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§

*RHR Consulting Services, 4105 Chelsea Court, Midland, Michigan 48674-3361; †McLaren/Hart, ChemRisk Division, Cleveland, Ohio; ‡ICF Kaiser, K. S. Crump Division, Research Triangle Park, North Carolina; and §Zeneca Central Toxicology Laboratory, Macclesfield, England

Received November 21, 1995; accepted December 6, 1995

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

[1] To whom correspondence should be addressed. Fax: (517) 631-7089.

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.

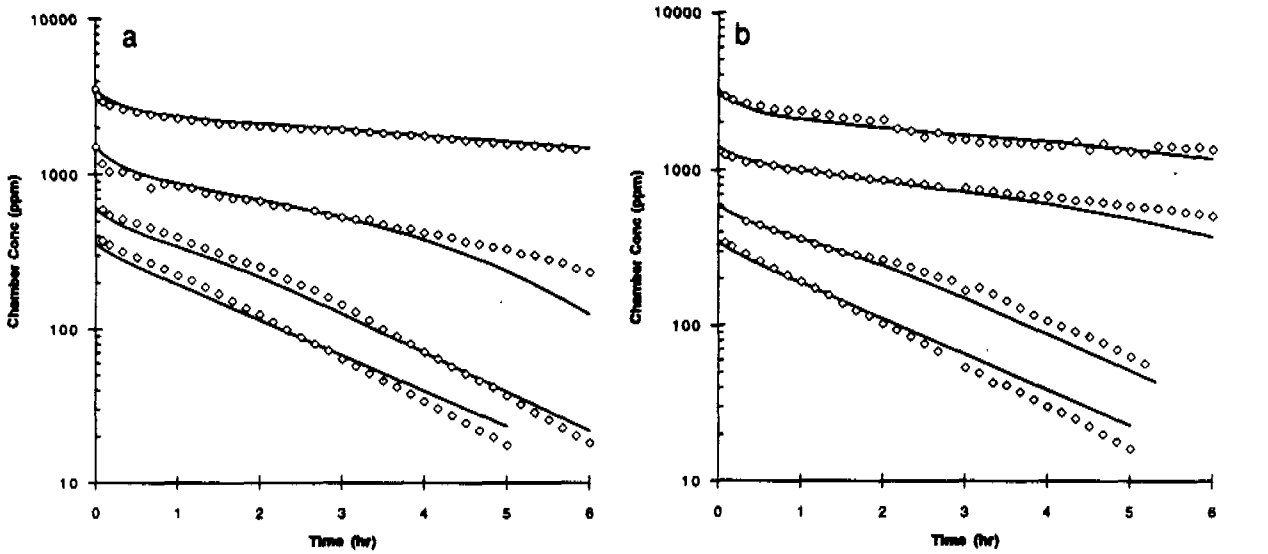

FIG. 1. Predicted (solid line) and observed (open symbols) concentrations of vinyl chloride in a 9.1-liter recirculating exposure chamber containing 3 male rats (a) or 3 female rats (b).

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}/g of liver (V_{max}/VL). Then the characteristic interspecies ratios of V_{max}/VL or 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

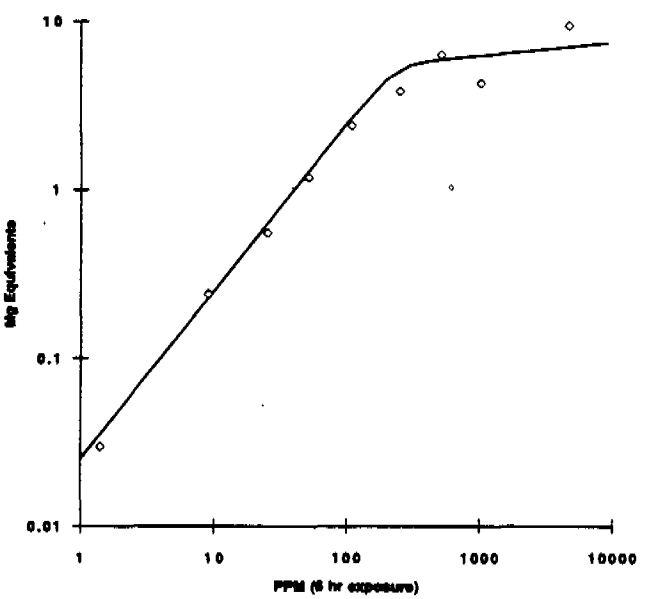

FIG. 2. Predicted (solid line) and observed (open symbols) amounts of radioactive metabolites derived from exposure of male rats to the indicated concentration of [^{14}C]vinyl chloride gas for 6 hr. Data taken from Watanabe *et al.* (1976).

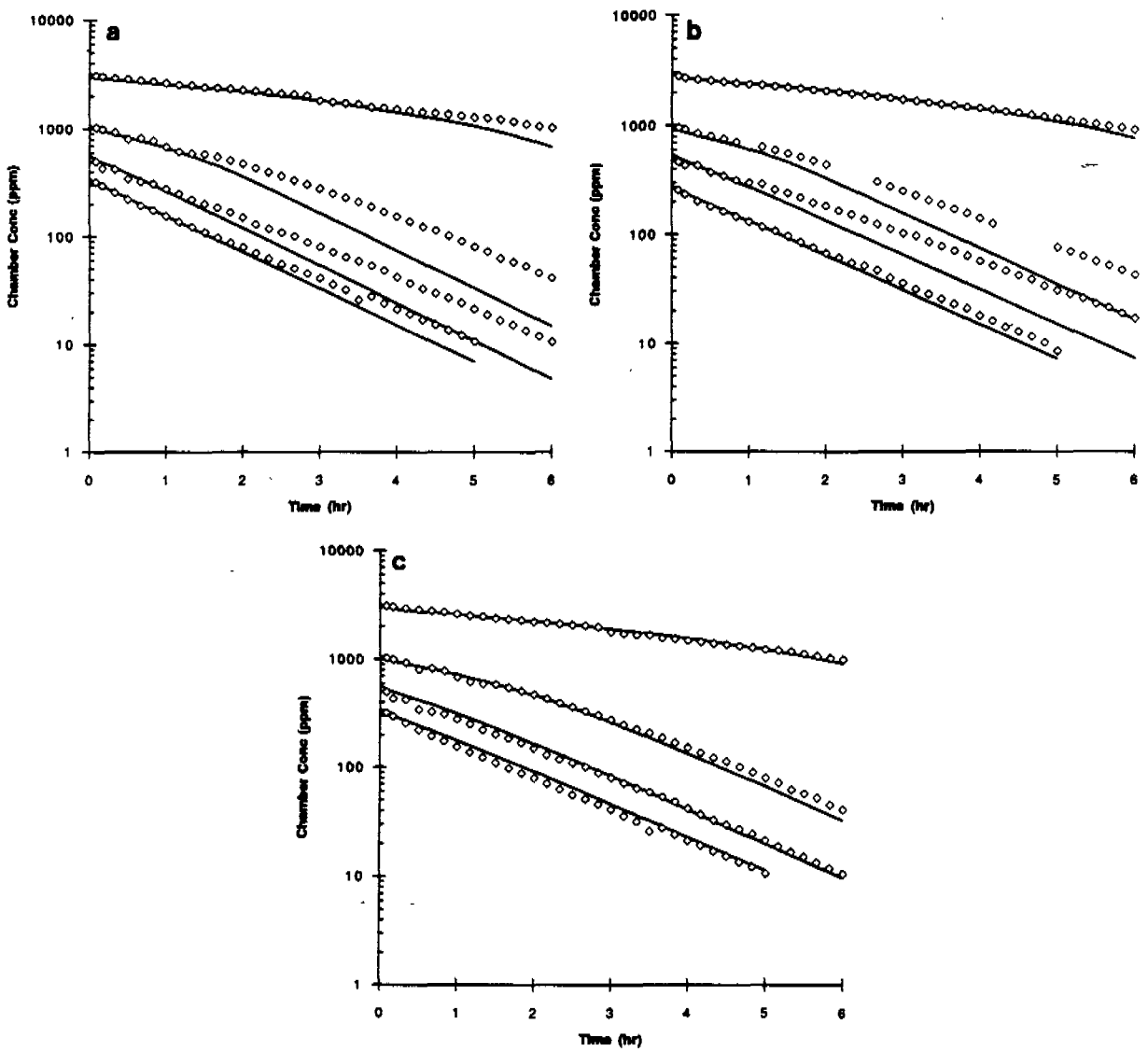

FIG. 3. Predicted (solid line) and observed (open symbols) concentrations of vinyl chloride in a 9.1-liter recirculating exposure chamber containing 14 male mice (a) or 14 female mice (b) with values of V_{maxC} and K_m calculated from *in vitro* studies (V_{maxC} = 9.04, K_m = 0.04). (c) Same data for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

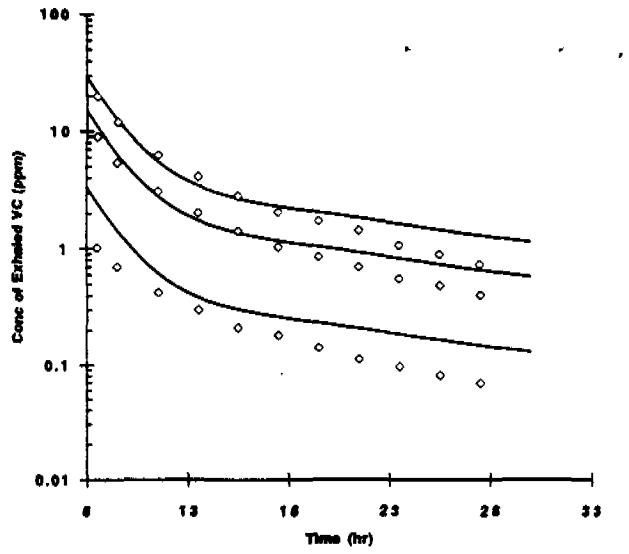

FIG. 4. Predicted (solid line) and observed (open symbols) concentrations of vinyl chloride exhaled by human subjects following 7.5 hr of exposure to vinyl chloride concentrations of 59, 261, or 492 ppm. Data are taken from Baretta *et al.* (1969).

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.

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).

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.

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-

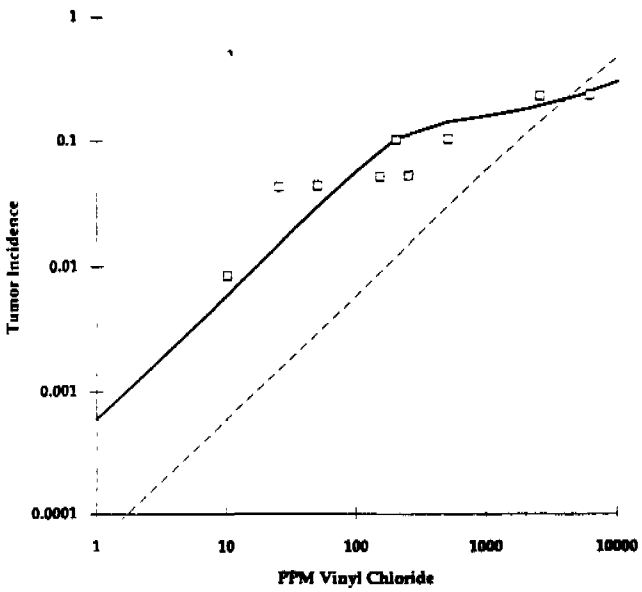

FIG. 5. Predicted (solid line) and observed (open symbols) incidences of liver angiosarcoma in rats following exposure to various concentrations of vinyl chloride for 4 hr/day, 5 days/week, for 12 months (animals held until death for observation of tumor incidence). The dotted line represents the type 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.

REFERENCES

Andersen, M. E., Gargas, M. L., and Ramsey, J. C. (1984). Inhalation pharmacokinetics: Evaluating system extraction, total *in vivo* metabolism, and the time course of enzyme induction for inhaled styrene in rats based on steady-state blood:air concentration ratios. *Toxicol. Appl. Pharmacol.* **73,** 176–187.

Andersen, M. E., Clewell, H. J., Gargas, M. L., Smith, F. A., and Reitz, R. H. (1987). Physiologically-based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* **87,** 185–205.

Andersen, M. E., Clewell, H. J., Gargas, M. L., MacNaughton, M. G., Reitz, R. H., Nolan, R. J., and McKenna, M. J. (1991). Physiologically based pharmacokinetic modeling with dichloromethane, its metabolite, Carbon Monoxide, and blood Carboxyhemoglobin in Rats and Humans. *Toxicol. Appl. Pharmacol.* **108,** 14–27.

Andersen, M. E., Clewell, H. J., III, Mahle, D. A., and Gearhart, J. M. (1994). Gas uptake studies of deuterium isotope effects on dichloromethane metabolism in female $B_6C_3F_1$ mice in vivo. *Toxicol. Appl. Pharmacol.* **128,** 158–165.

Baretta, E. D., Stewart, R. D., and Mutchler, J. E. (1969). Monitoring exposures to vinyl chloride vapor: Breath analysis and continuous air sampling. *Am. Ind. Hyg. Assoc. J.* **30,** 537–544.

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.

Corley, R. A., Mendrala, A. M., Smith, F. A., Staats, D. A., Gargas, M. L., Conolly, R. B., Andersen, M. E., and Reitz, R. H. (1990). Development of a physiologically based pharmacokinetic based model for chloroform. *Toxicol. Appl. Pharmacol.* **103,** 512–527.

Creech, J. L., and Johnson, M. N. (1974). Angiocarcoma of the liver in the manufacture of PVC. *J. Occup. Med.* **16,** 150–151.

Drew, R. T., Boorman, G. A., Haseman, J. K., McConnell, E. E., Busey, W. M., and Moore, J. A. (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.

ECETOC (1988). *The Mutagenicity and Carcinogenicity of Vinyl Chloride. A Historical Review and Assessment.* Technical Report No. 31, ISSN 0773-8072-31, Brussels, Belgium.

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.

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. Pharmacol.* **98,** 87–99.

Gargas, M. L., Clewell, H. J., III, and Andersen, M. E. (1990). Gas uptake inhalation techniques and the rates of metabolism of chloromethane, chloroethanes, and chloroethylenes in the rat. *Inhalation Toxicol.* **2,** 295–319.

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

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.

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

Health Effects Assessment Summary Tables (HEAST) (1995). United States Environmental Protection Agency, Office of Solid Waste and Emergency Response, 9200.6-303 (95-1), EPA/540 R-95/036, PB95-921199, May 1995.

Howe, R. B. (1983). GLOBAL83: An Experimental Program Developed for the U.S. Environmental Protection Agency as an update to GLOBAL82.

Howe, R. B., and Crump, K. S. (1982). *GLOBAL83: A Computer Program to Extrapolate Quantal Animal Toxicity Data to Low Doses (May 1982)* OSHA Contract No. 41USC252C3.

International Commission on Radiation Protection (1975). *Report of the Task Group on Reference Man* (W. S. Snyder *et al.*, Eds), ICRP Publication 23, Pergamon Press, New York.

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

Lee, C. C., Bhandari, J. C., Winston, J. M., Jouse, W. B., Dixon, R. L., and Woods, J. S. (1978). Carcinogenicity of vinyl chloride and vinylidene chloride. *J. Toxicol. Environ. Health* **4,** 15–26.

Maltoni, C. (1974). *Vinyl Chloride Carcinogenicity: An Experimental Model for Carcinogenesis Studies.* Monograph from the Institute of Oncology and Tumour Center, Bologna, Italy 40138.

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

Müller, G., Spassovski, M., and Henschler, D. (1974). Metabolism of trichloroethylene in man. II. Pharmacokinetics of metabolites. *Arch. Toxicol.* **32,** 283–295.

Nakajima, T., Wang, R. S., Murayama, N., and Sato, A. (1990). Three forms of trichloroethylene-metabolizing enzymes in rat liver induced by ethanol, phenoparbital, and 3-methylcholanthrene. *Toxicol. Appl. Pharmacol.* **102,** 6449–6455.

National Academy of Sciences (NAS) (1987). *Pharmacokinetics in Risk Assessment: Drinking Water and Health,* Vol. 8, National Academy Press, Washington, DC.

Ramsey, J. R., and Andersen, M. E. (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* **73,** 159–175.

Raucy, J. L., Kraner, J. C., and Lasker, J. M. (1993). Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. *Crit. Rev. Toxicol.* **23,** 1–20.

Reitz, R. H., McDougal, J. N., Himmelstein, M. W., Nolan, R. J., and Schumann, A. M. (1988). Physiologically based pharmacokinetic modeling with methylchloroform: Implications for interspecies, high dose/low dose and dose/route extrapolations. *Toxicol. Appl. Pharmacol.* **95,** 185–199.

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.

Reitz, R. H., Mendrala, A. M., Corley, R. A., Quast, J. F., Gargas, M. L., Andersen, M. E., Staats, D. A., and Conolly, R. B. (1990a). Estimating the risk of liver cancer associated with human exposures to chloroform using physiologically based pharmacokinetic modeling. *Toxicol. Appl. Pharmacol.* **105,** 443–459.

Reitz, R. H., McCroskey, P. S., Park, C. N., Andersen, M. E., and Gargas, M. L. (1990b). Development of a physiologically based pharmacokinetic model for risk assessment with 1,4-dioxane. *Toxicol. Appl. Pharmacol.* **105,** 37–54.

Sato, A., and Nakajima, T. (1979). Partition coefficients of some aromatic hydrocarbons and ketones in water, blood, and oil. *Br. J. Ind. Med.* **36,** 231–234.

Simonato, L., L'Abbe, K. A., Andersen, A., Belli, S., Comba, P., Engholm, G., Ferro, G., Hagmar, L., Langard, S., Lundberg, I., Perastu, R., Thomas, P., Winkelmann, R., and Saracci, R. (1991). A collaborative study of cancer incidence and mortality among vinyl chloride workers. *Scand. J. Work Environ. Health* **17,** 159–169.

Viola, P. L. (1970). Pathology of vinyl chloride. *Med. Lav.* **61,** 174.

Viola, P. L., Bigotti, A., and Caputo, A. (1971). Oncogenic response of rat skin, lungs, and bones to vinyl chloride. *Cancer Res.* **31,** 516–522.

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