DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Agency for Toxic Substances
and Disease Registry
Atlanta GA 30333

November 8, 1995

Hasmukh C. Shah, Ph.D.
Manager, Vinyl Chloride Panel
Chemical Manufacturers Association
2501 M Street, N.W.
Washington, DC 20037

Dear Dr. Shah:

This is in response to your October 23 letter in which you enclosed (1) a revised study protocol, "Vinyl chloride: Combined inhalation two-generation reproduction and developmental toxicity study in CD rats," and (2) the Chemical Manufacturers Association's (CMA) response to the Agency for Toxic Substances and Disease Registry's (ATSDR) peer reviewers' comments on the protocol. The study protocol was submitted by CMA to ATSDR for the purpose of conducting voluntary research to address ATSDR's priority data needs for vinyl chloride.

We have reviewed the CMA responses and the revised study protocol and found them to be satisfactory. Also, we agree with CMA's rationale for reducing the number of animals in the developmental study to 25 per group from 30 per group as described in the original protocol. With regard to a neurotoxicity component for this study, we confirm that the Environmental Protection Agency does not require additional neurotoxicity data at this time.

Therefore, we ask that you complete a memorandum of understanding (MOU) for the combined inhalation two-generation reproduction and developmental toxicity study and forward it to ATSDR. A hard copy and an electronic version of the ATSDR MOU are enclosed for your use.

In addition to reproductive and developmental toxicity studies via inhalation, I would like to bring to your attention two other ATSDR priority toxicity data needs for vinyl chloride, specifically, dose-response data in animals exposed via inhalation for acute- and chronic-duration. This was described in the Agency's March 10, 1994, Federal Register notice, "Status of the Superfund Substance-Specific Applied Research Program; Notice" (59 FR 11434), and Priority Data Needs Document for Vinyl Chloride.

Recently, we reevaluated the toxicity database for inhalation exposure for acute-duration. We determined that, at the present time, there is no need to obtain additional data as originally stated in the ATSDR Federal Register notice and priority data

needs document. This is reflected in the updated Toxicological Profile for Vinyl Chloride that is available for public comment.

With regard to chronic-duration studies via inhalation, we believe that the available data do not provide a suitable lowest-observed-adverse-effect level (LOAEL) or a no-observed-adverse-effect level for deriving ATSDR's Minimal Risk level (MRL). The MRL is defined as an estimate of daily human exposure to a dose of a chemical that is likely to be without an appreciable risk of adverse noncancerous effects over a specified duration of exposure.

The lowest LOAEL identified in a chronic-duration study was for a serious end point (testicular necrosis) in a rat study. However, MRLs are not derived using a serious end point. In addition, carcinogenicity was observed at concentrations equal to and less than that for testicular necrosis. Therefore, we have identified a priority data need to conduct additional animal studies via the inhalation route, the most relevant exposure route for populations living in the vicinity of hazardous waste sites. These studies are needed for determining exposure concentrations of vinyl chloride that establish dose-response relationships and defining threshold levels for chronic adverse health effects.

In light of the leadership role of CMA in conducting research on vinyl chloride, and the Agency's need to obtain additional data on vinyl chloride, we would also be interested in discussing opportunities for collaborative research to address this need. Please let me know of your interest in discussing this potential research.

We look forward to signing the MOU with CMA and to a continuing dialogue with CMA leading to additional successful voluntary research efforts to address ATSDR's data needs for vinyl chloride. If you have any questions, please call me at 404-639-6306.

Sincerely yours,

William Cibulas

William Cibulas, Ph.D.
Chief, Research Implementation Branch

Enclosures

cc:
Dr. Christopher T. DeRosa
Mr. Caffey Norman

MEMORANDUM OF UNDERSTANDING FOR VOLUNTARY RESEARCH PROGRAM

Under Section 104(i)(5) of CERCLA

An agreement between

THE AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY
Division of Toxicology
Research Implementation Branch

and

(Name of Participating Company)

(Date of signing this Memorandum of Understanding)

BFG40987

Table of Contents

I. PURPOSE

This Memorandum of Understanding (MOU) is entered into by the Agency for Toxic Substances and Disease Registry (ATSDR) and the private sector organization(s) identified in Paragraph I below (hereinafter referred to as the "company") in order to implement Section 104(i)(5) of the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), as amended by the Superfund Amendments and Reauthorization Act of 1986 (SARA). These Congressional acts direct ATSDR to assure the initiation of a program of research designed to determine the health effects of hazardous substances for which adequate health effects information is not available. In order to facilitate the discharge of ATSDR's responsibilities under Section 104(i)(5) of CERCLA, and in recognition of the fact that the company includes manufacturers and/or processors, or registrants of the hazardous substance(s) that is the subject of this MOU, ATSDR and the company hereby agree as follows:

II. IDENTIFICATION OF THE COMPANIES THAT ARE PARTIES TO THIS MEMORANDUM OF UNDERSTANDING

The following companies are parties to this MOU and shall be responsible for ensuring that the obligations and undertakings of the companies under this MOU are discharged and carried out as provided herein:

Names and Addresses of Participating Companies

III. IDENTIFICATION OF THE SUBSTANCE(S) SUBJECT TO RESEARCH REQUIREMENTS UNDER THIS MEMORANDUM OF UNDERSTANDING

The chemical substance(s) that is the subject of this MOU is _____ (CAS No._____). The chemical substance to be tested shall be as pure as reasonably can be attained. However, under certain circumstances, ATSDR recognizes that it may be more desirable to test mixtures or technical grade products. [Note: Substitute alternative language when the subject of the research is a human population as in epidemiologic studies].

IV. IDENTIFICATION OF THE EFFECTS OR CHARACTERISTICS FOR WHICH RESEARCH IS TO BE CONDUCTED

The health effects, environmental fate or other characteristics for which research is to be conducted by the company under this MOU are listed below:

[To be listed by the company]

V. IDENTIFICATION OF STUDY PLANS AND TESTING PROTOCOLS AGREED TO BY ATSDR AND THE COMPANY PRIOR TO SIGNING OF MOU

The research to be conducted on (name of chemical substance) pursuant to this MOU is identified in Table 1 below. The study plan, guidelines and protocols that were agreed to by ATSDR and the company are listed in Table 1 and described in detail in an Attachment to this MOU. The company agrees to perform (or sponsor and fund the performance of) the research identified in Table 1 in accordance with the guidelines and schedules established pursuant to the study plan and testing protocols agreed to prior to signing of this MOU.

TABLE 1
IDENTIFICATION OF STUDY PLANS AND TESTING PROTOCOLS AGREED TO BY ATSDR AND THE COMPANY PRIOR TO SIGNING OF MOU

COMPANY _____

TEST SUBSTANCE _____

IDENTIFICATION OF STUDY PLAN

Title _____

ID # _____

TEST TO BE CONDUCTED	GUIDELINES		
	TSCA*	Other EPA Guidelines	Alternate Guidelines

* Citation to 40 C.F.R. where appropriate

VI. SUBMISSION OF STUDY PLANS AND ESTABLISHMENT OF SCHEDULE FOR INITIATION OF RESEARCH AND SUBMISSION OF INTERIM AND FINAL REPORT

A. Prior to signing of this MOU the company shall submit to ATSDR the study plan for each test that is to be conducted pursuant to this MOU (see Appendix 1).

B. Prior to entering into this MOU, the study plan including all testing protocols and guidelines shall be reviewed by an ATSDR appointed peer review panel. Consistent with CERCLA section 104(i)(13), the peer review panel will consist of no fewer than three nor more than seven peer reviewers who a) are selected by the Administrator of ATSDR; b) are disinterested scientific experts; c) have a reputation for scientific objectivity; and d) lack institutional ties with any person involved in the conduct of the study under review.

C. The study shall be initiated within 8 weeks of the date on which ATSDR and the company have signed this MOU. Written notification of the starting date of the test will be submitted to ATSDR by the company. The completion date of the study will be established from the approved study plan.

D. Unless modified pursuant to Paragraph VII, a final draft report on the results of testing conducted pursuant to the approved study plan and signed into agreement under this MOU

shall be submitted to ATSDR within 20 weeks of the end of the study for ATSDR's peer review, consistent with CERCLA section 104(i)(13). Following acceptance by ATSDR, upon recommendation by the peer review panel, the company will submit a final report of the study to ATSDR within 4 weeks. Final reports will not be accepted if the data is designated Confidential Business Information (CBI) or otherwise restricted from public disclosure with the exception of personally identifiable information on study subjects. [Note: When the MOU covers multiple tests, different final report periods could be established for the different tests].

E. Unless modified pursuant to Paragraph VII, interim progress reports on each testing program conducted pursuant to a study plan approved by ATSDR under this MOU shall be submitted to ATSDR within 6 months after the initiation of testing, and thereafter, within 6 months after the submission of each previous interim report. If the study is scheduled to be completed in one year, an interim brief letter addressing the status of the research must be submitted to ATSDR within 6 months of the initiation of the study.

VII. MODIFICATION OF STUDY PLANS, GUIDELINES, AND SCHEDULES

A. If the company seeks to modify a study plan, guidelines, or schedules that have been approved by ATSDR pursuant to this MOU, the company shall notify ATSDR in writing of the proposed modifications and the reasons therefor. ATSDR shall respond in writing to the proposed modifications within 2 to 6 weeks either: (i) approving the modifications as proposed, (ii) approving the modifications as revised by ATSDR, or (iii) disapproving the modifications entirely. If ATSDR does not approve the modifications as proposed, the company will have 2 weeks within which to: (i) accept ATSDR's decision and proceed in accordance therewith, (ii) request that ATSDR reconsider its decision, or (iii) withdraw from the MOU. ATSDR will respond to request for reconsideration within 2 weeks (see Figure 1).

B. If the company submits a request for modification to ATSDR pursuant to Paragraph VII. A., the time schedule established for completion of these tests shall be extended by the length of time required by ATSDR and the company to respond to and approve the modifications.

VIII. OBSERVANCE OF GOOD LABORATORY PRACTICES

All research agreed to in this MOU shall be conducted in accordance with the Good Laboratory Practice (GLP) standards

REQUEST FOR MODIFICATION OF STUDY
ATSDR Review of Request
Approval
Disapproval
ATSDR revision
Approval
Continue Research
Company proceeds with original MOU
Company Review of Disapproval
Company requests reconsideration
ATSDR Re-review
Approval
Disapproval
Company elects to terminate MOU
Company requests reconsideration
Company elects to terminate MOU
Company Review of Revision
Withdraw from MOU

Figure 1.

codified in 40 C.F.R. Part 792, Subparts B, C, D, E, F, G, J, and L, to the extent that such GLP standards apply. Should Good Epidemiology Practices ("e.g., Guidelines for Good Epidemiology Practices for Occupational and Environmental EPidemiologic Research"--The Chemical Manufacturers Association's Epidemiology Task Group, Journal of Occupational Medicine, Volume 33, 1221-1229, 1991) be relevant to a research project, those Practices should be affixed to the study plan.

IX. INSPECTIONS

The company shall ensure that an authorized employee or duly designated representative of ATSDR is permitted, at reasonable times and in a reasonable manner, to (i) inspect any research or testing facility that is conducting research pursuant to this MOU, and (ii) inspect (and, in the case of records, copy) any records and specimens required to be maintained in connection with research performed pursuant to this MOU.

X. PAYMENT OF COST AND EXPENSES

The company agrees to pay all costs, direct and indirect, associated with the research programs. ATSDR will assume responsibility for administrative costs including the cost of peer review as part of its overall program.

XI. EVENTS CONSTITUTING A BREACH OF THIS MEMORANDUM OF UNDERSTANDING

Failure by the company to:

i) initiate any test agreed to in the approved study plan, appended to this MOU, by the date established pursuant to the study plan;

ii) adhere to GLP's or established test procedures to the extent that these standards apply;

iii) submit any interim report required under this MOU by the date established pursuant to this MOU; or

iv) submit any final report which receives ATSDR's approval following the peer reviewers' recommendations

shall constitute a breach of this MOU. In the event of a breach, ATSDR will not impose any claim to damages, but at the Agency's discretion may terminate the MOU.

Since this MOU is entered into voluntarily by both parties, termination by ATSDR is not considered reviewable agency action pursuant to the Administrative Procedures Act or any other applicable federal law, and there will be no appeal process beyond that set out in the agreement or otherwise mutually agreed to by the parties.

XII. FINAL REPORT - SUBMISSION AND PUBLICATION OF DATA

All data and reports submitted to ATSDR pursuant to this MOU shall be sent to ATSDR, in duplicate, at the address indicated in Paragraph XIV below. Acceptance of the final report is contingent upon approval by ATSDR following the peer review panel's recommendations, consistent with CERCLA peer review requirements. The company maintains all rights to publication of data and results, however all results of research conducted pursuant to this MOU and all supporting data associated with the final research report will be made available by ATSDR to the public as part of its implementation of Section 104(i)(5) of CERCLA. The final report will not be accepted if the data is designated Confidential Business Information (CBI) or otherwise restricted from public disclosure with the exception of personally identifiable information on study subjects.

XIII. STATUTORY COMPLIANCE

Nothing in this MOU shall be construed to delay or otherwise affect or impair the authority of the President, the Administrator of ATSDR, or the Administrator of EPA to exercise any authority of the President, the Administrator of ATSDR, or the Administrator of EPA under any other provision of law, including TSCA and FIFRA, or the response and abatement authorities of CERCLA.

XIV. ADDRESSES

Any notifications, reports, or other written statements required to be submitted or sent to a party to this MOU shall be sent by certified mail to the parties at the following addresses:

Agency for Toxic Substances and Disease Registry
Division of Toxicology, Research Implementation Branch
Mail Stop E-29
1600 Clifton Road, N.E.
Atlanta, GA 30333

Attention: Dr. William Cibulas

Company I
Address

Attention: _____

Company II
Address

Attention: _____

XV. SIGNATURES

Agency for Toxic Substances and Disease Registry

Date:_____ By:_____

Company I.

Date:_____ By:_____

Company II.

Date:_____ By:_____

Appendix 1

Study Plan and Testing Protocols

Prior to study plan negotiations, ATSDR and the company shall sign a Letter of Intent indicating the good faith intention of both parties to achieve a mutually acceptable study plan. The study plan shall be negotiated and agreed upon prior to the signing of the MOU by ATSDR and the company. The following describes minimal requirements of the study plan. The attached time schedule (Table 2) reflects only the time line contained within the MOU. Other scheduling will be negotiated prior to signing of the MOU

The study plan will consist of (1) the identity of the MOU under which testing will be performed; (2) the specific tests to be performed; (3) the name(s) and address(es) of the company which will conduct the study; (4) the test protocol, including, where appropriate; (i) the rationale for any combination of test protocols, (ii) the rationale for species/strain selection, (iii) dose selection (and supporting data), (iv) route(s) or method(s) of exposure, (v) description of diet to be used and its source, including nutrients and contaminants and their concentrations, (vi) for in vitro test systems, a description of culture medium and its source, (vii) and a summary of expected spontaneous chronic disease (including tumors), genealogy, and life span; (5)

a schedule, with reasonable timetables and deadlines, for initiation and completion of each short-term test and of each major phase of long-term tests, and submission of interim progress report and final report to ATSDR; and (6) supporting data on the chemical substance(s) being tested, including physical constants, spectral data, chemical analysis, and stability under test and storage conditions, as appropriate. In some cases, the obligation to conduct research is contingent upon the results of certain tests that are to be performed first.

Prior to a company entering into an MOU with ATSDR, the study plan including all testing protocols and guidelines shall be reviewed by an ATSDR appointed peer review panel. Consistent with CERCLA section 104(i)(13), the peer review panel will consist of no fewer than three nor more than seven peer reviewers who a) are selected by the Administrator of ATSDR; b) are disinterested scientific experts; c) have a reputation for scientific objectivity; and d) lack institutional ties with any person involved in the conduct of the study under review.

TABLE 2

TIME SCHEDULE FOR STUDY PLANS					
Action		Secondary Action	Weeks to Implement Action	Weeks (Total)	Additional Review (Weeks)
Submit Statement of Interest for TASARC review		----------	----	----	----
Submit Letter of Intent		----------	0	0	----
Negotiation of study plan		----------			----
ATSDR study plan peer review		----------			----
Signing of approved study plan		----------			----
Signing of MOU		----------			----
Begin study		----------	8		----
	Request to modify study plan	ATSDR's response to modified study plan			2-6
		Disapproval	Company requests reconsideration		2
			ATSDR response to request		2
Interim Report		----------	Due every 6 months	----	----
End of study		----------			----
	Final Draft Report	----------	20		----
	ATSDR's Peer Review	----------			----
Final Report		----------	4		----

CONSIDERING PHARMACOKINETIC AND MECHANISTIC INFORMATION IN CANCER RISK ASSESSMENTS FOR ENVIRONMENTAL CONTAMINANTS: EXAMPLES WITH VINYL CHLORIDE AND TRICHLOROETHYLENE

H. J. Clewell*, P. R. Gentry, J. M. Gearhart, B. C. Allen, and M. E. Andersen

K. S. Crump Group, ICF Kaiser International

Ruston, Louisiana 71270 USA

ABSTRACT

Risk assessments for vinyl chloride (VC) and trichloroethylene (TCE) are presented as examples of approaches for incorporating chemical-specific pharmacokinetic and mechanistic information into a more scientifically plausible cancer risk assessment. For VC, the evidence regarding mode of action includes direct reaction of a metabolite with DNA, resulting in DNA adducts and mistranscription, and cross-species target-tissue correspondence of a rare tumor type. Risk estimates for human exposure to VC predicted with a physiologically-based pharmacokinetic (PBPK) model and the linearized multistage (LMS) model were lower than those currently used in environmental decision-making by a factor of 30 to 50, and were more consistent with human epidemiological data. For TCE, there is evidence of increased cell proliferation due to receptor interaction or cytotoxicity in every instance in which tumors are observed, and the tumors typically represent an increase in the incidence of a commonly observed, species-specific lesion. Virtually safe exposure estimates for human exposure to TCE predicted with a PBPK model and a margin of exposure (MOE) approach were higher than those obtained by the conventional LMS approach by roughly a factor of 100. The MOE approach is recommended as an alternative to the LMS approach for chemicals with a carcinogenic mode of action which entails increased cell proliferation, leading to the expectation of a highly nonlinear cancer dose-response.

* To whom correspondence should be sent

INTRODUCTION

Assessing the potential risk associated with human exposure to carcinogenic environmental contaminants represents an uncomfortable admixture of scientific evaluation and political policy, with the potential for enormous impact on both the public health and the economic well-being of the nation. The principal challenge facing cancer risk assessors today is to realistically consider the implications of the chemical's mechanism(s) of carcinogenicity

in developing a risk assessment approach for a particular carcinogenic effect. It is becoming increasingly difficult to justify the use of the same standard risk assessment approach with chemicals that act through a purely radiomimetic, genotoxic mechanism, as well as with chemicals for which carcinogenicity is mediated by increased cell proliferation secondary to cytotoxicity or receptor interaction. Mechanism-dependent risk assessment approaches are the only alternative for maintaining the credibility of cancer potency estimates in the face of increasing sophistication in the understanding of the mechanisms of carcinogenicity. The new draft revisions to the U.S. Environmental Protection Agency (USEPA) guidelines for cancer risk [1] would appear to provide the flexibility necessary to move forward in this area.

Risk assessments for chemical carcinogens must necessarily be iterative in nature. It is in the nature of scientific inquiry that understanding develops slowly, as experimental information accumulates and theories can be tested and refined. Risk assessments, however, cannot be postponed indefinitely until an adequate understanding of the carcinogenicity of a particular chemical has been achieved. Therefore, it is necessary to attempt to perform the most scientifically defensible assessment possible, given the information available at that time, and to be ready to revise the estimate, repeatedly, whenever important new information is developed. In the last few years there has been a significant improvement in the level of understanding regarding chemical carcinogenesis in general and the mechanisms of carcinogenicity of vinyl chloride (VC) and trichloroethylene (TCE) in particular. The purpose of the study reported here was to attempt to perform state-of-the-science risk assessments for VC and TCE, using to as great an extent as possible the information currently available on pharmacokinetics, metabolism, and carcinogenic mechanism of action.

VINYL CHLORIDE

When it became evident that VC was carcinogenic both in animals and in humans, many of its uses were discontinued; the current use of VC is limited to serving as a chemical precursor in the production of such materials as polyvinyl chloride (PVC) and copolymer resins. However, VC is also produced from the biodegradation of trichloroethylene by bacteria in the soil. Thus past spills of trichloroethylene may lead to current or future exposures of the public to VC in drinking water or other environmental media. The current potency estimates for VC published by the USEPA do not quantitatively incorporate pharmacokinetic information on VC into the risk calculations [2]. To provide a more accurate assessment of human risk from exposure to VC, a physiologically-based pharmacokinetic (PBPK) model was developed which describes the uptake, distribution and metabolism of VC in the mouse, rat, hamster, and human following inhalation or oral exposure. The PBPK model was used to predict the total production of reactive metabolites from VC both in the animal bioassays and in human exposure scenarios. These measures of internal exposure were then used in the linearized multistage (LMS) model [3] to predict the risk associated with lifetime exposure to VC in air or drinking water.

Evidence for carcinogenicity: The carcinogenicity of VC has been well established in several animal species by a number of routes of exposure [2]. Of the many different tumor types which have been reported in animal bioassays of VC, four are of greater concern because they have been seen reproducibly at low concentrations (250 ppm and below): liver angiosarcoma, hepatocellular carcinoma, nephroblastoma, and mammary gland adenocarcinoma. Of these, two are particularly notable in that they are rarely seen in unexposed animals: liver angiosarcoma and nephroblastoma. Greater than expected incidences of angiosarcoma of the liver have also been reported in a number of cohorts of workers occupationally exposed to VC [2]. Angiosarcoma of the liver is considered to be a very rare type of cancer, with only 20-30 cases per year reported in the U.S. [4]. Increased death due to cancer associated with human VC exposure has also been reported for brain, lung, and hematopoietic systems, as well as for other tissues, but several analyses have concluded that liver angiosarcomas show the clearest evidence for causal association and also demonstrate the highest relative risk [5]. The correspondence across species for liver hemangiosarcoma is quite striking and has made this tumor the primary focus for VC risk assessments in recent years.

Metabolism: Based on the elimination of VC observed following administration by various routes of exposure, the metabolism of VC appears to be a dose-dependent, saturable process. The primary route of metabolism of VC is by the action of the mixed function oxidase (MFO) system, now referred to as Cytochrome P450 or CYP, on VC to form chloroethylene oxide. Chloroethylene oxide (CEO) is a highly reactive, short-lived epoxide that rapidly rearranges to form chloroacetaldehyde (CAA), a reactive α-halocarbonyl compound [6]. The main detoxification of these two metabolites is conjugation binding with glutathione (GSH), as evidenced by the observation of decreased non-protein sulfhydryl concentrations at high VC exposure concentrations [7].

Mechanism of carcinogenicity: It has long been a tenet of carcinogenic risk assessment that the mechanism of carcinogenicity for "genotoxic" carcinogens (sometimes referred to as initiators) involves reaction with DNA, leading to mistranscription during subsequent cell division, causing a loss or change in heritable information which results in a neoplastic daughter cell. As early as 1978, it was demonstrated that binding of VC to liver macromolecules following inhalation exposure of rats correlated well with both total metabolism and the observed incidence of angiosarcoma [8]. It was suggested that the carcinogenicity of VC was due to binding of a reactive metabolite with DNA and subsequent miscoding during cell reproduction. The *in vivo* formation of four etheno-DNA adducts have since been demonstrated following exposure of animals to VC: 1,N^2-ethenoguanine; N^2,3-ethenoguanine; 1,N^6-etheno-2'-deoxyadenosine, and 3,N^4-etheno-2'-deoxycytidine [9]. These etheno-adducts are highly persistent and can lead to defective transcription [10].

Selection of a risk assessment approach: Based on the information described above on the metabolism and mechanism of carcinogenicity of VC, it is necessary to determine the appropriate approach for conducting a human risk assessment. The evidence is strong that the carcinogenicity of VC is related to the production of

reactive metabolic intermediates. The most appropriate pharmacokinetic dose metric for a reactive metabolite is the total amount of the metabolite generated divided by the volume of the tissue into which it is produced [11]. In the case of VC, a reasonable dose metric for angiosarcoma would be provided by the total amount of metabolism divided by the volume of the liver. The assumption underlying the use of this dose metric is that the concentration of the actual carcinogenic moiety, or the extent of the crucial event associated with the cellular transformation, is linearly related to this pseudo-concentration of reactive intermediates, and that the relationship of the actual carcinogenic moiety or crucial event to the dose metric is constant across concentration and species. Specifically, the average amount generated in a single day is used, averaged over the lifetime (i.e., the lifetime average daily dose, or LADD). The use of a dose rate, such as the LADD, rather than total lifetime dose, has been found empirically to provide a better cross-species extrapolation of chemical carcinogenic potency [12]. Subsequent steps in the carcinogenic mechanism related to specific adduct formation, detection, and repair, as well as to the consequences of DNA mistranscription and the potential impact of increased cell proliferation, have not yet reached the point where they can be incorporated into a risk assessment in any quantitative form. However, there appears to be sufficient evidence to justify the assumption that VC acts as a classic initiator, producing genetic transformations through direct reaction of its metabolites with DNA. Therefore the traditional assumption of low-dose linearity of risk appears to be warranted, and the LMS model would seem to be the most appropriate approach for low-dose extrapolation.

Description of PBPK model: The PBPK model for VC used in this study is an adaptation of a previously described PBPK model for vinylidene chloride [13]. For a poorly soluble, volatile chemical like VC, only four tissue compartments are required: a richly perfused tissue compartment which includes all of the organs except the liver, a slowly perfused tissue compartment which includes all of the muscle and skin tissue, a fat compartment which includes all of the fatty tissues, and a liver compartment. The physiological parameters used in the model are the current USEPA reference values [14]. The model assumes flow-limited kinetics, or venous equilibration; that is, that the transport of VC between blood and tissues is fast enough for steady state to be reached within the time it is transported through the tissues in the blood. The partition coefficients are based on in vitro studies with tissue suspensions [15]. All metabolism is assumed to occur in the liver, which is a good assumption in terms of the overall kinetics of VC, but which would have to be revised to include target-tissue-specific metabolism if a serious attempt were to be made to perform a VC risk assessment for a tissue other than the liver [11]. Metabolism of VC is modeled by two saturable pathways: one high affinity, low capacity, representing P450 2E1, and one low affinity, high capacity, representing the other P450 isozymes (e.g., 2C11/6 and 1A1/2). The parameters for the two oxidative pathways in the mouse, rat, hamster, and human were estimated by fitting the model to data from closed-chamber inhalation exposures with each of the species and strains of interest [16].

In the model, the reactive metabolites produced by these pathways (whether CEO, CAA, or other intermediates) may then either be metabolized further, leading to CO_2, react with GSH, or react with other

cellular materials, including DNA. Because exposure to VC has been shown to deplete circulating levels of GSH, a simple description of GSH kinetics was also included in the model [13]. Initial estimates for the subsequent metabolism of the reactive metabolites and for the glutathione submodel in the rat were taken from the model for vinylidene chloride [13]. These parameter estimates were then refined for the case of VC with data on glutathione depletion [17,7], total metabolism [18], and CO_2 elimination [19]. The parameters obtained for this portion of the model in the rat were used for the other species with appropriate allometric scaling (i.e., the first-order rate constants were scaled by body weight raised to the -1/4 power).

Pharmacokinetic risk assessment: The model just described was used to calculate the pharmacokinetic dose metrics for angiosarcoma in the most informative of the animal bioassays [20,21,22], as well as for human inhalation exposure. The 95% upper confidence limits (UCLs) on the human risk estimates for lifetime exposure to 1 part per billion (ppb) VC were then calculated on the basis of each of the sets of bioassay data, using the LMS model, and the resulting risk estimates are shown in Table 1.

Table 1: Human risk estimates (per million) for lifetime exposure to 1 ppb vinyl chloride in air based on the incidence of liver angiosarcoma in animal bioassays

Animal Bioassay Study	95% UCL Risk / million / ppb	
	Males	Females
Maltoni *et al.* - Mouse Inhalation [20,21]	1.52	3.27
Maltoni *et al.* - Rat Inhalation [20,21]	5.17	2.24
Feron *et al.* - Rat Diet [22]	3.05	1.10
Maltoni *et al.* - Rat Gavage [20,21]	8.68	15.70

The risk estimates based on inhalation studies with mice (1.5×10^{-4} and 3.3×10^{-4}) agree very well with those based on inhalation studies with rats (5.17×10^{-4} and 2.24×10^{-4}) , demonstrating the ability of pharmacokinetics to integrate dose-response information across species. The risks estimated from the dietary administration of VC (3.05×10^{-4} and 1.1×10^{-4}) are also in good agreement with those obtained from the inhalation bioassays, showing good route-to-route correspondence of potency based on the pharmacokinetic dose metric. However, the estimates based on oral gavage of VC in vegetable oil (8.68×10^{-4} and 15.7×10^{-4}) are about 6-fold higher than either dietary or inhalation exposure. Incorporation of corn oil into the diet increased the yield of aflatoxin B_1-induced tumors in rats [23]; a similar phenomenon could be responsible for the apparently higher potency of VC when administered by oil gavage compared to incorporation in the diet.

Epidemiological analysis: In order to evaluate the plausibility of the risks predicted on the basis of the animal data, risk calculations were also performed on the basis of the best available epidemiological data [24,25,26]. A linear relative risk dose-response model was used for analysis of the human data. To obtain pharmacokinetic, human-based risk estimates, the PBPK model was run for the exposure scenario appropriate to each of the selected subcohorts from each of the studies. The resulting internal dose metrics were multiplied by the appropriate durations to obtain the cumulative internal doses, which were then input into the relative risk model, along with the observed and expected liver cancer deaths for each subcohort, to obtain an estimate of the carcinogenic potency. Then, to determine the risk associated with a continuous lifetime exposure to 1 ppb for comparison with the animal results, the PBPK model was run for a 1 ppb continuous exposure and the average daily value of the internal dose metric was calculated. Using the 95% upper bound on the estimate for the potency provides a 95% upper confidence limit on the lifetime risk per ppb of vinyl chloride for comparison with the animal-based results obtained with the LMS model.

Table 2: Human risk estimates (per million) for lifetime inhalation of 1 ppb vinyl chloride in air based on the incidence of liver angiosarcoma in human epidemiological studies

Epidemiological Study	95% UCL Risk / million / ppb
Fox & Collier [24]	0.71 - 4.22
Jones *et al.* [25]	0.97 - 3.60
Simonato et al. [26]	0.40 - 0.79

A comparison of the results of the analyses of the three sets of data, shown in Table 2, gives some indication of the consistency of the human results, even before the comparison with the animal predictions. It is encouraging that the lifetime risk of liver cancer per ppm VC exposure estimated from the three studies only ranges over about one order of magnitude: from 0.4×10^{-4} to 4.2×10^{-4}. Moreover, these estimates are in remarkable agreement with the estimates based on animal data shown in Table 1. However, any confidence produced by this agreement should be tempered by the likelihood that misclassification of exposure in the human studies tends to underestimate the true risk at lower doses. Nevertheless, the agreement of the pharmacokinetic animal-based risk estimates with the pharmacokinetic human-based risk estimates provides strong support for the assumption used in this study: that cross-species scaling of lifetime cancer risk can be performed on a direct basis of lifetime average daily dose (without applying a body surface area adjustment) when the risks are based on biologically appropriate dose metrics calculated with a validated PBPK model.

Conclusions: Giving priority to the animal studies most closely approximating the human route of exposure, the best conservative estimate of the carcinogenic risk of angiosarcoma from lifetime exposure to 1 ppb

VC in air is 5.2×10^{-4}, or 2.0×10^{-4} $(\mu g/m^3)^{-1}$, based on inhalation studies in male rats [20,21]. This value is consistent with the range of estimates from epidemiological studies of 0.4×10^{-4} to 4.2×10^{-4} risk per ppm VC, but is roughly a factor of 30 below the currently published inhalation unit risk of 8.4×10^{-3} $(\mu g/m^3)^{-1}$. The model was also used to estimate the daily internal dose for human drinking water consumption. The resulting best conservative estimate of the carcinogenic risk of angiosarcoma from lifetime exposure to 1 $\mu g/L$ VC in drinking water is 1.14×10^{-4} $(\mu g/L)^{-1}$, based on studies with male rats of the dietary administration of VC [22]. This value is roughly a factor of 50 below the currently published unit risk of 5.4×10^{-3} $(\mu g/L)^{-1}$.

Although VC has often been cited as a chemical for which saturable metabolism should be considered in the risk assessment, saturation appears to become important only at very high exposure levels (greater than 250 ppm by inhalation or 25 mg/kg/day orally) compared to the lowest tumorigenic levels, and thus has little impact on the quantitative risk estimates. The important contribution of pharmacokinetic modeling is to provide a more biologically plausible estimate of the effective dose: total production of reactive metabolites at the target tissue. The ratio of this biologically effective dose to the administered dose is not uniform across routes and species. Therefore any estimate of administered dose is less adequate for performing route-to-route and interspecies extrapolation of risk. The risk estimates obtained for VC using the pharmacokinetic dose metric are lower than those obtained with conventional external dose calculations by a factor of 30 to 50, and appear to be more consistent with human epidemiological data.

TRICHLOROETHYLENE

TCE has been widely used in industry for many years because of its excellent solvent properties and its nonflammability. The ACGIH has recently announced its intention of classifying TCE into a new carcinogenicity group, A5 (not suspected as a human carcinogen), based on a well-conducted, negative epidemiological study performed in an aircraft maintenance facility at Hill Air Force Base by the National Cancer Institute [27,28]. The USEPA, on the other hand, has for a number of years regulated TCE on the basis of its carcinogenicity, although it has wavered between group 2B (sufficient evidence in animals) and C (limited evidence) in trying to classify the likelihood of carcinogenicity from TCE [29,30,31]. However, in 1989 the International Agency for Research on Cancer classified TCE as a group 3 animal carcinogen (limited evidence) [32], and the USEPA has since withdrawn the classification of TCE from its IRIS database for consideration. Nevertheless, regardless of the formal classification the USEPA cancer risk estimates for TCE [30,31], calculated on the basis of metabolized dose with the LMS model, have continued to be used for environmental decision-making since 1985. In contrast to the case of VC, the most recent potency estimates for TCE published by the USEPA do attempt to incorporate pharmacokinetic information on TCE into the risk calculations [30,31]. The current USEPA unit risks for TCE, 1.7×10^{-6} $(\mu g/m^3)^{-1}$ and 0.32×10^{-6} $(\mu g/L)^{-1}$, are based on total metabolized dose in mg/kg/day, adjusted by body surface area (i.e., by the ratio of the body weights raised to the negative 1/3 power), which provides a reasonable

approximation to the internal exposure (area under the concentration curve) for the metabolites. However, it is disconcerting to note that these pharmacokinetically based potencies for TCE are very similar to those shown above for VC, in spite of the strong epidemiological evidence suggesting that VC is a more potent human carcinogen than TCE. Clearly, pharmacokinetics alone is inadequate to provide a reasonable comparison of the human risk for cancer from these two chemicals. Just as the pharmacokinetics of a chemical must always be considered in order to obtain a realistic measure of internal exposure to the chemical, the pharmacodynamics of the chemical (that is, the mechanism by which the chemical causes cancer) must also be considered in order to obtain a realistic measure of the response to the chemical.

Several steps are involved in performing a risk assessment for TCE that considers both pharmacokinetics and mechanism. Information must first be gathered on the pharmacokinetics and metabolism of TCE, as well as on each of its key metabolites: chloral (CHL), trichloroacetic acid (TCA), trichloroethanol (TCOH), dichloroacetic acid (DCA), and dichlorovinylcysteine (DCVC). This pharmacokinetic and metabolism data can then be used in a PBPK model to provide a prediction of the concentration profiles for TCE and its metabolites in each of the target tissues, whether associated with exposure to TCE in the animal bioassays or in potential human exposure scenarios. Mechanistic information specific to each of the tumors of concern must then be incorporated to provide a link between target tissue chemical exposure and biological or biochemical effects in the target tissue leading to the observed cancer response. The specific mode of action associated with the production of a particular tumor provides the basis for expectations regarding both the dose-response for tumor incidence and the nature of cross-species scaling. These expectations, in turn, should drive decisions concerning the most appropriate risk assessment approach and the assumptions to be made where chemical-specific data are lacking.

Evidence for carcinogenicity: By far the most common carcinogenic outcomes associated with TCE exposure are liver and lung tumors in several strains and both sexes of mice [33]. Statistically increased tumor outcomes observed in only a single study include malignant lymphoma in HAN:NMRI mice exposed by inhalation, renal tubular cell adenoma and carcinoma in male F344 rats exposed by oral gavage, and benign testicular (leydig cell) tumors in Sprague-Dawley rats exposed by inhalation. Of these, the kidney tumors have raised the greatest concern since they were not observed in control animals. Direct human evidence of carcinogenicity from TCE exposure is equivocal at best: epidemiological studies have generally been negative, although most are limited by problems due to small cohorts, inadequate latency periods, and co-exposure to other contaminants [33]. The largest study, mentioned above [27,28], was unable to link TCE exposure with increased cancer incidence in any tissue. A few epidemiological studies have, however, tentatively linked TCE exposure with increased incidence of urinary tract tumors and lymphoma in workers, as well as with childhood leukemia [33].

Metabolism: Based on both *in vitro* and *in vivo* studies, the metabolism of TCE has been suggested to consist of saturable oxidation of TCE to CHL by the MFO system, followed by either oxidation of CHL to TCA by an aldehyde oxidase or reduction to TCOH by alcohol dehydrogenase (ADH) with subsequent glucuronidation; oxidation of TCOH to TCA was also proposed [34]. DCA has been identified as a minor urinary metabolite of TCE (on the order of 1%) in both rats and mice, but has not been detected as a metabolite of TCE in the human. Significantly, the clearance of DCA in humans appears to be much more rapid than would be expected from allometric scaling of animal data; the extremely high rate of clearance of DCA in humans is probably responsible for the failure of investigators to detect it as a metabolite of TCE.

Mechanism of carcinogenicity in the liver: It has been suggested that both TCA and DCA play a major role in the tumor incidence observed in mice dosed with TCE [35]. Both compounds have been shown to produce focal hyperproliferative lesions, adenomas, and carcinomas on chronic administration. The typical sequence of events for tumorigenicity can be described as follows: initially upon treatment with DCA or TCA, there is evidence of a slight, but generalized liver hyperplasia, consistent with the induction of a mitogenic signal by the chemical. However, this increased cell proliferation soon returns to a normal liver turnover rate in spite of continued exposure to the mitogen, presumably in response to the expression of endogenous negative growth factor (TGF-β) by stromal cells. Upon repeated exposure for about 30 weeks, however, there is a sudden appearance of hyperplastic nodules, which eventually progress to neoplastic lesions. This sequence of events is entirely consistent with a "suppression escape" mechanism for promotional carcinogenicity [36]. The sudden appearance of rapidly dividing cells, representing an escape from cytostatic suppression, produces a greatly increased probability of mutational events leading to an increased tumorigenicity.

The observation that similar concentration-time profiles of DCA and TCA produce the hyperplastic and tumorigenic responses in the mouse but not in the rat apparently reflects a difference in the susceptibility of the two species to the onset of hyperplasia. Since *in vitro* studies with rat hepatocytes have demonstrated the mitogenic response, the most likely possibility for the observed difference in susceptibility is a differential genetic predisposition for the escape from suppression. The maternal imprinting of the gene for a negative growth factor receptor in the mouse [37] provides one such possible explanation, if the rat is not similarly predisposed genetically. Since the human appears to have both alleles for this gene [37], it is possible that the much lower potency of TCA and DCA in the rat provides a more realistic estimate of the potency that could be expected in the human.

Mechanism of carcinogenicity in the lung: Tumors have also been observed in the lungs of mice exposed to TCE by inhalation. The mechanism in this case appears to be entirely different from that just described for the liver. In a well-designed experimental effort [38], which provides an excellent example of the kind of studies needed to support biologically-based risk assessments, investigators at ICI combined *in vivo* and *in vitro* experiments to

elucidate the mechanism of TCE carcinogenicity in the mouse lung. In the *in vivo* studies, female mice and rats were exposed to TCE at a range of inhaled concentrations at and below the concentrations at which tumors are observed in mice, and the effects of TCE in the lung were determined. A specific lesion, characterized by vacuolization of lung Clara cells, was observed in mice, but not rats. There was evidence of a threshold for the Clara cell effects at about 20 ppm. Mice exposed to 100 ppm CHL by inhalation displayed Clara cell lesions similar to those observed with 1000 ppm TCE. In contrast to these results, only mild effects were observed with TCOH inhaled at 100 ppm, and none were observed with 500 mg/kg TCA given intraperitoneally (the effects had been observed with intraperitoneally administered TCE at 2000 mg/kg). These results suggested that CHL was responsible for the toxicity.

In the *in vitro* studies, mouse lung Clara cells were shown to metabolize TCE to CHL, TCOH, and TCA, with CHL being the major metabolite. Significantly, no TCOH glucuronide was detected. In comparison with mouse Clara cells, mouse hepatocytes were shown to produce primarily TCOH and its glucuronide. In both cell preparations, a steady state concentration of CHL was achieved. Separate *in vitro* studies demonstrated that mouse Clara cells possess a relatively low activity for the glucuronidation of TCOH as compared either to the glucuronidation of other substrates in the lung or to the glucuronidation of TCOH in the liver. It has also been determined that ADH, the enzyme which converts CHL to TCOH, has a low activity in the mouse lung, consistent with the relatively low production observed in the Clara cells. On the basis of this evidence, the investigators concluded that the observed acute toxicity in the lung was a result of accumulation of CHL in Clara cells resulting from a limitation in the formation of TCOH and its glucuronide. The specificity of this lesion for the Clara cells can be rationalized in terms of their relatively high Cytochrome P450 activity, coupled with limited ADH and UDP glucuronosyl transferase (UGT) activities.

The implications of these results for the lung tumorigenicity of TCE are two-fold. First, the accumulation of CHL, if it does occur *in vivo*, has clear carcinogenic implications, since CHL has been shown to be genotoxic in a number of studies [38]. Secondly, the recurrent toxicity observed with intermittent exposure is likely to produce compensatory cell proliferation, exacerbating the genotoxic effect. The fact that the lung tumors were generally benign is also significant: the production of primarily benign tumors is more consistent with a nongenotoxic, cell-proliferative mechanism.

Mechanism of carcinogenicity in the kidney: While both of the tumors discussed thus far are observed in the mouse but not in the rat, the reverse is true for the kidney tumors produced by TCE. A mechanism for the induction of these tumors has been proposed, in which direct conjugation of TCE with glutathione (GSH) in the liver is followed by further metabolism in the kidney to a cysteine conjugate which can then be cleaved to a reactive intermediate in the kidney tubular cells [39]. The cysteine conjugate formed from TCE, dichlorovinylcysteine (DCVC), has been shown to be highly nephrotoxic as well as mutagenic in the Ames test.

Detoxification and clearance of DCVC takes place by urinary excretion of the N-acetyl derivative; the fact that N-acetyl-DCVC has been identified in the urine of humans exposed to TCE occupationally [39], indicates that exposure of the kidney to DCVC does occur in the human.

As with the two previous cases, cell proliferation also appears to play a role in this tumor outcome. In the only bioassay that reported a significant increase in kidney tumors from TCE, cytotoxicity was observed in the kidney at both the low and high doses, while tumors were observed only at the high dose. Kidney cytotoxicity was also reported in association with a non-statistically-significant incidence of kidney tumors in the only other study demonstrating the tumor response.

Selection of a risk assessment approach: With regard to the use of a cancer dose-response model, the highly nonlinear dose-response expected for the receptor-mediated, promotional mechanism suggested for the liver carcinogenicity of TCE argues against the use of the usual linear extrapolation to low-dose risk associated with the use of the LMS model [3]. In the case of the lung and kidney, although genotoxicity may lead to a small but finite residual risk component which is linear at low dose, there is also evidence for cytotoxicity at the high doses where tumors are actually observed. The extreme nonlinearity of the impact of cytotoxicity driven, compensatory cell-proliferation on risk at the doses where tumors are observed is incompatible with the behavior and underlying assumptions of the LMS model, even if a pharmacokinetic dose metric is used. It has frequently been suggested that the LMS model may simply be inappropriate for use with chemicals whose carcinogenicity is mediated by changes in cell proliferation, and that a promising alternative in such cases is a biologically based dose-response (BBDR) model of cancer which incorporates cell proliferation; it is also possible to link such cancer models to PBPK descriptions of target tissue exposure to provide a more complete description of the carcinogenic process for cytotoxic or mitogenic chemicals [40,41]. However, there are at least two difficulties associated with the use of these alternatives to the LMS model. First, the parameters for the cell proliferation models are often not available from direct experiment, but must be estimated by fitting bioassay data. Unfortunately, it has been shown that the parameters in the BBDR model are not independently identifiable under such conditions, and therefore the estimates of risk at low dose could vary widely depending on the specific parameterization chosen [42]. Secondly, even when experimental data on hyperplastic nodules or altered hepatic foci permit a more independent estimate of the cancer model parameters, low-dose risk estimates with the typical 2-stage BBDR model are exquisitely sensitive to the estimated dose-response for the model parameters [43].

Another alternative which has been suggested for risk assessments with non-genotoxic carcinogens is the use of a threshold approach based on the underlying process which is required for carcinogenicity [44]. The rationale for the expectation of a threshold in non-genotoxic carcinogenicity is that, unlike the case for direct reaction with DNA, the nonlinear process underlying carcinogenicity in these cases (e.g., cytotoxicity or response to receptor binding) is not one which would be expected to be active at very low doses. The greatest difficulty in

gaining acceptance for the threshold approach is that, strictly speaking, it implies that no increased risk is incurred from exposures below the threshold, but only an apparent (experimentally observable) threshold can be determined. Residual carcinogenic activity below the apparent threshold for the nonlinear process could result from insufficient experimental power to detect the effect at lower incidence, from secondary mechanisms (e.g. from the mutagenic activity of a chemical which is also cytotoxic at higher concentrations), or from the inherent nature of the dose-response for the effect (e.g. for receptor-mediated effects possessing a linear dose-response in the low-dose regime).

The margin of exposure (MOE) approach is similar in practice to the threshold approach, but the assumptions underlying its use are not as constraining. Rather than estimating a human exposure threshold below which no risk is expected, the MOE approach merely estimates the human exposure producing a dose-metric value which is a specified factor ("margin") below the value of the dose metric at which a minimal tumor response (e.g. 10% -- the ED_{10}) was observed in animals. The MOE approach admits the possibility that in spite of the presence of a highly nonlinear dose-response in the experimental regime there may still be residual risk, and even low-dose linear behavior, below the apparent threshold for the nonlinear process. However, it relies on the nonlinearity of the process underlying the carcinogenic mode of action to assure that the margin of risk between the human and animal exposures is much greater than the MOE. That is, an MOE of 100 might be expected to provide a risk reduction of greater than 1000, while an MOE of 1000 might be expected to provide a risk reduction of greater than 100,000 (since it is expected that risk falls off at a much faster rate than exposure).

Description of PBPK model: The PBPK model for TCE used in this study is an expansion of a previously published model of TCE and its metabolite TCA [45], to include the other key metabolites: DCA, TCOH (which is the principal source of DCA), DCVC in the kidney, and CHL in the lung. The parent chemical portion of the model includes individual tissue compartments for the liver, gut tissue, fat, and tracheo-bronchial region of the lungs. All other tissues are lumped into rapidly perfused (kidney, brain, alveolar region of lungs, etc) and slowly perfused (muscle, skin, etc) compartments. The model includes both inhalation and oral routes of exposure. Oral gavage is modeled using a two-compartment description of the GI tract. Allometric scaling is used throughout the model (flows and capacities scaled by body weight to the three-quarters power, rate constants scaled by body weight to the negative one-quarter power) to simplify intraspecies and interspecies extrapolation.

The model includes three target tissues: lung, kidney, and liver. The dose metrics provided in the lung are the instantaneous concentration and area under the curve (AUC) for CHL in the tracheo-bronchial region, which is assumed to be produced by saturable production and clearance of CHL in Clara cells. The dose metric in the kidney is total production of the thioacetylating intermediate from DCVC divided by the volume of the kidney. The model implicitly assumes that all glutathione conjugation of TCE leads eventually to the appearance of DCVC in the kidney. Clearance of TCE by N-acetyl-transferase into the urine is also modeled. Two dose metrics are

included in the description of the liver: AUC for DCA and AUC for TCA. The model assumes that all oxidative metabolism proceeds through CHL, which is further metabolized to TCA and TCOH. TCOH can subsequently be oxidized to TCA, conjugated with glucuronic acid, or reduced to DCA. DCA is also produced from the reduction of TCA. Biliary excretion of TCOH glucuronide and enterohepatic recirculation of free TCOH is described, with only the glucuronide being excreted in the urine. The model is able to reproduce data on TCE, TCOH and TCA kinetics in the mouse, rat, and human, as well as DCA kinetics in mice, for both inhalation exposure and oral gavage.

Table 3: Comparison of virtually safe lifetime exposure levels (ppb in air or μg/L in water) for TCE based on the Margin of Exposure (MOE) approach and the Linearized Multistage (LMS) approach

MOE[a] = 1000	ED_{10} / MOE	MOE Level	10^{-6} Risk Level[b]
- Inhalation (ppb):			
Lung	0.009	6000 (9)[c]	41 (0.06)
Kidney	9.02	15000 (36)	300 (0.64)
Liver	3.59	88 (12.5)	0.35 (0.05)
- Drinking Water (μg/L):			
Lung	0.009	600,000 (900)	4000 (6.0)
Kidney	9.02	225,000 (540)	4500 (9.6)
Liver	3.59	390 (56)	5.6 (0.8)

[a] Margin of exposure below ED_{10} (dose corresponding to an extra risk of 10%)
[b] Lifetime extra cancer risk based on the Linearized Multistage Model
[c] Alternate (worst-case) calculation -- see text

Pharmacokinetic risk assessment: The results of the dose metric calculations with the PBPK model are summarized in Table 3. In this table, the most plausible estimates of acceptable exposure levels are shown for each target tissue and human exposure scenario of concern. The numbers in parentheses represent alternative, worst-case risk estimates. The purpose for including these alternative estimates is to demonstrate the broad uncertainty in the current risk estimates. In every case the discrepancy between the best estimate and worst-case estimate could be greatly reduced by experiments which are well within the state of the science. In the case of lung tumors, the numbers in parentheses represent the calculations which assume that the cross-species scaling for the clearance of CHL in the lung parallels that of P450 (which falls off dramatically) rather than following allometric expectations. In the case of kidney tumors, the numbers in parentheses represent the calculations which

assume an extremely low GST pathway production in the rat compared to the human (based on one animal study) rather than assuming a production more in keeping with allometric expectations (based on another animal study). Both of these uncertainties can readily be addressed by *in vitro* studies similar to those which have been performed with methylene chloride [47]. In the case of liver tumors, the numbers in parentheses represent the calculations which assume that the human susceptibility to mitogenic carcinogens is similar to that of the mouse, as opposed to the most plausible estimates, which assume that the human susceptibility is more similar to the rat. The studies needed to resolve this question are more difficult to define, but the importance of this question goes well beyond TCE, and the benefits of such studies would be significant.

Table 3 also demonstrates two different approaches for estimating acceptable exposure levels for the public. In the traditional quantitative risk estimate approach, the LMS model is used to obtain quantitative estimates of the risk associated with a given human exposure scenario, based on the bioassay dose-response data. Typically, USEPA considers an increased lifetime risk of cancer on the order of 10^{-6} to be acceptable for the public [29,30,31]. Depending on the target tissue, the TCE exposure levels associated with an increased lifetime risk of 10^{-6} range from 0.35 to 300 ppb in air or 5.6 to 4500 μg/L in water. For comparison, the most recently published risk estimates from USEPA would equate to lifetime 10^{-6} risk exposure levels of 0.11 ppb and 3.1 μg/L.

A second approach for estimating acceptable levels is to simply relate the human exposure level to the animal bioassay results by determining the ratio between the ED_{10} in the animals (calculated from the bioassay data using the multistage model) and the dose metric for the human exposure. Alternatively, an acceptable ratio, or MOE, can be set and the corresponding human exposure can be calculated directly from the ED_{10} and the MOE. This is the approach shown in the left side of Table 3. In each case, the acceptable dose metric levels were calculated by dividing the appropriate ED_{10} by the desired MOE. The model was then used to translate the acceptable dose metric level into an acceptable exposure level. Based on an analogy between nongenotoxic carcinogenicity and noncancer toxicity, a minimum MOE of 100 would seem to be justified on the basis of 10 for human variability (particularly for variability in the activities of the key metabolizing enzymes) and 10 for uncertainty in the animal to human extrapolation. For exposures of the public, it might sometimes be appropriate to add an additional margin of 10 because of the potentially large number of individuals exposed. For the purpose of this illustration an MOE of 1000 was used in obtaining the public exposure levels in Table 3. Depending on the target tissue, the TCE exposure levels which provide an MOE of 1000 range from 88 to 15000 ppb in air or 390 to 600,000 μg/L in water. In general, the MOE approach results in acceptable levels which are higher than those obtained by the LMS approach by roughly two orders of magnitude.

Conclusions: The basis for determining which of the two approaches is the most appropriate is the mode of action of the chemical carcinogenicity being considered. For example, it would seem clear that the use of the LMS approach is both justified and preferable in the case of a carcinogen such as vinyl chloride for which the evidence regarding mode of action includes (a) direct reaction of a metabolite with DNA that results in DNA

adducts and mistranscription; (b) no evidence of enhanced cell proliferation, receptor interaction, or cytotoxicity at clearly tumorigenic doses; and (c) cross-species target-tissue correspondence of a rare tumor type. The cancer risk from a genotoxic chemical like vinyl chloride is very likely to fall off linearly with dose, even to very low exposure levels. On the other hand, the MOE approach seems quite applicable, and more appropriate than the LMS approach, for a carcinogen such as TCE for which (a) there is no similar evidence of direct interaction with DNA, (b) there is evidence of enhanced cell proliferation due to receptor interaction or cytotoxicity associated with every target tissue, and (c) there is little evidence of cross-species correspondence or the production of rare tumor types (the kidney tumors providing the possible exception). The cancer risk from a chemical like TCE, for which the mode of action appears to involve the highly nonlinear impact of enhanced cell proliferation, is very likely to fall off much faster than dose, producing an incremental reduction in risk far exceeding the reduction in exposure.

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