CHEMICAL MANUFACTURERS ASSOCIATION

May 2, 1996

Ms. Joan Dollarhyde
Integrated Risk Information System (IRIS) Submission Desk
NCEA (MS-190)
U.S. Environmental Protection Agency (EPA)
26 Martin Luther King Drive
Cincinnati, OH 45268

Dear Ms. Dollarhyde:

In accordance with the Federal Register notice of Tuesday, April 4, 1996, this is to inform you of our intent to submit information about Vinyl Chloride (CAS. NO. 75-01-4) for the IRIS Pilot Project. The purpose of our submission is to identify cancer and non-cancer risk assessment materials which may not be readily accessible to EPA's internal and external peer review groups. Specifically, we intend to submit information on the following:

- Reitz, R.H.,et al, "Predicting Cancer Risk from Vinyl Chloride Exposure with a Physiologically Based harmacokinetic Model." *Toxicology and Applied Pharmacology* 137, 253-267 (1996)

- Clewell, H.J., et al, "Considering Pharmacokinetic and Mechanistic Information in Cancer Risk Assessments for Environmental Contaminants: Examples with Vinyl Chloride and Trichloroethylene." In Press.

In addition, through a Memorandum of Understanding with the Agency for Toxic Substances and Disease Registry , CMA is conducting a combined inhalation two-generation reproductive and developmental toxicity study of Vinyl Chloride in rats. The results of this study also will be made available to EPA for the IRIS Pilot Project.

CMA's interest in the IRIS Pilot Project represents the concern of its Vinyl Chloride Health Committee which is composed of representatives from all U.S. manufacturers of Vinyl Chloride. The Committee also will be gathering information on Vinyl Chloride in the near future which may not be easily accessible to EPA.

We believe that the organization of this material is of key importance to a comprehensive characterization of Vinyl Chloride risk assessment. We look forward to working with EPA on this issue. Any questions about this material should be referred to me at 703/741-5639.

Sincerely,

Robert A. Venezia

Robert A. Venezia, Dr.P.H.
Manager, CHEMSTAR

CMA 115686

1300 WILSON BOULEVARD, ARLINGTON, VA 22209 • TELEPHONE 703-741-5000 • FAX 703-741-6000

URGENT FAX

RUSH TO: Dr. Robert Venezia, Chem Manu Assoc

FAX: 703 741-6091

FROM: RHR Toxicology Consulting

PAGES (INCLUDING THIS COVER): 3

May 7, 1996

Dear Dr. Venezia,

Here is a copy of the EMAIL which Ie submitted to IRIS in connection with the project which Mike Gargas and I have proposed to carry out for CMA's VC panel.

I apologize for submitting material prematurely --- I did NOT want to miss the submission deadline and took the liberty of preparing a list for IRIS. I understand from Mike that CMA had planned to ask for an extension of the submission deadline. Sorry about that!

None of the materials have been submitted to IRIS; just the list as requested in the Federal Register Notice. Hope this has not caused you any inconvenience, and we look forward to your response to our proposal.

Dick Reitz

IRIS SUBMISSION --- to IRIS.comments@epamail.epa.gov, on May 2, 1996
RESPONSE TO REQUEST FOR SUPPLEMENTAL INFORMATION (VINYL CHLORIDE)
Fed ral Register Notice: Vol. 61, No. 64, April 2, 1996 (pages 14570-14571)

Gentlemen,

We believe you may find the following documents/items useful during your re-evaluation of the carcinogenic potency of vinyl chloride (CAS 75-01-4).

(1). Reitz, R.H., Gargas, M.L., Andersen, M.E., Provan, W.M., and Green, T.L. (1996). Predicting cancer risk from Vinyl Chloride exposure with a Physiologically Based Pharmacokinetic model. Toxicol. Appl. Pharmacol. 137, 253-267.

(2). VCDOSE2.CSL --- The extensively commented source code for a Physiologically Based Pharmacokinetic model capable of describing the uptake, distribution, and conversion of vinyl chloride to its reactive and genotoxic metabolites. This source code may be compiled to an executable model with the software packages ACSL-PC and/or SimuSolv.

(3) VCDOSE2.CMD --- The associated parameter definitions and procedures used simulate different species and exposure conditions with the PBPK model described above.

(4) Baretta, E.D., Stewart, R.D., and Mutchler, J.E. (1969) Monitoring exposures to vinyl chloride vapor: Breath analysis and continuous air sampling. Amer. Indust. Hygiene Assoc. Journal, 30, 537- 544.

(5) Buchter, A., Bolt, H.M., Filser, J., Goergens, H.W., Laib, R.J., and Bolt, W. (1978). Pharmakokinetik and karzinogenese von vinylchlorid abreitmedizinische risikobeurteilung. Verh. Dtsch. Ges. Arbeitsmed., 18, 111-124 <ENGLISH TRANSLATION>.

(6) Creech, J.L., & M.N. Johnson (1974), Angiocarcoma of the liver in the manufactur of PVC.J. Occup. Med., 16, 150-151.

(7) Drew, R.T., G.A. Boorman, J.K. Haseman, E.E. McConnell, W.M. Busey, & J.A. Moore (1983) The effect of age and exposure duration on cancer induction by a known carcinogen in rats, mice, and hamsters. Toxicol. Appl. Pharmacol., 68, 120-130.

(8) ECETOC (1988), Technical Report No. 31 "The mutagenicity and carcinogenicity of vinyl chloride: A historical review and assessment." ISSN 0773-8072-31, Brussels, Belgium.

(9) Gargas, M.L., Andersen, M.E. and Clewell, H.J. III. (1986). A physiologically-based simulation approach for determining metabolic constants from gas uptake data. Toxicol. Appl. Pharmacol. 86, 341-352.

(10) Gargas, M.L., Burgess, R.J., Voisard, D.E., Cason, G.H., and Andersen, M.E. (1989) Partition coefficients of low molecular weight volatile chemicals in various liquids and tissues. Toxicol. Appl. Pharamcol., 98, 87-99.

(11) Gehring, P.J., P.G. Watanabe, & C.N. Park (1978) Resolution of dose-response toxicity data for chemicals requiring metabolic activation. Toxicol. Appl. Pharmacool., 44, 581-591.

(12) Guengerich, F.P. & P.G. Watanabe (1979) Metabolism of (14C) and (36Cl)-labeled vinyl chloride in vivo and in vitro. Biochem. Pharmacol, 28, 589-596.

(13) Guengerich, F.P., Kim, D.H., and Iwasaki, M. (1991) Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem. Res. Toxicol., 4, 168-179.

(14) Kappus, H., H.M. Bolt, A. Buchter, & W. Bolt (1976) Liver microsomal uptake of 14C-VC and transformation to protein alkylating metabolites in vitro. Toxicol. Appl. Pharmacool., 37, 461.

(15) R.L., and Woods, J.S. (1978) Carcinogenicity of vinyl chloride and vinylidene chloride. J. Toxicol. Envir. Hlth., 4, 15-26.

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(16) Maltoni, C., C. Lefemine, P. Chieco, & D. Carrettu (1974), Vinyl chloride carcinogenesis: Current results and perspectives. Med. Lav., 65, 421.

(17) Raucy, J.L., J.C. Kraner, & J.M. Lasker (1993) Bioactivation of halogenated hydrocarbons by Cytochrome P4502E1. Critical Reviews in Toxicology, 23, 1-20.

(18) Reitz, R.H., Mendrala, A.L. and Guengerich, F.P. (1989). In Vitro Metabolism of Methylene Chloride in Human and Animal Tissues: Use in Physiologically-Based Pharmacokinetic Models. Toxicol. Appl. Pharmacol. 97, 230-246.

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(22) Watanabe, P.G., G.R. McGowan, E.O. Madrid, & P.J. Gehring (1976) Fate of 14C-vinyl chloride following inhalation exposure in rats. Toxicol. Appl. Pharmacol., 37, 49-59.

TOXICOLOGY AND APPLIED PHARMACOLOGY 137, 253–267 (1996)
ARTICLE NO 0079

Predicting Cancer Risk from Vinyl Chloride Exposure with a Physiologically Based Pharmacokinetic Model

RICHARD H. REITZ,*[,1] MICHAEL L. GARGAS,† MELVIN E. ANDERSEN,‡ W. M. PROVAN,§ AND TREVOR L. GREEN§

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Predicting Cancer Risk from Vinyl Chloride Exposure with a Physiologically Based Pharmacokinetic Model. REITZ, R. H., GARGAS, M. L., ANDERSEN, M. E., PROVAN, W. M., AND GREEN, T. L. (1996). *Toxicol. Appl. Pharmacol.* 137, 253–267.

A physiologically based pharmacokinetic (PBPK) model capable of describing the metabolism of vinyl chloride (VC) in rats, mice, and humans has been developed and validated by comparison with experimental data from experiments not used in model development. This PBPK model has been used to predict measures of delivered dose (reactive VC metabolites produced in the livers of the affected species) hypothesized to be involved in the induction of liver angiosarcoma in rats, mice, and human populations exposed to VC. Measures of delivered dose in rats were fit to an empirical dose–response model (the linearized multistage model of Crump *et al.*) and used to make predictions of liver angiosarcoma incidence in mice and human populations exposed to VC. This procedure gave a good prediction of angiosarcoma incidence in mice. Predictions of angiosarcoma incidence in humans were more than two orders of magnitude lower than risk estimations which did not utilize pharmacokinetic data, but were still almost an order of magnitude higher than actually observed in exposed human populations.

Vinyl chloride (1-chloroethylene, VC) is a colorless, explosive gas. VC is only slightly soluble in water but dissolves readily in fats and organic solvents. VC is most commonly used as a precursor for the production of polyvinyl chloride (PVC) plastics, and the highest potential for human exposures exists at the sites where PVCs are manufactured. Because this material has relatively low acute toxicity, occupational exposure standards for VC (OEL) were typically 500 ppm (ECETOC, 1988) until 1970 when Viola discovered that rats exposed to VC vapor developed an increased incidence of tumors (Viola, 1970; Viola *et al.*, 1971). Viola's results were confirmed by Maltoni *et al.* in 1974, and Maltoni also reported that a rare form of liver cancer (angiosarcoma) was induced in rats by VC (Maltoni *et al.*, 1974).

In that same year Creech and Johnson (1974) reported that a search of the medical files of employees exposed to VC at a Goodrich plant in the United States revealed three cases of death from the same rare type of liver cancer (angiosarcoma). Since that time, VC has been the subject of numerous animal studies and epidemiological surveys, and it is clear that VC induces angiosarcomas of the liver in both animals and humans (see ECETOC, 1988, for a review). Other types of tumors (nonliver) have been associated with VC exposure in animals, but the epidemiological data have not linked exposure to VC to induction of other types of tumors in humans (ECETOC, 1988).

VC is metabolically activated to a reactive species (probably chloroethylene oxide) which is capable of binding to DNA and causing genotoxicity *in vivo* (ECETOC, 1988). This activation is catalyzed by cytochrome P450 enzymes, and there is good evidence that the metabolism of VC is saturable *in vivo* and *in vitro* (Kappus *et al.*, 1976; Gehring *et al.*, 1978; Guengerich and Watanabe, 1979). A large body of data relating the tumorigenic response in the livers of animals and humans to biochemical events taking place in the various species is available. The purpose of this paper is to discuss methods for using this database to prepare estimates of cancer risk for human populations exposed to VC.

VC is worthy of consideration for another reason. In most cases where estimations of the human cancer risk have been based on animal studies, it is not possible to know whether the projections of risk are realistic (epidemiological data are not precise enough to either confirm or deny the risk projections). In the case of vinyl chloride, a rather large body of epidemiological data indicates that significant increases in human cancer have occurred as a result of past practices which resulted in high human exposures to VC. This provides a unique opportunity to test the ability of current risk assessment practices to provide reliable estimates of human cancer risk from animal data.

Objectives

Physiologically based pharmacokinetic models of chemical disposition have been developed for a variety of chemicals, including the chlorinated ethylenes (NAS, 1987). These

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0041-008X/96 $18.00

models are particularly well suited for risk extrapolations because they are based on specific physiological and biochemical properties of the different species and dose routes as well as physical chemical information about the solubilities and vapor pressures of the different compounds (Andersen *et al.*, 1987). Our objectives in this project were:

1. To develop a PBPK model capable of predicting the metabolism of VC in both rodents and humans.
2. To validate this model with existing data sets for rodents and humans.
3. To develop a quantitative risk assessment procedure based on the predictions of the validated PBPK model for VC.
4. To compare the PBPK-based risk assessment procedure with the existing EPA risk assessment procedure (HEAST, 1995).
5. And finally, to compare the results from this PBPK-based risk assessment with the actual incidence of liver angiosarcomas in human populations exposed to VC in the workplace. (Simonato *et al.*, 1991).

METHODS

Construction of the PBPK Model

The PBPK model for VC was based on a PBPK model developed by Ramsey and Andersen (1984) to describe the kinetics of inhaled styrene in rats and humans. In this model a series of simultaneous differential equations describing the distribution, elimination, and metabolism of chemical was incorporated into a computer program using an integrated software package containing routines for numerical integration, optimization, sensitivity analysis, and graphical display. This software package (SimuSolv)[2] is commercially available from Mitchell and Gauthier Associates (200 Baker Ave., Concord, MA 01742-0013).

The VC model contains four tissue groups (fat, muscle, rapidly perfused tissues, and liver) and assumes that all metabolism takes place in the liver where the rate of metabolism is described by the Michaelis–Menten equation. Detailed descriptions of this type of model are given elsewhere (Ramsey and Andersen, 1984; Andersen *et al.*, 1987). An annotated copy of the source code for this model is available from the corresponding author.[3]

Physiological parameters in the model (blood flows, ventilation rates, organ sizes) appropriate for rats, mice, and humans were identical to those used by Andersen *et al.* (1987) in a multispecies PBPK model for methylene chloride with two changes: (1) the size of the liver compartment for rodents was based on historical data for control animals from the Toxicology Laboratory of the Dow Chemical Company and (2) the allometric constants for alveolar ventilation and cardiac flow in rats used by Andersen *et al.* (1987) were increased from 15 to 18 in order to provide a more consistent description of the gas uptake data sets.

Blood/air partition coefficients for rat, mouse, and humans and tissue/air partition coefficients for rat liver, rat muscle, and rat fat were determined using the vial equilibration method of Sato and Nakajima (1979) as modified by Gargas *et al.* (1989). Tissue/blood partition coefficients for rats were obtained by dividing the tissue/air partition coefficients by the blood/air partition coefficient. No direct measurements were available for the tissue/air partition coefficients in the rapidly perfused group of tissues in this model, so this partition coefficient was set equal to the partition coefficient for liver, a technique that has proven successful in the development of PBPK models for other halogenated, volatile materials (Andersen *et al.*, 1987; Reitz *et al.*, 1988, 1990a,b). Tissue/blood partition coefficients for mice and humans were estimated by dividing the tissue/air partition coefficients for rats by the blood/air partition coefficients for mice or humans, respectively. All of the partition coefficients used in the PBPK model for VC are listed in Table 1.

TABLE 1
Parameters Used in the Physiologically Based Pharmacokinetic Model for Vinyl Chloride for Humans, Rats, and Mice

	Human	Rat	Mouse
	Weights (% of body weight)		
Liver	3.14%	2.53%	5.86%
Rapidly perfused	3.71%	5.0%	5.0%
Slowly perfused	62.1%	76.47%	76.14%
Fat	23.1%	7.0%	4.0%
	Flows (allometric constants)		
Alveolar ventilation	15	18	28
Cardiac output	15	18	28
	% of cardiac output		
Liver	24.0%	24.0%	24.0%
Rapidly perfused	52.0%	52.0%	52.0%
Slowly perfused	19.0%	19.0%	19.0%
Fat	5.0%	5.0%	5.0%
	Partition coefficients		
Blood/air	1.16	1.68	2.41
Liver/air	1.60	1.60	1.60
Rapidly perfused/air	1.60	1.60	1.60
Slowly perfused/air	2.10	2.10	2.10
Fat/air	20.0	20.0	20.0
	Metabolic constants		
V_{maxC} (allometric)	3.97	2.75	8.13
K_m (mg/liter)	0.04	0.04	0.28

Note. Alveolar ventilation and cardiac output are calculated from the allometric constants by multiplying the constant by the body weight (kg) of the animal raised to the 0.74 power. V_{max} is calculated from the allometric constant V_{maxC} by multiplying the constant by body weight raised to the 0.70 power.

Metabolic constants for rats. Metabolic parameters for male and female Sprague–Dawley rats (body weight 200–400 g) were obtained by computer optimization of gas uptake data sets (four experiments for each sex) according to procedures previously described by Gargas *et al.* (1986). Basically, given the physiology of the animals and the solubilities of VC in rat blood and tissues, the metabolic rate constants V_{maxC} and K_m were varied until a satisfactory description of data gathered in several independent gas uptake experiments was obtained for both male and female rats (Figs 1a and 1b).

[2] SimuSolv is a registered trademark of the Dow Chemical Co.

[3] To receive a copy of the source code, please send a self-addressed stamped envelope. If a copy on magnetic media is desired, please include a formatted 3.5" DOS diskette with your request.

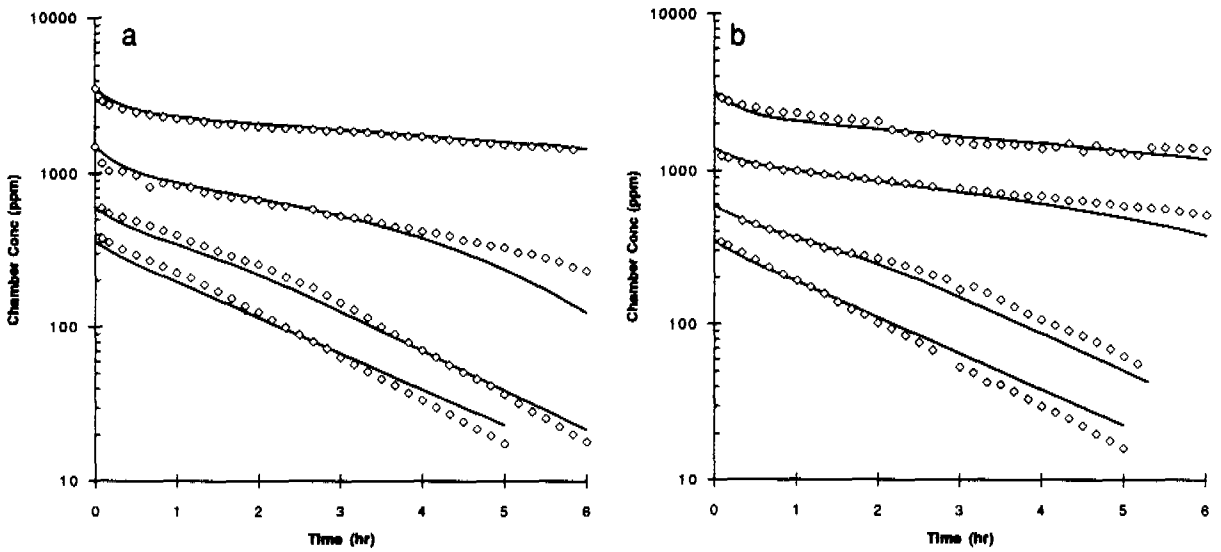

FIG. 1. Predicted (solid line) and observed (open symbols) concentrations of vinyl chloride in a 9.1-liter recirculating exposure chamber containing 3 male rats (a) or 3 female rats (b).

Metabolic constants for mice and humans. Vinyl chloride belongs to a large class of low molecular compounds (including benzene, styrene, CCl_4, $CHCl_3$, CH_2Cl_2, CH_3Cl, CH_3CCl_3, 1,2-dichloropropane, ethylene dichloride, ethylene dibromide, vinyl bromide, acrylonitrile, vinyl carbamate, ethyl carbamate, and trichloroethylene) which are readily metabolized by cytochrome P450, IIE1 (P450 2E1). Guengerich *et al.* (1991) observed that metabolism of these substrates by microsomal preparations from liver (a) showed the same relative activity for different preparations of microsomes with all the substrates, (b) was sensitive to inhibition by known inhibitors of P450 2E1 metabolism such as diethyldithiocarbamate and disulfiram with all the substrates, and (c) showed inhibition by specific antibodies to P450 2E1 raised in rabbits, but was not sensitive to addition of antibodies specific for other forms of P450 such as P450 IIIA or $P450_{MP}$. Guengerich also reported that purified preparations of P450 2E1 were also active on all these substrates.

Based on these and other studies, oxidation of this broad group of substrates by P450 2E1 has been identified as the primary means of biotransformation in both animals and humans (Nakajima *et al.*, 1990; Guengerich *et al.*, 1991; Raucy *et al.*, 1993). Consequently, the extensive *in vivo* and *in vitro* studies carried out with CH_2Cl_2 (Andersen *et al.*, 1987, 1991) and $CHCl_3$ (Corley *et al.*, 1990; Reitz *et al.*, 1990a) provide a reliable basis for estimating *in vivo* metabolic rates for other members of this class (including VC) in humans. The process by which these data were used to estimate *in vivo* metabolic rate constants for humans and mice for VC is outlined below:

1. *In vivo* maximum rates of metabolism (V_{max}'s) in rats are obtained by experimentation (VC) or from the literature (CH_2Cl_2 $CHCl_3$). These values are listed in Table 2.

2. The weight of the liver in animals used in the *in vivo* studies is calculated for each chemical and each species from the percentage liver and the body weight (Table 2).

3. The V_{max} for each species is divided by the weight of liver to give an *in vivo* rate per gram of tissue for each chemical. These V_{max}/g are normalized to the rat for each chemical, and the ratio to rat is listed in the last row of Table 2 for each chemical.

4. For chloroform and methylene chloride, the *in vivo* ratio to rat is nearly constant (2.570, 2.707) suggesting that after normalization, the ratio to rat does not depend upon the chemical being studied (at least within this limited series of chemicals all metabolized by P450 2E1).

5 *In vivo* studies by Andersen *et al.* (1991) with CH_2Cl_2 provide a basis for calculating the ratio to rat for humans of 0.208; Table 2. (Comparable *in vivo* studies for $CHCl_3$ in humans were not available.)

6. Finally, the experimentally determined *in vivo* V_{max} for VC in rats and the ratio to rat for mice and humans were used to calculate *in vivo* V_{max}'s for VC by multiplying V_{max}/g (rat) by the ratio to rat and weight of liver in each species.

For example the *in vivo* V_{max} for VC in humans is calculated as: (0.968/5.69) × 0.208 × 2198 = 77.7 mg/hr (Table 2).

RESULTS

Model Validation

Validation in rats. As noted under Methods, metabolic rate constants were obtained from *in vivo* gas uptake experiments previously conducted at Wright Patterson AFB (Gargas *et al.*, 1990). To verify that the model using these metabolic rate constants was broadly descriptive of metabolism in the rats, independent experiments performed by Watanabe *et al.* (1976) were evaluated. Watanabe and co-workers exposed rats to a series of concentrations of radiolabeled VC for 6 hr, and then collected radioactive excreta from these animals for up to 72 hr. These studies allowed the estimation of the total amounts of VC metabolized by the rats (Watanabe *et al.*, 1976; Gehring *et al.* (1978). The amounts of radioactive metabolites observed by Watanabe *et al.* were compared to the predictions of the PBPK model for rats. Other than changing the body weights and exposure concentrations to reflect the different experimental conditions, no changes were made in the model developed from gas uptake experiments. The results are shown in Fig. 2.

The model gave an excellent simulation of Watanabe *et*

TABLE 2
In Vivo Metabolic Rate Constants for PBPK Model for Vinyl Chloride

	Rat	Mouse	Human
Methylene chloride			
Body wt (kg)	0.233	0.0275[a]	83.0
Percentage liver	2.53%	5.86%	3.14%
Liver wt (g)	5.895	1.612	2606
V_{max} (mg/hr)	1.500	1.054	138
Ratio to rat	1.000	2.570	0.208
Chloroform			
Body wt (kg)	0.230	0.0285	—
Percentage liver	2.53%	5.86%	—
Liver wt (g)	5.819	1.670	—
V_{max} (mg/hr)	2.431	1.889	—
Ratio to rat	1.000	2.707	—
Vinyl chloride			
Body wt (kg)	0.225	0.0285	70.0
Percentage liver	2.53%	5.86%	3.14%
Liver wt (g)	5.69	1.67	2198
Ratio to rat (ave)	1.000	2.639	0.208
V_{max} (mg/hr)	0.968	0.749[b]	77.7[b]
V_{maxC} (allometric)	2.75	9.04	3.97

Note. *In vivo* metabolic rate constants for the PBPK model for vinyl chloride were estimated from data reported by Andersen *et al.* (1987, rats and mice; 1991, humans) for methylene chloride and Corley *et al.* (1990) for chloroform. Published *in vivo* maximum rates (V_{max}) of oxidative metabolism (catalyzed by P450 enzymes) and historic organ weight data from subchronic studies at Dow Chemical Co. were used to calculate the V_{max}/kg of liver (V_{max}/VL). Then the characteristic interspecies ratios of V_{max}/VL for oxidative metabolism of these typical halogenated hydrocarbons were used to estimate in the *in vivo* V_{max}'s for VC in mice and humans.

[a] Andersen *et al.* (1987) listed 34.5 g as the body weight for mice in their Table 1 since this was the body weight for the mice in the NTP bioassay of methylene chloride and their PBPK model was used for risk assessment. However, the average body weight of the mice used in the gas uptake studies was actually 27.5 g and this body weight is used to calculated the V_{max}/VL ratio.

[b] Calculated by multiplying the average mouse or human ratio of V_{max}/VL to rat (methylene chloride and chloroform), the *in vivo* V_{max}/VL value for VC in rats and the VL for mice or humans.

al.'s data. Over a range of concentrations from 1.4 up to 4600 ppm, the PBPK model accurately predicted the levels of radioactive metabolites produced and successfully identified the region where saturation of VC metabolism occurs (200–500 ppm; Fig. 2).

Validation in mice. Metabolic rate constants for $B_6C_3F_1$ mice were estimated by extrapolation from *in vivo* results in $B_6C_3F_1$ mice obtained with model substrates as outlined under Methods. In order to test whether these estimated rate constants accurately reflected the *in vivo* metabolism of VC in mice, an independent set of gas uptake experiments conducted in male and female $B_6C_3F_1$ mice was used for validation.

For this validation, the basic PBPK model for rats was adapted to mice by (a) incorporating the known physiol cal differences between rats and mice (see Andersen *et* 1987), (b) changing the blood/air partition coefficient VC to that measured in the laboratory with samples $B_6C_3F_1$ mouse blood (Gargas *et al.*, 1989), and (c) settin the allometric constant describing the maximum rate of metabolic oxidation of VC to the value estimated from *in vivo* studies with other volatile, low-molecular-weight halogenated hydrocarbons (Table 2). Other than these changes, the structure of the model was not altered.

Simulated and actual data from four experiments with male $B_6C_3F_1$ mice (initial concentrations of 345, 570, 1065, and 3190 ppm) and four experiments with female $B_6C_3F_1$ mice (initial concentrations of 280, 550, 975, and 2950 ppm) are shown in Figs. 3a and 3b. As can be seen from inspection of these figures, the model gives a reasonable (but not perfect) simulation of the gas uptake data.

The highest concentrations of VC (~3000 ppm where metabolism is probably saturated) were well described by the PBPK based on the metabolic rate constants estimated from model substrates. These data depend primarily upon the value chosen for V_{max} in the model and suggest that the extrapolation procedure employed for estimating the maximum *in vivo* rate of metabolism in $B_6C_3F_1$ mice was successful.

The low concentrations (~300 ppm, where metabolism is presumably first order) are also well described by the model. For rapidly metabolized substances, the uptake is largely flow-limited (i.e., depends upon how rapidly the material is delivered to the liver rather than the specific values

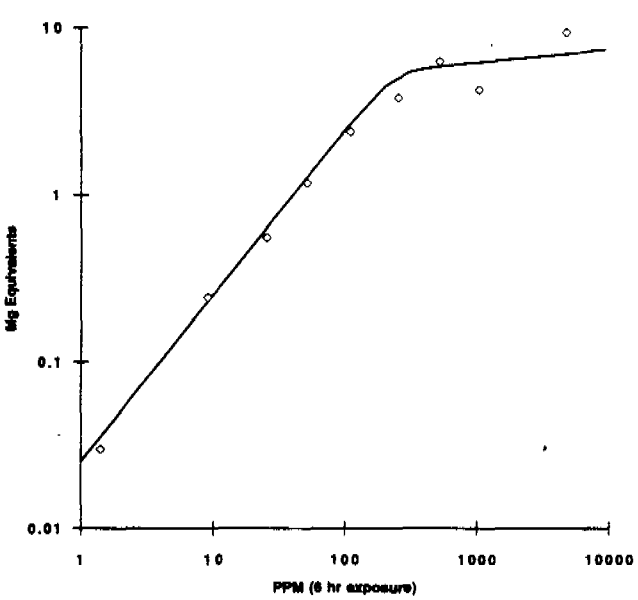

FIG. 2. Predicted (solid line) and observed (open symbols) amounts of radioactive metabolites derived from exposure of male rats to the indicated concentration of [^{14}C]vinyl chloride gas for 6 hr. Data taken from Watanabe *et al.* (1976).

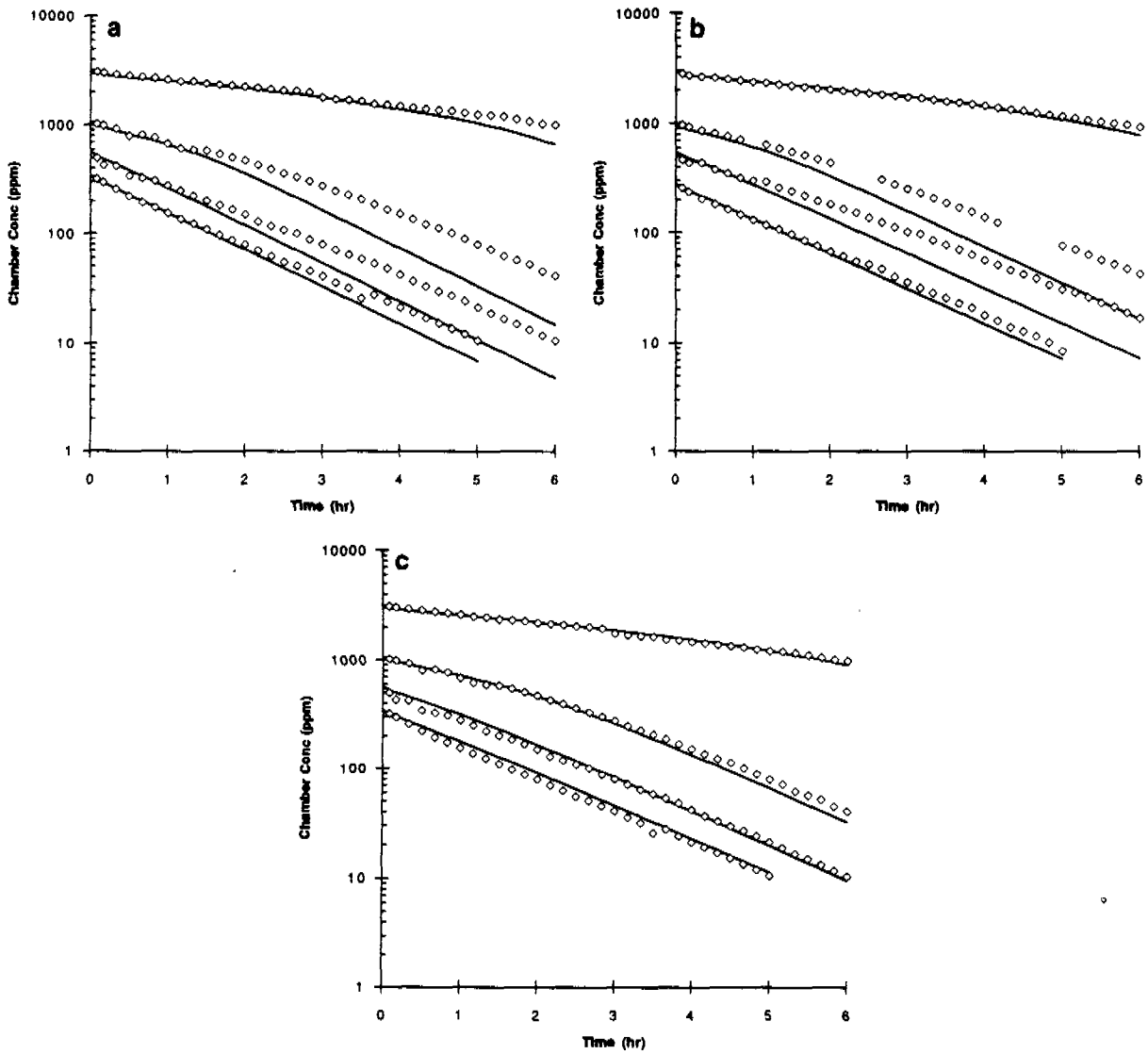

FIG. 3. Predicted (solid line) and observed (open symbols) concentrations of vinyl chloride in a 9.1-liter recirculating exposure chamber containing 14 male mice (a) or 14 female mice (b) with values of V_{maxC} and K_m calculated from *in vitro* studies (V_{maxC} = 9.04, K_m = 0.04). (c) Same data for 14 male mice after computer optimization of V_{maxC} and K_m (V_{maxC} = 8.13, K_m = 0.28).

of V_{max} and K_m). Correspondence of model simulations with experimental data at low concentrations suggests that the physiological parameters for mice (flow rates and partition coefficients) used in the PBPK models for $B_6C_3F_1$ mice are appropriate.

However, for the experiments involving intermediate concentrations of VC (550–1065 ppm), the model predicts slightly more metabolism (uptake from the chamber) than was experimentally observed. These results were obtained in the region where metabolism of VC changes from flow-limited conditions to zero order (enzyme saturation), and the simulation of these concentrations is sensitive to the value chosen for K_m in the Michaelis–Menten equation.

To explore the possibility that a different value of K_m might give a better simulation of the experimental results, a computer optimization was conducted with SimuSolv. The results of this optimization (in which the computer varied both V_{maxC} and K_m are shown in Fig. 3c. In this optimization, it was found that a K_m of 0.28 gave a much better simulation of the gas uptake data than the K_m obtained from the rat experiments (K_m = 0.04 in rats). However, the optimum value for V_{maxC} remained relatively constant (the optimum value of V_{maxC} was 8.13; quite close to the extrapolated value of 9.04).

Given that the same enzyme (P450-2E1) is responsible for oxidation of VC in both species, it may seem surprising

that the apparent K_m's differ by this amount. However, based on studies of deuterium isotope effects on CH_2Cl_2 metabolism, Andersen *et al.* (1994) have suggested that the apparent K_m's for oxidation of P450-2E1 substrates likely reflect a more complicated series of events than simple "binding" of a substrate to an enzyme's active site. Andersen speculated that the apparent K_m's for P450-2E1 substrates may be ". . . a measure of reactivity of activated oxygen species with available C—H bonds." If this hypothesis proves to be correct, then the K_m for VC oxidation in humans would be expected to be more like the K_m of rats than the K_m of mice, since the mouse liver has an usually high capacity for oxidation of these substrates (Andersen *et al.*, 1987; Corley *et al.*, 1990).

The preceding validation exercise indicates that the procedure used to estimate *in vivo* metabolic rate constants for VC should give accurate representations of human VC metabolism at either high or low concentrations of VC. The extrapolation procedure would be less precise in identifying the region where transition from first order to zero order kinetics with VC occurs when extrapolating to species other than the rat.

Validation in humans. We also attempted to locate independently gathered human data for validation of the human PBPK model for VC. Validation of human models for other solvents (CH_2Cl_2, 1,1,1-trichloroethane, styrene) has been previously reported (Andersen *et al.*, 1991; Reitz *et al.*, 1988; Ramsey and Andersen, 1984), providing support for the techniques for estimating partition coefficients and physiological constants in humans. Consequently, the primary emphasis was on evaluation of the metabolic rate constants for VC estimated by the techniques outlined under Methods. No attempt was made to "curve fit" experimental data by adjustment of model parameters in validating the human PBPK model.

Ideally, we hoped to find measurements of the rate of production of VC-specific metabolites in human subjects (e.g., excretion of VC-specific metabolites in the urine of humans exposed to VC similar to data gathered in humans exposed to trichloroethylene; Müller *et al.*, 1974). Unfortunately, we were unable to locate this type of dataset for VC. However, we did locate a dataset in which exhaled air concentrations of VC were reported for human volunteers following VC exposure and this dataset was evaluated with the human PBPK model.

In these studies, Baretta *et al.* (1969) exposed groups of human volunteers to 59, 261, or 492 ppm VC for 7.5 hr in a carefully controlled laboratory setting. Workers entered the chamber and were exposed to VC at the indicated concentrations for approximately 3.5 hr. Then they left the chamber to have lunch in an area free of VC for approximately 0.5 hr, following which they returned to the chamber for another 4 hr (7.5 hr exposure to VC). This exposure pattern was simulated by the PBPK model during the validation exercises.

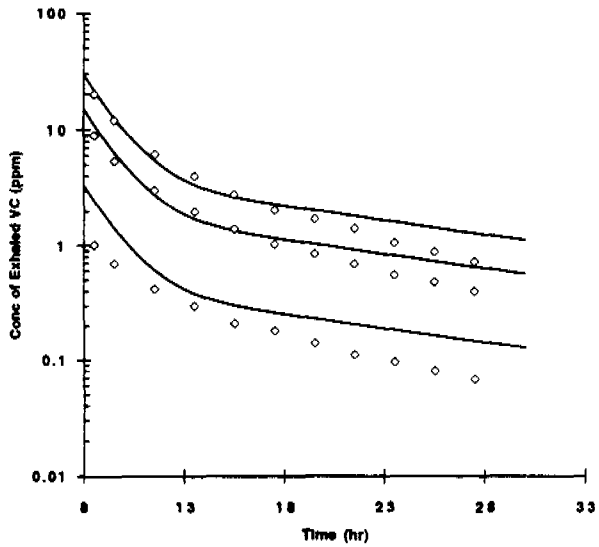

FIG. 4. Predicted (solid line) and observed (open symbols) concentrations of vinyl chloride exhaled by human subjects following 7.5 hr of exposure to vinyl chloride concentrations of 59, 261, or 492 ppm. Data are taken from Baretta *et al.* (1969).

Samples of exhaled breath were collected from these volunteers (4–7 subjects in each exposure) at different times postexposure for up to 20 hr. The sampling procedure involved giving each person several glass tubes (20 mm in diameter, approximately 23 cm in length) capped with screw-cap septa. Each worker was asked to inhale through his nose and exhale by mouth into the glass tube four times. After the fourth breath, the workers quickly capped the glass tubes with impermeable septa and returned the tubes for analysis. A comparison of observed and simulated results for these exposures is presented in Fig. 4. The model for VC gave an good simulation of expired air data for all three concentrations over the period from 1 to 20 hr postexposure. It is noteworthy that these experiments included exposures at concentrations up 500 ppm, a region where metabolic saturation occurs in rats (Fig. 2).

A sensitivity analysis was conducted to determine whether this fit was dependent upon selection of the proper values for the metabolic rate parameters (V_{max} and K_m) or whether other model parameters were more influential in the prediction of expired air concentrations of VC (CEX) as described elsewhere (Reitz *et al.*, 1990b). Predicted values of CEX 1 and 10 hr postexposure were compared to the same values predicted with "baseline" values for all model parameters with VC concentrations of 59 and 492 ppm (the lowest and highest VC concentrations studied by Baretta *et al.*, 1969).

The sensitivity analyses revealed that model parameters associated with flow rates (fraction cardiac output directed

to fat compartment, alveolar ventilation, and cardiac output) and partitioning within the body (blood/air, fat/air, and, at the 1-hr postexposure period, muscle/air) had 3- to 30-fold more influence on the predicted values of CEX than the metabolic rate parameters V_{max} and K_m. In fact, doubling or halving the values of V_{max} or K_m did not appreciably change the fit to the data collected by Baretta *et al.* (simulations not shown).

Buchter *et al.* (1978) also studied the pharmacokinetics of VC in human subjects. Human volunteers inhaled VC vapor from (and exhaled back to) a closed system containing 10 ppm and the removal of VC from this system for periods up to 30 min was evaluated. In other studies, volunteers inhaled a constant concentration of VC (~2.5 ppm) for 15–30 min. Data reported by Buchter *et al.* were also well simulated by the PBPK model (simulations not shown), but a sensitivity analysis revealed that simulations of these data were even less sensitive to the values chosen for V_{max} and K_m than the data of Baretta *et al.* (1969).

It is reassuring that the limited human data for VC are consistent with the PBPK model developed here. However, it must be conceded that the validation procedures described above neither support nor refute our procedure for estimating VC metabolic rate constants in humans. Unfortunately, since VC is a known human carcinogen, it is considered unlikely that definitive studies of VC metabolism capable of rigorously establishing metabolic rate constants for the human PBPK model will be conducted in the foreseeable future.

Risk Estimation

The carcinogenicity of VC in animals has been studied extensively (in fact it may be the most extensively studied of any of the animal carcinogens known). Exposure paradigms include single exposures, high exposures for short periods (days or weeks), exposures early in the natural life span versus late in the natural life span, etc. Given the wealth of data available for preparation of risk estimations, we were forced to select a subset of the data for illustrative purposes. We have chosen to focus on studies in which VC was administered for a substantial fraction of the animal's lifetime (12 months exposure in each of the cases evaluated) with follow-up until the animals' death wherever possible. The reader is referred to the publications of Maltoni, Drew, and Lee for further details of these and other studies.

We are aware that the pattern of exposure (i.e., whether a given exposure occurs early or late in life) may influence the carcinogenic potency of VC, but we have not attempted to consider this factor in our analyses. Similarly, we are aware that the life expectancy of mice (but not rats) exposed to VC in the first 12 months of their life is significantly shorter than the life span of control mice (cf., Drew *et al.*, 1983 who reported a mean survival time of 780 days for control female $B_6C_3F_1$ mice compared to 301 days in the group exposed to 50 ppm VC for 6 hr/day). However, we have not attempted to apply any "less than lifetime" correction factors to the cancer potencies obtained from the mouse studies.

TABLE 3
Incidence of Angiosarcomas of the Liver Observed in Sprague–Dawley rats.

Exposure concentration (ppm)	Males	Females	Males + females
0	0/173	0/239	0/412
1	0/58	0/60	0/118[a]
5	0/59	0/60	0/119[a]
10	0.59	1/60	1/119
25	1/60	4/60	5/120
50	2/174	13/180	15/354
100	0/60	1/60	1/120[a]
150	1/60	5/60	6/120
200	7/60	5/60	12/120
250	1/29	2/30	3/59
500	0/30	6/30	6/60
2500	6/30	7/30	13/60
6000	3/29	10/30	13/59

Note. Data are from experiments BT1, BT2, BT9 and BT15 conducted by Maltoni (1974; Maltoni *et al.*, 1974). Data are given as number of angiosarcomas/number of animals examined for males, females, and combined males and females. Control animals from several experiments are combined in the 0 ppm group. Animals were exposed 4 hr/day, 5 days/week for 52 weeks and then held until they died (typically at least another year). Tumors were scored at the time of death.

[a] Eliminated from GLOBAL83 analysis because of mathematical limitations of the PC version of the computer fitting program (only 10 dose/response groups allowed).

Deriving rat potency estimates. Maltoni conducted a series of inhalation bioassays of VC in male and female Sprague–Dawley rats at concentrations ranging from 1 to 30,000 ppm (Maltoni, 1974). These animals were exposed to VC for 4 hr/day, 5 days/week, with exposures beginning in young adult animals and continuing until the animals reached 1 year of age. After the first year of exposure, animals were held until they died and then examined for the presence of tumors. Survival of the animals was compromised at the highest concentrations, so these results were not employed in derivation of a rat potency for VC. Results from exposures conducted at 0, 1, 5, 10, 25, 50, 100, 150, 200, 250, 500, 2500, and 6000 ppm were selected as the basis for fitting a dose–response curve for induction of liver angiosarcoma by VC metabolites. Tumor incidence data used in constructing this curve are listed in Table 3.

The "dose surrogate" (measure of dose delivered to the target organ) chosen for risk analysis was the average daily amount of metabolite produced per day per liter of liver tissue. This type of dose surrogate is appropriate for risk analysis when the metabolite is highly reactive (as the chlo-

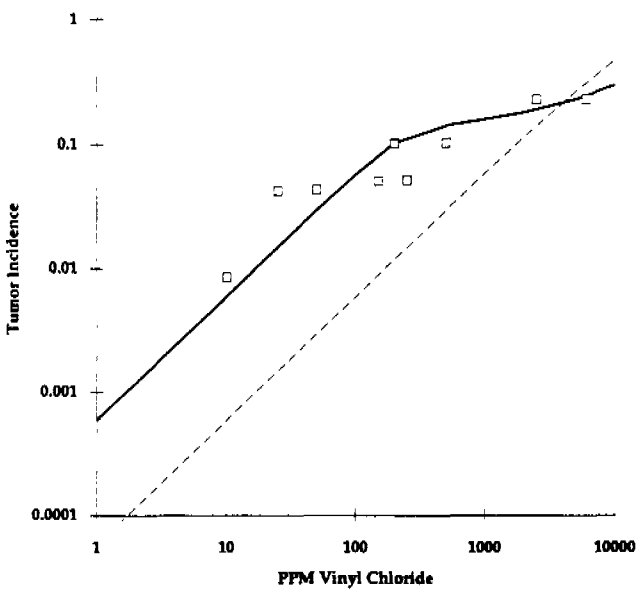

FIG. 5. Predicted (solid line) and observed (open symbols) incidences of liver angiosarcoma in rats following exposure to various concentrations of vinyl chloride for 4 hr/day, 5 days/week, for 12 months (animals held until death for observation of tumor incidence). The dotted line represents the type of risk extrapolation that might have been prepared by EPA if the only data available for VC had been from two high VC concentrations but does not correspond to the actual EPA risk assessment (HEAST, 1993). Data are taken from Maltoni *et al.* (1974).

roethylene oxide formed from VC would be) and either reacts with DNA or water in the target organ with a very short half-life (i.e., would not be expected to persist long enough to circulate to other organs in the body). Rationale for the selection of dose surrogates from PBPK models have been discussed extensively elsewhere (Andersen *et al.*, 1987) and the reader is referred to this publication for further details.

The PBPK model for VC in rats was then used to calculate the amount of metabolites produced during a typical day of exposure (4 hr exposure to the selected VC concentration). Maltoni exposed rats to VC for 5 days/week and for 1 year, so the lifetime average daily doses (LADD) were calculated by multiplying the values obtained from the computer by 5/7 (to correct for less than daily exposure) and 1/2 (to correct for less than lifetime exposure). Results from male and female rats were combined and empirically fitted to a metabolic dose/tumorigenic response curve with the computer program GLOBAL83 (Howe and Crump, 1982; Howe, 1983). The predicted (maximum likelihood estimate) and observed results are depicted graphically in Fig. 5.

The risk estimation based on the PBPK model (curved, heavy line in Fig. 5) describes the tumor incidences observed by Maltoni over a broad range of doses, showing the ability of the PBPK model to compensate for the effects of metabolic saturation in the activation of VC. For illustrative purposes, a hypothetical risk estimation based on administered dose (ppm VC) at the two highest concentrations (2500, 6000 ppm) is also shown with a dotted line in Fig. 5. This represents the type of risk estimation that EPA might have conducted if the VC data were from a "typical" bioassay (i.e., the only tumor incidences reported were for MTD and MTD/2), and it is noteworthy that such a procedure would have significantly *underpredicted* the tumor incidences seen in rats by Maltoni at lower concentrations (e.g., 10–100 ppm). This line does not correspond to the actual EPA risk assessment for VC (HEAST, 1995).

For the purposes of illustration in this paper, the dose–response model relating tumor incidence in rats and levels of VC metabolites in liver was obtained from the GLOBAL83 computer program. Other mathematical models relating tumor incidence to doses of carcinogenic species have been developed and could certainly have been employed in addition to or instead of the multistage model. However, since the extrapolation range evaluated was relatively small, it was not considered necessary to explore these other models (they all give basically the same results when used for regions where experimental data are available as is the case here).

Extrapolation from the fitted dose/response curve in rats indicates that a LADD of 0.177 mg equivalents of VC metabolites/day/liter of liver is associated with a lifetime increase of 1×10^{-4} in the cancer incidence of rats (MLE estimate). This risk-specific dose (RSD) may now be used to estimate the excess risk of cancer in mice and humans exposed to VC under the assumption that equal average concentrations of VC metabolites in the liver of these species produce equal lifetime risks of cancer.

This assumption is precisely the same as that used by most U.S. regulatory agencies when performing risk assessments based on administered LADD (e.g., doses in mg/kg/day) except that an interspecies scaling factor related to body surface area is also employed by those agencies. Andersen *et al.* (1987) suggested that since the PBPK model already contains provisions for considering metabolic and physiological differences between species, the body surface area factor should be eliminated from risk assessments based on PBPK models. As will be seen later, comparisons of predicted and observed incidences of angiosarcoma in humans exposed to VC are consistent with the proposal of Andersen *et al.* (1987).

Estimation of risk to mice from rat data. Maltoni also reported the effects of exposure to VC on the incidence of angiosarcomas in Swiss albino mice exposed to VC 4 hr/day, 5 days/week for 30 weeks with the experiment terminated at 81 weeks (Maltoni's experiment BT 4 summarized in ECETOC, 1988). This experiment contained exposure groups of 0, 50, 250, 500, 2500, 6000, and 10000 ppm VC with approximately 30 male or female animals/group (approximately 60 total mice/group) except for the control group which contained 150 male and female mice.

TABLE 4
Incidence of Angiosarcomas of the Liver Observed in Swiss Albino Mice

Exposure concentration (ppm)	Males	Females	Males + females	PB-PK LADD
0	0/80	0/70	0/150	0
50	1/30	0/30	1/60	38.4
250	9/30	9/30	18/60	173.1
500	6/30	8/30	14/60	265.2
2,500	6/29	10/30	16/59	331.0
6,000	2/30	11/30	13/60[a]	—
10,000	1/26	9/30	10/56[a]	—

Note. Data are from experiment BT4 conducted by Maltoni (1974; Maltoni *et al.*, 1974) and summarized in ECETOC, 1988. Data are given as number of angiosarcomas/number of animals examined for males, females, and combined males and females. Animals were exposed 4 hr/day, 5 days/week for 30 weeks and then held until they reached 81 weeks of age. To calculate the dose surrogates for mice, the PBPK model was configured according to Table 1 and a 4-hr exposure with 20 hr exposure free was simulated by the model. The simulated values of the dose surrogate were converted to lifetime average daily doses by multiplying by 5/7 (days/week) and 30/104 (fraction of lifetime exposed). Potency values were estimated with GLOBAL83 as described under Methods.

[a] Eliminated from dose–response regression because of the likelihood of poor survival at this dose.

VC was seen to increase the incidence of angiosarcoma of the liver (and angiosarcoma at other sites) in exposed mice in a dose-related fashion (Table 4). As with the rats, the tumor response at very high concentrations of VC reached a plateau and then declined. It is presumed that the decrease in tumor incidence is related to the fact that the animals in the highest dose groups showed very poor survival.

Lifetime average daily dose surrogate measures (LADD) were calculated for the mice and these doses and the tumor incidences were subjected to processing by GLOBAL83 to determine the parameters for the LMS, with the MLE and extra risk options selected. When this was done, the RSD (MLE estimate of dose associated with a lifetime increase in risk of 1×10^{-4} to mice) was found to be 0.0797 mg equivalents of metabolite per liter of liver per day, in fairly good agreement with the RSD previously calculated for rats (0.177 mg equivalents/liter liver/day).

Two other studies of the effect of VC exposure on development of liver angiosarcoma have been reported. Lee *et al.* (1978) exposed CD-1 mice to VC for 6 hr/day, 5 days/week for 12 months, at which time the experiment was terminated (no holding period after exposure). Lee reported combined incidences of hepatic angiosarcoma of 0, 4.8, 36.5, and 44.9% after exposure to 0, 50, 250, and 1000 ppm of VC, respectively. When subjected to GLOBAL83 calculations, the RSD associated with a lifetime increase in risk of 1×10^{-4} to mice (based on Lee *et al.*'s studies) was found to be 0.120 mg equivalents of metabolite per liter of liver per day, intermediate in potency between the RSD previously calculated for rats (0.177 mg equivalents/liter liver/day) and the RSD based on Maltoni's experiments in Swiss albino mice (0.0797 mg equivalents of metabolite per liter of liver per day). Thus these two mouse studies and the rat study gave quite consistent estimates of the RSD for VC metabolites.

However, when a bioassay of VC in $B_6C_3F_1$ and Swiss CD-1 female mice (and F344 rats and Syrian golden hamsters) conducted by the National Toxicology Program (NTP; Drew *et al.*, 1983) was evaluated, quite different results were seen. In these studies, only one exposure concentration was studied (50 ppm, 6 hr/day, 5 days/week), but exposures were begun at different points in the animals life spans and were conducted for different durations (6 months, 12 months, etc.). One of the exposed groups of mice was subjected to an exposure paradigm similar to that employed by Maltoni *et al.* (1974) in that exposure began when the animals were 9 weeks old, they were exposed for almost 12 months. Drew *et al.* reported that none of the treated animals survived more than 1 year, and the cause of death in the treated animals was frequently considered to be ". . . due to the development of neoplasms . . . ."

In this group of female $B_6C_3F_1$ mice VC increased the incidence of angiosarcoma in the NTP study from 5.8% in controls (4/69 animals) to 76.7% (69/90 animals). In contrast, at this exposure concentration and paradigm, Maltoni *et al.* (1974) and Lee *et al.* (1978) observed a 2–5% incidence of angiosarcomas in treated mice with no angiosarcomas seen in control animals. Thus the NTP has reported a considerably higher incidence of angiosarcomas in both control and treated animals than either Lee or Maltoni and this difference is not due to differences in survival, since the treated mice studied by Lee and Maltoni all survived longer than in the NTP study.

The RSD for VC in $B_6C_3F_1$ mice (calculated in the same manner as the RSDs for Maltoni's and Lee's studies) was 0.0032 mg equivalents of VC metabolites/day/liter of liver. This RSD is significantly lower (25- to 55-fold, implying more risk associated with a fixed concentration of VC) than the RSDs calculated from the Lee and Maltoni studies.

The discrepancy in the carcinogenic potency of VC appears to be laboratory rather than strain specific because NTP also studied another strain of mouse (CD-1 mice) and again observed a much higher incidence of angiosarcomas in the liver (63.8% in treated vs 1.4% in controls) than seen by either Maltoni or Lee. It is noteworthy that the incidence of liver angiosarcomas reported by NTP (Drew *et al.*, 1983) in rats exposed to 100 ppm VC (20%) was also significantly higher than reported by Maltoni (1–2% incidence).

Estimation of human risk. Risk estimates for humans occupationally exposed to VC were prepared by the following procedure:

TABLE 5
Lifetime Average Delivered Doses (LADDs) of VC Metabolites in Humans Exposed to VC for 5 Days/Week, 50 Weeks/Year, for the Indicted Numbers of Years

ppm	Years exposed	ppm · years	PBPK LADD	PBPK prediction per 100,000	Observed cases per 100,000
10 years exposure					
50	10	500	3.33	188	—
100	10	1,000	6.63	374	(6.2)[a]
200	10	2,000	13 06	736	—
—	—	4,000	—	—	42.2
500	10	5,000	26 68	1,497	—
—	—	8,000	—	—	152.3
1000	10	10,000	31.28	1,753	—
—	—	>10,000	—	—	(280.0)[b]
2000	10	20,000	36.03	2,532	—
20 years exposure					
50	20	1,000	6.66	376	(6.2)[a]
100	20	2,000	13.26	747	—
200	20	4,000	26.11	1,465	42.2
—	—	8,000	—	—	152.3
500	20	10,000	53.35	2,971	
—	—	15,000	—	—	(280.0)[b]
1000	20	20,000	62.57	3,476	
2000	20	40,000	72.07	3,993	

Note. The LADDs are calculated from the PBPK model for humans constructed as outlined under Methods, correcting for the fraction of a year exposed (50/52) and the fraction of a lifetime exposed (10, 20, or 30/70). Estimated lifetime risks (incidences/100,000) predicted for these exposures based on the rat potency factor are obtained from the GLOBAL83 computer program (specifying the maximum-likelihood estimate, *not* the 95% upper confidence limit). Observed cases per 100,000 at different levels of exposure (cumulative ppm · years) for the group having more than 25 years since first employment are obtained from Table 10 of Simonato *et al.* (1991).

[a] Group listed as having <2000 cumulative ppm years by Simonato *et al.*, entered at 1000 ppm · years for comparison

[b] Group listed as having >10,000 cumulative ppm · years by Simonato *et al.*, entered between 10,000 and 20,000 ppm · years for comparison

1. The validated PBPK model for humans was used to construct a table of LADDs expressed in the same terms as used in the rat model: milligram VC metabolites formed/day/liter of liver tissue for conditions thought likely to have been present in the workplace in past years (i.e., TWAs of 50–2,000 ppm and employment for 10–20 years).

In performing these calculations, milligram equivalents of metabolites were adjusted for the fraction of the day that workers were exposed (8/24), the days/week that the workers were at their jobs (5/7), and the fraction of a lifetime that exposure took place (years/70).

2. Once these estimates of dose had been prepared, the GLOBAL83 program was used to estimate the likelihood that tumors would be produced, based on the potency number derived from rats (RSD = 0.177).

The results of these estimations are presented in Table 5. The predictions range from about 200 cases per 100,000 (for workers employed 10 years at a plant where the TWA was 50 ppm) to almost 4000 cancers per 100,000 in workers employed for 20 years in a plant where TWAs were 2000 ppm.

The predictions of human risk may be compared with results reported by Simonato *et al.* (1991) based on the worldwide vinyl chloride tumor registry. Simonato's results are based on the evaluation of 12,706 individuals selected from a population of 14,351 subjects from 19 factories where VC was used industrially. The completeness of follow-up in this study was stated by the authors to be 97.7%, and the average length of follow-up was 17 years (with 36% of the population followed for >25 years). The total number of person years at risk in this study was 222,746.

Simonato *et al.* (1991) reported a clear association between both duration of employment and ranked level of exposure. They estimated the absolute risk of angiosarcoma in exposed population with >25 years since first employment to be between 6.2 cases/100,000 for exposures less

than 2000 ppm · years to 280 cases/100,000 for individuals with more than 10,000 ppm · years. Relative risks in highly exposed populations were as high as 45.4:1. Absolute risks observed in the VC cohort with >25 years since first employment are listed in Table 5 for comparison with risks predicted by the linearized multistage model (LMS) using maximum likelihood estimates (MLE) from the PBPK model.

In each case, the estimates from the procedure employing the PBPK model are substantially higher than actually observed in humans. For example, based on the PBPK prediction, workers exposed to 200 ppm TWA VC for 20 years (4000 ppm · years) would be expected to develop 1465 cases of angiosarcoma/100,000. However, the incidence of angiosarcomas observed in the group with >25 years since first employment and 2000–5999 estimated ppm · years was reported by Simonato *et al.* to be 42.2, which is about 35-fold lower than the PBPK prediction. At higher levels of human exposure (e.g., 6000–9999 ppm · years, >10,000 ppm · years) the PBPK predictions are higher than the observed rates by a factor of 10 or so (Table 5).

Potency factors derived from the studies of Drew *et al.* (1983) were also used to predict human risk (data not shown). When these potency factors were used, the discrepancy between predicted risk and observed incidence was much greater. Predictions based on the Drew studies were almost three orders of magnitude (1000-fold) higher than the actual (observed) incidences of liver angiosarcoma in exposed workers, so these studies are clearly less consistent with human experience than the studies conducted by Maltoni and Lee.

It is noteworthy that we did not employ the "body surface area factor" employed by the U.S. Environmental Protection Agency for cancer risk extrapolation between rats and humans in our risk estimations. If the surface area factor had been employed, the predicted risks would have increased by a factor of approximately 5- to 6-fold for rat to human extrapolations (or 12- to 13-fold for mouse to human). Inclusion of the surface area factor in the PBPK-based risk estimation for VC would clearly have made the risk estimations that we produced less consistent with the reported incidences of angiosarcomas in the exposed workers.

DISCUSSION

A multispecies PBPK model capable of quantitatively describing the metabolic activation of inhaled VC was developed from pharmacokinetic principles and then validated with independent data sets for rats, mice, and humans. Only minor modifications of the model described by Ramsey and Andersen (1984) for inhaled styrene were necessary to accomplish this.

VC is metabolized in mammals by the cytochrome P450 enzymes (likely by the 2E1 subclass) to produce reactive, short-lived intermediates (chloroethylene oxide). These reactive intermediates alkylate DNA and are genotoxic (mutagenic) to both bacterial and mammalian cells. The reactive metabolites of VC are generally assumed to be responsible for the induction of angiosarcomas and other tumors in mammals exposed to VC. Since these intermediates are too short-lived (reactive) to circulate in the bloodstream very far from the organ of their formation, the carcinogenic effects of VC on a particular organ system are assumed to be related to the rates of metabolic activation occurring in that organ. We estimated the rates of induction of liver cancer in various species under different exposure regimens by using the PBPK model to predict the rates of metabolism in the liver of the treated animals.

Tumor Induction in Rats

The results presented in Fig. 5 indicate that accurate predictions of the risk of developing angiosarcoma must consider the dose dependency of VC metabolism. When risk assessments are based on the concentration of VC inhaled by the animals (instead of the amount of reactive metabolite formed by the animals), the predictions deviate widely from incidences observed by Maltoni and others.

For example, if a risk assessment were prepared from a cancer study which contained only high doses of VC (i.e., doses where metabolic activation was saturated), extrapolation to low doses would significantly *underestimate* the incidence of tumors in rats (shown by the dotted line in Fig. 5). On the other hand, if risk estimations were based doses of VC below the level of metabolic saturation, linear extrapolation to high doses would greatly *overestimate* the incidence of tumors in rats exposed to high concentrations of VC. As Gehring *et al.* (1978) pointed out, basing risk estimation on VC metabolites instead of VC itself produces a consistent estimate of carcinogenic potency across the entire dose range (Fig. 5).

Tumor Induction in Mice

In addition to increasing the reliability of high-dose/low-dose extrapolations, PBPK models provide an scientific basis for extrapolations between different species, considering physiological as well as metabolic differences. Since VC has been extensively studied in the mouse as well as the rat, this provided an opportunity to test the ability of the PBPK model to accomplish interspecies extrapolations.

Mice are generally known to contain higher levels of the cytochrome P450 enzymes than either rats or humans and this is reflected by the higher rates of *in vivo* metabolism seen in mice versus rats for the substrates listed in Table 2 (CH_2Cl_2, $CHCl_3$, VC). Based on this knowledge, mice would be expected to be more sensitive to the tumorigenic effects of exposure to a given concentration of VC than rats, and

this has been widely verified (Maltoni *et al.*, 1974; Lee *et al.*, 1978; Drew *et al.*, 1983; ECETOC, 1988). The RSDs (1×10^{-4} lifetime risk) calculated for the different species/strains of mice were:

Maltoni (rat potency)	0.177
Maltoni (Swiss mouse potency)	0.0797
Lee (CD-1 mice)	0.120
Drew ($B_6C_3F_1$ mice)	0.0032

It is noteworthy that the duration of the mouse experiments differed from those of the rat; Maltoni's experiments in rats were "entire life span" (generally >100 weeks), while Maltoni's experiments in mice were terminated at 81 weeks of age. In the case of mouse studies conducted by Lee *et al.* and Drew *et al.*, all animals had either been euthanized or had died by 52 weeks of age (Lee *et al.*, 1978; Drew *et al.*, 1983). Thus it might be argued that if the mouse experiments had been of longer duration, higher cancer potencies for mice might have been calculated (or that application of a "less than lifetime" correction factor was warranted, reducing the similarity of the RSD values in rats and mice).

However, since the authors of at least one study (Drew *et al.*) attributed the early mortality to ". . . induction of neoplasms (in the treated animals) . . .," it is not clear that application of a factor intended to correct for loss of animals from *nonneoplastic causes before they had a chance to develop cancer* would be appropriate. In any case, the incidence of angiosarcoma was very high in the Drew *et al.* and Lee *et al.* studies (77 and 45% respectively), so holding the animals for another year could not have increased the tumor incidences by more than a factor of 2. The mouse studies of Maltoni, by contrast, were nearly lifetime studies (81/104) so that only a small "less than lifetime" correction factor would have been required (~2-fold).

Thus, with the exception of the RSD estimated from the Drew *et al.* (1983) data set, the estimated cancer potencies in rats and mice are remarkably close. This suggests that when the physiological and biochemical differences in rats and mice are properly considered, these species have similar sensitivities to the carcinogenic action of VC metabolites and provides support for the hypothesis that reliable estimates of human liver cancer can be produced by this technique.

The reason for the discrepancy in potency factors derived from the Drew *et al.* study is not clear, since even within the same species (mouse) and experimental paradigm (12 months exposure, tumors evaluated at or before 12 months; Lee *et al.*, 1978; Drew *et al.*, 1983) dramatically different potencies were obtained. One possibility is that diagnostic criteria in this bioassay may have differed from those employed by other investigators, since angiosarcomas of the liver (a rare tumor) was not reported in any of the control mice from other groups but were reported in 2–5% of the control animals at NTP. This possibility could be evaluated by an expert committee of veterinary pathologists with access to slides from the archives of the different organizations.

It is also possible that the relatively high background incidence of liver tumors in the $B_6C_3F_1$ mouse has made it abnormally sensitive to the influence of liver carcinogens such as VC. This suggests that rodent strains with high background tumor incidences may not be good models to use when estimating human risk (if humans have much lower background incidences). In any case, as will be discussed later, the results from Maltoni's and Lee's groups appear to be much more consistent with the data from humans exposed to VC than the results of Drew *et al.* (1983).

Comparison of Potency Factors (PBPK and Conventional)

Maltoni's studies on VC carcinogenicity in rodents probably are the most extensive animal carcinogenicity data set in the world and were chosen as the most appropriate basis for estimations of human risk. Using the potency factor previously calculated for rats, it is possible to calculate the "unit risk" for humans continuously exposed to VC. The fitted dose–response curve indicates that lifetime exposure to 1.77×10^{-3} mg equivalents of VC metabolites/day/liter of liver tissue is associated with an increase in liver cancer risk of 1×10^{-6} (MLE). The 95% lower confidence limit on dose for this risk would be 1.40×10^{-3} mg equivalents of metabolite per day per liter of liver tissue.

It may be calculated with the PBPK model for humans that continuous exposure to 0.869 ppb of VC would be predicted to increase lifetime cancer risk by one in a million (MLE), or that continuous exposure to 1 μg VC/m^3 would increase lifetime cancer risk by 4.51×10^{-7} (MLE). The corresponding 95% upper confidence limits (UCL) obtained from GLOBAL83 are 0.687 ppb (for one in a million risk) or 5.70×10^{-7} increase in lifetime excess risk for continuous exposure to 1 μg VC/m^3.

The numbers calculated with the PBPK model may be contrasted with the value reported in HEAST (1995). In each case the calculations represent the UCL for excess lifetime risk associated with continuous inhalation of 1 μg/m^3 of VC:

HEAST Value	8.4×10^{-5} risk
PBPK Based Value	5.7×10^{-7} risk

Thus the value calculated from the PBPK-based approach described here suggests that the potency factor currently listed in HEAST should be reduced approximately 147-fold. In performing a risk estimation such as this one, there are many points where use of different assumptions/data (e.g., type of tumor modeled, selection of most sensitive species/bioassay as sole source of data, application of "less than lifetime" correction factors) can impact the cancer potency factor. Nevertheless, since PBPK modeling predicts roughly an order of magnitude less VC metabolism in humans than

rodents at equivalent atmospheric concentrations and does not include a surface area "correction" factor predicting that humans are always more sensitive than rodents by another order of magnitude. changes in these two factors appear to account for most of the differences (two orders of magnitude) in the two risk estimations.

The HEAST value is taken from a current issue of the EPA publication, but the entry for VC bears the note that the recommended values ". . . do not incorporate considerable information that is now available." The Office of Health and Environmental Assessment goes on to state that "One unpublished physiologically-based pharmacokinetic model prediction results in a 100-fold *increased* risk" (emphasis added). If the EPA were to increase the value in HEAST by 100-fold, then the procedures outlined here would differ from those in HEAST by 14,700-fold (more than four orders of magnitude).

Comparison with Human Epidemiology

It was noted above that considerable variation exists in the potency factors available for estimating the incidence of angiosarcomas in human populations exposed to VC. One of the most important questions, therefore, is: "Which of the alternative potency factors gives the most accurate description of the actual human experience?"

To answer this question, we have consulted the epidemiological literature generated on VC during the past 30–40 years. All of these studies share, to some extent, the common problems of not having complete follow-ups for the total lifetimes of the individuals, the possibility of missing a tumor when another cause of death is present, imprecise measures of exposure, etc. After surveying the avilable literature, we chose to compare our predicted risks to data gathered by Simonato *et al.* (1991). This epidemiology study was chosen because we believe that it represents one of the most robust analyses available, both with regard to the number of individuals followed and the quality and length of follow-up procedures employed.

A weakness in this database is the absence of a precise measure of the magnitude of VC exposures in the workplace. However, Simonato *et al.* (1991) have attempted to characterize the magnitude of occupational exposure by subdividing workers into groups based on their ppm · years of exposure (calculated as years on the job times TWA ppm levels estimated to be present in the occupational setting during hours of work = ppm · years). This grouping permitted simulation of worker exposures with the PBPK model. The reader is referred to Simonato's manuscript for further details of the exposure estimations.

Simonato *et al.* had 24 cases of liver cancer in their cohort. The overall incidence of liver cancer was statistically different than expected, and the odds ratios as high as 45:1 were observed in some of the groups with the longest duration of exposure and highest exposure concentrations (Table 9 in Simonato *et al.*, 1991). For the purpose of this comparison, we selected a subgroup of workers with more than 25 years since first exposure, subdivided by Simonato *et al.* into four exposure categories: (1) <2000 ppm · years, (2) 2000–5999 ppm · years, (3) 6000–9999 ppm · years, and (4) >10,000 ppm · years. Although this exposure information is obviously imprecise, it allowed the calculation of a roughly equivalent exposure paradigm in Table 5 so that we could predict the approximate tumor incidence in the groups studied by Simonato to compare with the results he reported.

For example, in the subgroup estimated to have the lowest exposures by Simonato (0–2000 ppm · years), the "reported" incidence of angiosarcoma was 6.2 per 100,000. In contrast, the risk assessment procedure described in this article gave a maximum likelihood estimate (MLE) of between 188 and 736 cases per 100,000 for ppm · years between 500 and 2000 (Table 5). Thus, the PBPK model predicted almost two orders of magnitude more cancer cases than actually occurred.

Similarly, individuals with 2000–5999 ppm · years had a reported incidence of 42.2 cases per 100,000, while the PBPK-based procedure estimated the incidence in this group to be from 700 to 1500 cases per 100,000. A similar disparity existed for the two most highly exposed groups from Simonato *et al.* (153–280 cases per 100,000 versus 1500 to 4000 cases per 100,000 predicted by the PBPK-based extrapolation. It is noteworthy that in the higher exposure group, the degree of overprediction by the PBPK procedure seems to decrease (from almost two orders of magnitude overprediction to approximately one order of magnitude; Table 5). It also appears that the degree of overprediction (excess conservatism) is greatest at the lowest rates of VC exposure (below 6000 ppm · years in Table 5).

It should be noted that a large fraction of the cohort from Simonato is still alive, so it is possible that more tumors may be added to the 24 already reported. Nevertheless, in view of the long follow-up time in the subgroup selected for comparison, it is considered extremely unlikely that the incidence will double even when all the workers are followed to the end of their natural lives.

Consequently, it appears that risk assessments based on estimates of the amounts of reactive metabolites of VC delivered to the liver of the target species (calculated with a PBPK model) *still* significantly overestimate the potential of VC metabolites to induce liver cancer in humans. Since the PBPK has been well validated in several species, we do not believe that this is because the PBPK model has overpredicted the formation of VC metabolites in human liver. Rather, it appears that the livers of humans are less sensitive to the carcinogenic effect of reactive VC metabolites than the livers of the commonly used inbred laboratory rodents.

The reasons for this lower sensitivity of human livers to reactive metabolites are not clear, but it has been noted that longer lived species such as humans have higher levels of DNA repair enzymes than rodents. Thus production of a genotoxic lesion in humans may not have the same adverse consequences as in the relatively DNA repair-deficient rodents. A variety of other explanations are also possible, and clearly further research will be required before it is possible to choose between the different possibilities.

In summary, the procedures we have described here (based on a quantitative description of the metabolism of VC in different species and a well-characterized oncogenic response to VC in different species) suggest that current estimates of the carcinogenicity of VC based on rodent studies significantly overestimate its oncogenic potential in humans.

Furthermore, there has been considerable discussion as to whether it is appropriate to include a "surface area factor" when using a PBPK model to extrapolate the results of rodent cancer studies to humans. We believe that the results of these studies suggest that inclusion of such a factor in a PBPK-based risk assessment cannot be justified on either pharmacokinetic or pharmacodynamic grounds.

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

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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.

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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^{-6}, or 2.0×10^{-6} $(\mu g/m^3)^{-1}$, 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^{-6} to 4.2×10^{-6} risk per ppm VC, but is roughly a factor of 30 below the currently published inhalation unit risk of 8.4×10^{-5} $(\mu g/m^3)^{-1}$. 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^{-6} $(\mu g/L)^{-1}$, 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^{-5} $(\mu g/L)^{-1}$.

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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HEAST TABLE 3: CARCINOGENICITY

March 1993

CHEMICAL	ROUTE	EXPERIMENT LENGTH / SPECIES	TARGET	CANCER	[EPA GROUP]	[SLOPE FACTOR] ORAL (mg/kg/day)$^{-1}$	[SLOPE FACTOR] INHALATION (mg/kg/day)$^{-1}$	[UNIT RISK] ORAL (ug/L)$^{-1}$	[UNIT RISK] INHALATION (ug/cu m)$^{-1}$	REFERENCE
VINYL CHLORIDE		000075-01-4								
	ORAL: DIET	8001 DAYS			A	1.9E+0		5.4E-5		010368
		RAT	LUNG	TUMORS						
			LIVER	TUMORS						
	ORAL [SLOPE] COMMENT: UNDER REVIEW, NUMBER SUBJECT TO CHANGE.									
	INHALATION: INTERMITTENT	1 YEAR			A		3.0E-1		8.4E-5	010367
		RAT	LIVER	TUMORS						

INHALATION [SLOPE] COMMENT: SEE APPENDIX A-II, DOSE CONVERSIONS ON HEAST.
INHALATION [UNIT RISK] COMMENT: UNDER REVIEW, NUMBER SUBJECT TO CHANGE.
GENERAL COMMENT: THE MOST RECENTLY REVIEWED QUANTITATIVE TOXICITY VALUES LISTED HERE APPEAR IN EPA DOCUMENTS PUBLISHED IN 1984 AND 1985. USE OF THESE VALUES ON AN INTERIM BASIS WAS VALIDATED BY CRAVE (04/05/90). THE AGENCY IS AWARE THAT THESE VALUES DO NOT INCORPORATE CONSIDERABLE INFORMATION THAT IS NOW AVAILABLE. THE OFFICE OF HEALTH AND ENVIRONMENTAL ASSESSMENT'S POSITION IS THAT THESE TOXICITY VALUES DO NOT REFLECT STATE-OF-THE-ART SCIENCE FOR VINYL CHLORIDE. EPA NOW HAS INDIVIDUAL ANIMAL DATA, NOT AVAILABLE WHEN THE ORAL UNIT RISK WAS CALCULATED, THAT MAY INFLUENCE THIS VALUE. ADDITIONAL INFORMATION THAT MAY BE FACTORED INTO A REVISED QUANTITATIVE TOXICITY VALUE INCLUDES DATA ON INCREASED SENSITIVITY OBSERVED IN YOUNG ANIMALS AND DATA ON METABOLISM/PHARMACOKINETICS. A UNIT RISK FOR AIR THAT CONSIDERS INFORMATION ON YOUNG AGE EXPOSURE INCREASES THE RISK (I.E., LOWERS THE RISK SPECIFIC DOSE) BY AT LEAST 3-FOLD. THE CONSIDERATION OF METABOLISM PHARMACOKINETICS WILL FURTHER INCREASE THE RISK. ONE UNPUBLISHED PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL PREDICTION RESULTS IN A 100-FOLD INCREASED RISK.

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