for this chemical in the rat. Studies needed to validate the rat model and to develop and validate a mouse model necessary to fill the data gaps described in Section 3.0 are proposed.

4.1 Toxicokinetics of 1,1,2-Trichloroethane

Available studies indicate that 1,1,2-trichloroethane is readily absorbed following oral and inhalation exposures (ATSDR, 1989). Following absorption, 1,1,2-trichloroethane distributes to all organs of the body, with a tendency for higher concentrations in adipose tissue and tissues with a high lipid content (ATSDR, 1989).

1,1,2-Trichloroethane is metabolized by the cytochrome P-450 enzyme system via oxidative and reductive pathways. Chloroacetic acid is generated through oxidative metabolism and reductive metabolism generates free radicals. 1,1,2-Trichloroethane also reacts with glutathione, yielding S-carboxymethyl cysteine and thiodiacetic acid. Mice appear to have a greater capacity than rats in metabolizing 1,1,2-trichloroethane (Mitoma *et al.* 1985) and a greater capacity to generate reactive intermediates capable of binding DNA, RNA, and proteins (Mazzulo *et al.* 1986). Since cytochrome P-450 and glutathione are found in the greatest amounts in the liver, there is likely to be a first-pass metabolic effect for 1,1,2-trichloroethane following oral exposure which may alter its distribution and toxicity.

Following exposure to an inhaled dose of radiolabeled 1,1,2-trichloroethane, approximately 2.9% of the radiolabel was excreted in air after 1 hour in a volunteer (Morgan *et al.* 1970). Urinary excretion was approximately 0.01%/min. The half-life of 1,1,2-trichloroethane in various tissues has been determined in mice following inhalation exposure (Takahara, 1986) with overall half-life reported to be 49.3 minutes. Following oral exposure, approximately 7-10% of the dose was excreted unchanged by the lungs, 3-7% as exhaled CO_2, 72-87% as urinary metabolites and 1% in the feces (Mitoma *et al.* 1985; Yllner *et al.* 1971).

4.2 Existing PBPK Model for 1,1,2-Trichloroethane

A PBPK model for 1,1,2-trichloroethane dosimetry in the rat has been described (Gargas *et al.*, 1989a; Gargas *et al.*, 1990). This model accounted for inhalation of 1,1,2-trichloroethane, distribution to liver, fat, richly perfused and slowly perfused tissues, and elimination via exhalation and P-450 mediated metabolism in the liver. Partition coefficients were determined by vial equilibration techniques and the kinetic constants of P-450 metabolism were determined *in vivo* by modeling exhaled breath concentrations following inhalation exposures at constant concentrations of 1,1,2-trichloroethane.

Unfortunately, the model was not validated for inhalation exposure and oral absorption was not part of the original model description. In addition, no inhalation or oral model currently exists for 1,1,2-trichloroethane in mice. Validated PBPK models capable of describing inhalation and oral

exposures in rats and mice are necessary to perform the route-to-route extrapolations described in Section 3 above.

4.3 Proposed Model Development and Validation

The following studies are proposed to develop and validate the models described in the previous section. Detailed protocols are not described here, but rather, a general description of the types of studies necessary to develop and validate rat and mouse models are presented for USEPA consideration.

4.3.1 Validation of Rat Inhalation Route

Validation of a PBPK model involves testing how well a particular model performs at predicting experimental data not used for model development. Ideally, predictions of time courses of internal dose measures during and following exposure are preferable, although this is quite impractible for most reactive metabolites. Usually, blood and/or tissue concentration time course data for the parent chemical have been used for model validation (Corley *et al.*, 1990; D'Souza *et al.*, 1987; Reitz *et al.*, 1996) and are appropriate if it can be demonstrated that the dose measure of interest (*i.e.*, reactive metabolites) is dependent on the kinetic behavior of parent chemical.

To validate the inhalation portion of the rat PBPK model, the following experiments are proposed:

- Measure 1,1,2-trichloroethane blood concentration time courses during and following 4-6 hour inhalation exposures at several constant inhaled concentrations.

- Use existing inhalation rat model to predict these time courses.

- Compare model output to actual data

4.3.2 Develop Model and Validate Oral Exposures

Uptake of chemicals such as 1,1,2-trichloroethane from the gastrointestinal tract have been described using a first-order rate constant for absorption. The rate constants vary when chemical is delivered in water or in corn oil (Corley *et al.*, 1990; Gargas, 1990; Ramsey and Andersen, 1984). However, the rate constants seem to be about 1.0 hr^{-1} and 5.0 hr^{-1} for water and oil vehicles, respectively, for compounds similar to 1,1,2-trichloroethane (*i.e.*, chloroform, methylene chloride, 1,1,1-trichloroethane). Therefore, we propose to use these constants as default values for oral absorption and test their ability to predict oral uptake in the following validation experiments. If validation of this model fails, it will be necessary to more formally measure the rate constants, possibly using an exhaled breath technique (Gargas *et al.* 1989a).

To validate the rat oral absorption portion of the model, the following experiments are proposed:

- Deliver several concentrations of 1,1,2-trichloroethane in corn oil by gavage and measure blood time course concentrations of parent compounds.

- Compare data to model predictions.

- Deliver several concentrations of 1,1,2-trichloroethane in water by gavage and measure blood time course concentrations of parent chemical if technically feasible *(i.e.*, if enough chemical can be dissolved in water to result in measurable blood concentrations).

- Compare to model predictions.

4.3.3 Develop Initial Mouse Model

In vivo metabolic constants and *in vitro* blood:air partition coefficients are needed to develop an initial PBPK model for the mouse. It is proposed that metabolic constants be derived using the exhaled breath technique developed by Gargas *et al.* (1989a) and that blood:air partition coefficients be determined by vial equilibration techniques (Gargas *et al.* 1989b). Tissue:air partition coefficients are not usually necessary for mouse tissues if rat tissue:air values are available since it is technically difficult to obtain "clean" mouse tissue samples, and rat and mouse tissue:air partition coefficients

have been shown to be quite similar. The rat tissue:air partition coefficients can be divided by the mouse blood:air values to estimate the tissue:blood coefficients used in the model. This approach has been used successfully in previous work (Corley *et al.*, 1990; Reitz *et al.*, 1996).

4.3.4 Inhalation and Oral Route Validation

The experiments necessary to conduct the model validation for the mouse will be essentially the same as described above for the rat (Sections 4.3.1 and 4.3.2). Selection of inhalation concentration ranges and oral dose ranges may vary and will be determined using preliminary PBPK models as aids to experimental design.

It is anticipated that all model development and validation studies will be described in manuscripts that will be submitted to peer-reviewed journals for publication. These published models will then be used to conduct the dose-route extrapolations necessary to fill the data gaps for 1,1,2-trichloroethane identified in the test rule for subchronic toxicity, neurotoxicity, carcinogenicity and immunotoxicity. The studies, species, toxicological endpoints and internal dose measures proposed to fill the data gaps via PBPK modeling are summarized in Table 6.

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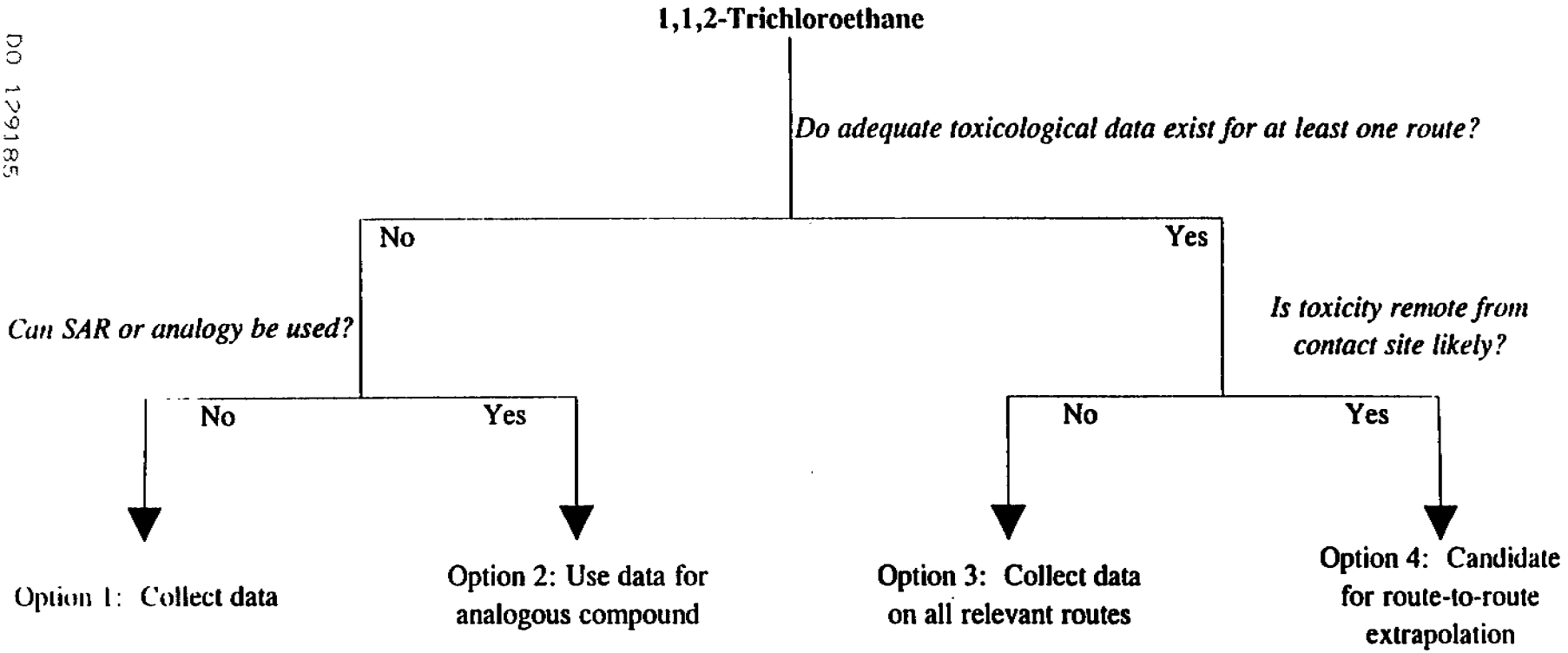
Figure 1
Decision Tree for Route-to-Route Extrapolation
1,1,2-Trichloroethane*



*Adapted from Gerrity and Henry (1990)

Figure 2
Comparison of Current and Proposed PBPK Models for 1,1,2-Trichloroethane

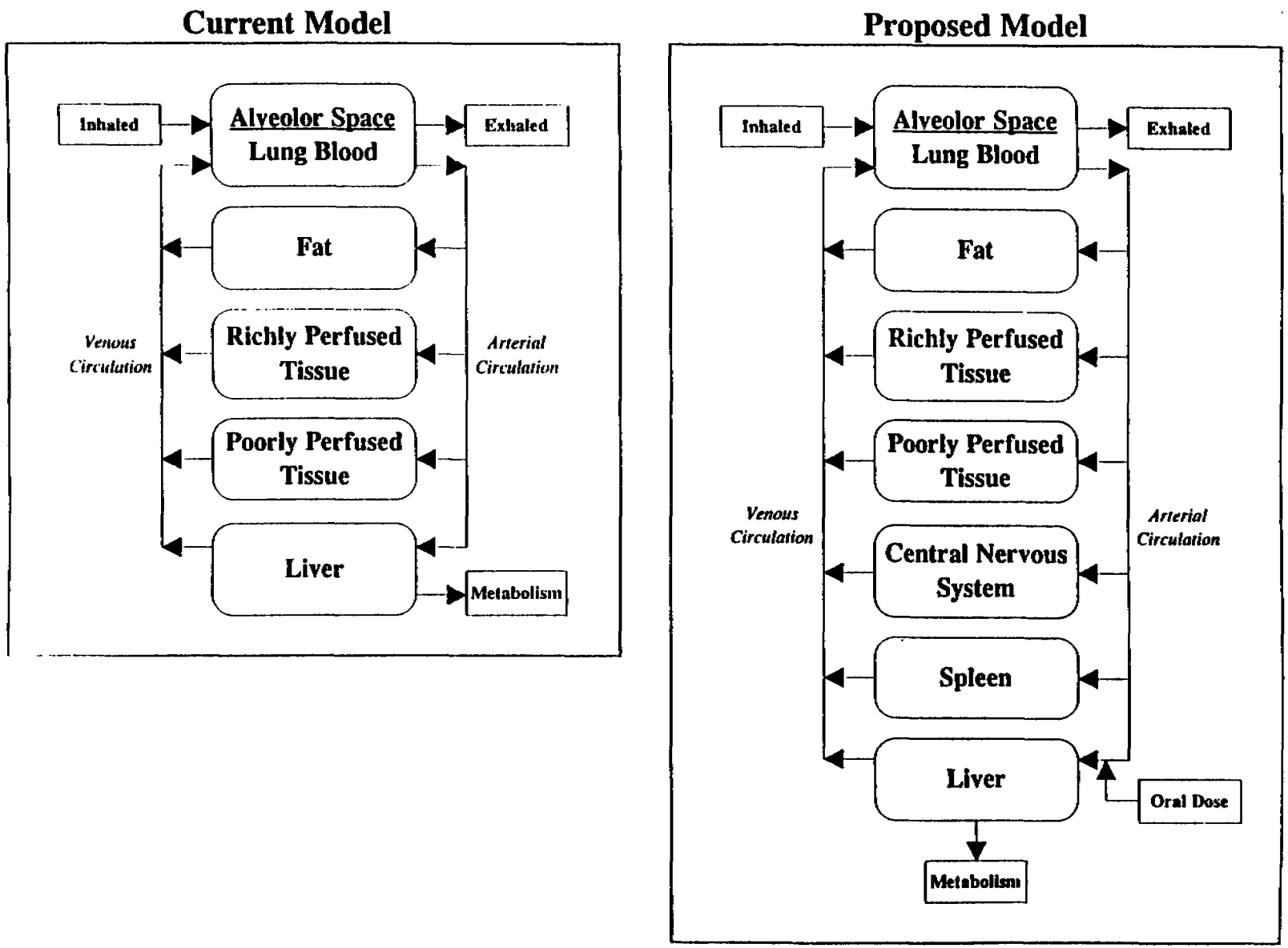


Table 1
Comparison of Candidate Studies to OPPTS Test Guidelines for Subchronic Toxicity

		Candidate Studies for Route Extrapolation	
	Study:	White et al. 1985	NCI 1978
	Subchronic effects observed:	Liver, hematological	Lack of histopathological changes
	LOAEL:	44-46 mg/kg-day	None
	NOAEL:	3.9-4.4 mg/kg-day	400 mg/kg-day [CHECK]
Parameter	**Recommended**		
Test species	Rat (other mammal)	Mouse	Mouse
Strain	Common lab	CD-1	B6C3F1
Age	Young, healthy	Young, healthy	Young, healthy
Sex	Both	Both	Both
Number of animals	10/sex/dose, more if interim sacrifice	32-48/sex/group	50/sex/dose
Husbandry	Standard	Adequate	Some limitations, but adequte overall
Control Groups	Concurrent sham or vehicle control	Vehicle control	Vehicle and untreated controls
Concentration level and selection	>=4 (including control)	Male (0, 4.4, 46, and 305 mg/kg-day); Female (0, 3.9, 44, and 384 mg/kg-day)	0, 195, 390 mg/kg-day
Limit dose	Maximum tolerable dose achieved	Range determined based on 1-day, and 14-day range finding studies	Range determined based on 6 week study
Intermediate dose	Provides gradiation of effects	Adequate	NA
Lowest dose level	Should provide NOAEL	Adequate	Adequate
Administration of the substance	6 hr/day, 7 day/week	Daily, 90 days	5 d/wk, 78 wks
Observation period	90 days	90 days	630 days
Exposure specifications and physical measurements	Nose only or whole body	Oral (drinking water)	Oral (oil gavage)
Observation of animals	Mortality (daily), clinical (weekly)	Adequate	Adequate
Clinical pathology	Hematology and clinical chemistry	Adequate	Not evaluated
Ophthalmological examinations	High-dose and control	Not applicable	Histopathological examination of ocular tissues
Gross pathology	Gross necropsy and organ weights	Adequate	Adequate
Histopathology	Histopathology, including respiratory tract	Not evaluated	Dated methods, but adequate overall
Results reporting	Tabular results, statistics	Tabular means & SDs, Duncan's multiple range test	Tabular incidences
Evaluation	Adequately described	Adequate	Adequate

Table 2
Comparison of Studies to OPPTS Guidelines for Developmental Toxicity

	Candidate for Route Extrapolation
	Seidenberg et al. (1986)
Effect:	No developmental effects observed
LOAEL:	None
NOAEL:	350 mg/kg-day

Parameter	Recommended	
Test Species	>2	Mouse
Strain	Common laboratory	ICR/SIM
Sex	Pregnant female	Pregnant female
Route	Inhalation	Oral (oil gavage)
Number of animals	>20/group (rats); >12/group (rabbits)	30/group
Control group	Filtered air/vehicle	Vehicle
Concentrations	>4 (including control)	0, 350 mg/kg-day
Dose Seletion	Characterizes dose response	Maternal toxicity observed (3/30 deaths)
Exposure Duration	6 hr/d	Daily
Observation Period	Gd 6-15 (rat); Gd6-18 (rabbit)	Gd 8-12
EnvironmentalConditions	Standard/monitored	Adequate
Observation	Daily	Adequate
Gross Necropsy	Uterus/embryo	Adequate
Data Reporting	Tabular/detailed	Adequate
Evaluation	Statistics/described	Adequate

Table 3
Comparison of Candidate Studies to OPPTS Test Guidelines for Neurotoxicity

		Candidate Studies for Route Extrapolation		
		Study: White et al. (1985) - Acute	NCI (1978) - Longer term	NCI (1978) - Longer term
		Neurological effects observed: Sedation, loss of righting reflex	Lack of histopathological changes for brain and nerves	Lack of histopathological changes for brain and nerves
		LOAEL: 450 mg/kg-day	None	None
		NOAEL: 400 mg/kg-day	390	92
Parameter	**Recommended**			
Test species	Rat (mice or dog)	Mouse	Mouse	Rat
Age	Young adult (>42 d)	Young adult	Young adult	Young adult
Sex	Both	Both	Both	Both
Number of animals	10/sex/dose	8/sex/dose	50/sex/dose	50/sex/dose
Control Groups	Concurrent sham or vehicle control	None	Vehicle and untreated controls	Vehicle and untreated controls
Concentration level and selection	>=4 (including control)	7 dose groups between 200 and 600 mg/kg	0, 195, 390 mg/kg-day	0, 46, 92 mg/kg-day
Acute studies	MTD; < 2g/kg	Adequate	NA	NA
Subchronic and chronic studies	MTD; <1 g/kg	NA	Range determined by 6 week study	Range determined by 6 week study
Administration of the substance	Appropriate route of exposure	Oral (water gavage)	Oral (oil gavage)	Oral (oil gavage)
Combined protocol	Combine with other endpoints	NA	NA	NA
Time of testing	Acute (0, 8hr, 7d, 14 d); subchronic (0, 4 wk, 8 wk, 13 wk)	up to 14 days	Functional tests not evaluated	Functional tests not evaluated
Functional observational battery	Standard evaluations for appearance, behavior, and functional integrity	Appearance and behavior	Appearance and behavior evaluated grossly	Appearance and behavior evaluated grossly
List of measures	Autonomic function, abnormal motor movements, response to general and sensory stimuli, alertness, grip strength, landing foot splay, body weight, behavioral changes	Abnormal motor movements	Not evaluated	Not evaluated
Motor activity	Individually assessed, automated	Not evaluated	Not evaluated	Not evaluated
Neuropathology	Neuropathological examinations	Gross necropsy only	Adequate	Adequate
Results	Tabular, per animal	Text only	Tabular incidences	Tabular incidences
Evaluation	Adequately described, statistics	Limited	Adequate	Adequate

Table 4
Comparison of Candidate Studies to OPPTS Test Guidelines for Carcinogenicity

		Candidate Studies for Route Extrapolation	
		Study: NCI (1978) - Mouse study	NCI (1978) - Rat study
		Carcinogenic effects observed: Liver, adrenal tumors	None
Parameter	**Recommended**		
Test species	Rats and mice	Mouse	Rat
Strain	Common lab strain	B6C3F1	Osborne-Mendel
Age	Young, healthy (<8 wks)	Young	Young
Sex	Both	Both	
Number of animals	50/sex/group	50/sex/group	50/sex/group
Control Groups	Concurrent sham or vehicle control	Vehicle and untreated controls	Vehicle and untreated controls
Concentration level and selection	> =4 (including control)	0, 195, 390 mg/kg-day	0, 46, 92 mg/kg-day
Limit dose	MTD; <1000 mg/kg-day	Adequate	May not have been achieved
Intermediate dose	Provides gradation of effects	Adequate	Adequate
Lowest dose level	Provides NOAEL	NA	NA
Route	Inhalation	Oral (oil gavage)	Oral (oil gavage)
Exposure	6 hr (inhalation), 5-7 d/wk for 18 (mice) or 24 (rat) months	5 d/wk, 78 wk	5 d/wk, 78 wk
Observation period	Lifespan	630 days	777 days
Observation of animals	Morbidity (daily), clinical (weekly)	Adequate	Adequate
Clinical pathology	at 12 and 18 months	Not evaluated	Not evaluated
Immunotoxicity screen	Optional	NA	NA
Gross necropsy	Complete	Adequate	Adequate
Histopathology	Complete	Adequate	Adequate
Results reporting	Tabular, per animal, statistics	Incidence	Incidence
Evaluation reporting	Adequately reported	Adequate	Adequate

Table 5
Comparison of Candidate Studies to OPPTS Test Guidelines for Immunotoxicity

		Candidate Studies for Route Extrapolation	
		Study: Sanders et al. (1985); White et al. 1985	NCI (1978)
		Immunological effects observed: Humoral immune system depression	Lack of histopathology
		LOAEL: 44-46 mg/kg-day	None
		NOAEL: 3.9-4.4 mg/kg-day	390 mg/kg-day
Parameter	**Recommended**		
Test species	Rat or mouse	Mouse	Mouse
Strain	Common lab strain	CD-1	B6C3F1
Age	Young, healthy (6-8 wks)	Young, healthy	Young, healthy
Sex	Both	Both	Both
Number of animals	6 - 10/sex/group	32-48/sex/dose	50/sex/dose
Husbandry	Standard	Adequate	Adequate
Control & Test Substances	Purity and vehicle evaluated	95%	92.70%
Control Groups	Concurrent sham or vehicle control	Vehicle control	Vehicle and untreated controls
Dose administration	Inhalation	Oral (drinking water)	Oral (oil gavage)
Concentration level and selection	> =4 (including control)	Male (0, 4.4, 46, and 305 mg/kg-day); Female (0, 3.9, 44, and 384 mg/kg-	0, 195, 390 mg/kg-day
Limit dose	MTD	Range determined based on 1-day, and 14-day range finding studies	Range determined based on 6 week study
Lowest dose level	Provides NOAEL	Adequate	Adequate
Administration of the substance	30-90 days; 5-7 days/week	90 days	5 d/wk, 78 wks
Observation period	30-90 days	90 days	630 days
Immunotoxicity tests	Functional (antibody plaque forming cell assay or immunoglobin quantification); Enumeration of splenic or peripheral blood T cells, B cells and NK cells	Complete	Histopathology of lymph nodes, spleen, bone marrow
Results reporting	Tabular, statistics	Tabular means & SD, Duncan's multiple range test	Tabular incidences
Evaluation reporting	Adequately described	Adequate	Adequate

Table 6
Summary of Pharmacokinetic Modeling Activities Proposed for 1,1,2-Trichloroethane

	Data Gap							
	Subchronic		Neurotoxicity		Carcinogenicity		Immunotoxicity	
Study	White et al. (1985)	NCI (1978)	NCI (1978)	NCI (1978)	NCI (1978)	NCI (1978)	White et al. (1985)	NCI (1978)
Species	Mouse	Mouse	Mouse	Rat	Mouse	Rat	Mouse	Mouse
Route	Oral (drinking water)	Oral (oil gavage)	Oral (oil gavage)	Oral (oil gavage)	Oral (oil gavage)	Oral (oil gavage)	Oral (drinking water)	Oral (oil gavage)
Endpoint	Liver, hematological	Lack of histopathological changes to the liver	Lack of histopathology to CNS	Lack of histopathology to CNS	Liver, adrenal tumors	Lack of tumors	Humoral immune system depression	Lack of histopathological changes
LOAEL	44-46 mg/kg-day	None	None	None	195 mg/kg-day	None	44-46 mg/kg-day	None
NOAEL	3.9-4.4 mg/kg-day	390 mg/kg-day	390 mg/kg-day	92 mg/kg-day	NA	NA	3.9-4.4 mg/kg-day	390 mg/kg-day
Dose Metric	Total metabolized (P 450)	Total metabolized (P 450)	Parent compound	Parent compound	Total metabolized (P 450)	Total metabolized (P 450)	Parent compound	Parent compound
Target Tissue	Liver	Liver	Central nervous system	Central nervous system	Liver	Liver	Lymphoid tissue (*i.e.*, spleen)	Lymphoid tissue (i.e., spleen)