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TOXICOLOGY AND APPLIED PHARMACOLOGY 95, 185–199 (1988)

Physiologically Based Pharmacokinetic Modeling with Methylchloroform: Implications for Interspecies, High Dose/Low Dose, and Dose Route Extrapolations

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Physiologically Based Pharmacokinetic Modeling with Methylchloroform: Implications for Interspecies, High Dose/Low Dose, and Dose Route Extrapolations. REITZ, R. H., MCDOUGAL, J. N., HIMMELSTEIN, M. W., NOLAN, R. J., AND SCHUMANN, A. M. (1988). *Toxicol. Appl. Pharmacol.* **95**, 185–199. A unified physiologically based pharmacokinetic (PB-PK) model was developed and used to describe the disposition of methylchloroform (1,1,1-trichloroethane, MC) in three different species (rats, mice, and humans) after four different routes of exposure (inhalation, intravenous injection, bolus gavage, and drinking water administration). Metabolism of MC followed Michaelis–Menten kinetics in each species. V_{max}'s were calculated from the allometric equation: $V_{max} = 0.419\ BW^{0.7}$, and K_m appeared to be identical in each species (5.75 mg equivalents/liter). Once the PB-PK model had been developed for young adult animals (1–3 months of age), it was used to study the disposition of MC in older rats and mice (approximately 18.5 months of age). Most of the changes in the pharmacokinetic behavior of MC in older rats could be simulated by increasing the size of the fat compartment in the PB-PK model from 7 to 18% of body weight. However, the pharmacokinetic behavior in older mice was more complex; increasing the size of the fat compartment in this species from 4 to 18% only accounted for part of the observed differences between old and young animals. An appropriate dose surrogate (average area under the liver concentration/time curve) was selected and the PB-PK model was used to make quantitative comparisons between "internal doses" of MC in long term animal studies and "internal doses" associated with human exposures to MC. Values of the dose surrogate in humans consuming 2 liters/day of water with typical levels of MC contamination (1–10 ppb) were four to six orders of magnitude lower than the dose surrogates in the rodent studies at levels of MC exposure which failed to produce adverse effects on the liver (875–1500 ppm, 6 hr/day, 5 days/week).

Lifetime bioassays in rodents are conducted to assess the potential of various substances to produce chronic toxicity, including carcinogenicity. When these studies are completed, they are used to estimate the risk that similar toxicity will be manifested in human populations exposed to the same agents. However, there are significant differences between the conditions in the lifetime rodent bioassay and conditions existing in the human environment, and these differences need to be considered in hazard evaluations.

Physiologically based pharmacokinetic

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(PB-PK) models provide a technique for eliminating some of the uncertainty in risk estimations (NAS, 1986; Andersen *et al.*, 1987). These models calculate the "internal dose" of relevant chemical species in target organs during specific exposure scenarios, and are useful for high dose/low dose extrapolations, dose route extrapolations, and interspecies extrapolations.

The utility of the PB-PK models stems from the fact that they realistically describe the sizes of organ compartments, partitioning of test material between blood and tissues, flow of blood and gases through the organs, and rates of metabolic transformation of the test chemical. The concept of incorporating this type of information into pharmacokinetic models was proposed 50 years ago by Teorell (1937), but computer hardware and software to implement his ideas were not available at that time. Examples of PB-PK models implemented with modern technology may be found in the works of Himmelstein and Lutz (1979), Dedrick (1973), and Fiserova-Bergerova (1975).

In order to demonstrate the versatility of this technique with a specific compound, we have developed a PB-PK model for 1,1,1-trichloroethane (methylchloroform, MC). This model was based on the model used by Ramsey and Andersen (1984) to describe the disposition of styrene, and details of the mathematical formulation of such models may be found in that publication.

Our objectives in this project were threefold:

(1) To demonstrate the ability of the PB-PK model to perform high dose/low dose, dose route, and interspecies extrapolations.

(2) To determine whether PB-PK models could be used to predict previously reported changes in MC disposition in older rats and mice (Schumann *et al.*, 1982b).

(3) To develop a mechanism for making quantitative comparisons between chronic animal inhalation studies and human exposures.

MATERIALS AND METHODS

Test materials. A sample of 1,1,1-trichloroethane (MC) was obtained from the Inorganic Chemicals Department of the Dow Chemical Co. This material was analyzed by gas chromatography (7.5% Oronite NIW plus 2.5% Carbowax 20 M TPA on 80/100 mesh Chromosorb W.H.P.) using a programmed temperature gradient of 4°C/min, starting at 70°C and finishing at 130°C. The test material was found to be more than 99.8% MC.

Radioactive MC [2-^{14}C], Lot No. 1119-293, (sp act 2.51 mCi/mmol) was obtained from New England Nuclear (Boston, MA) This material was repurified before use by preparative gas chromatography (10% SP 1000 on Chromosorb WAW, isothermal 100°C). The radiochemical purity after purification was found to be 97.9%. For administration to the animals, a saturated solution was prepared by stirring [^{14}C]MC in water for 8 hr (excess MC present). The sp act of the MC used in these experiments was 4.53×10^{-3} mCi/mmol and the concentration was 3.57 mg/ml ($2.66 \times 10^{+5}$ dpm/ml).

Animals. Male Fischer 344 rats (weight range 240–260 g) were obtained from Charles River Breeding Laboratories (Kingston, NY) and acclimated to the laboratory for at least 7 days before being placed on test. The animals were housed in stainless-steel cages in rooms designed to maintain 22°C, with relative humidity controlled between 40 and 60%, and a 12 hr/day lighting cycle from 7 AM to 7 PM. Animals were fed Certified Purina Chow (Ralston Purina, St. Louis, MO) and municipal water *ad libitum*.

Intravenous injections. Male F344 rats were anesthetized with ketamine/xylene and cannulas were implanted the day before the exposures (minimum 18 hr recovery time). MC was dissolved in heparinized rat plasma at a concentration sufficient to give doses of 8.84, 25.6, and 47.0 mg/kg when volumes of 1.0 ml plasma/kg were injected into an indwelling *femoral* cannula of rats. Blood samples (0.1 ml) were collected from an indwelling *jugular* cannula and immediately transferred to a sealed vial containing 1.0 ml hexane. After 45 min extraction, aliquots of the hexane layer were analyzed for MC by gas chromatography with an electron capture detector.

Oral gavage (MC in water). Solutions of MC in water were prepared by stirring overnight in a closed glass vessel. The concentration of MC in the water was determined by gas chromatography before administration to each rat, and was in the range of 0.8–1.0 mg/ml. MC was administered to rats (mean weight 250 g) in a volume of 4.0 ml to give a dose of 14.2 ± 1.9 mg/kg ($n = 6$). Blood samples were collected from indwelling *jugular* cannulas and analyzed for MC by gas chromatography as outlined above.

Drinking-water exposure. This exposure was carried out essentially as described by Frantz and Watanabe (1983). Animals were placed in all-glass metabolism

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cages designed to allow separate collection of urine, feces, expired air, and CO_2. Animals were allowed to acclimate to the cages for 32 hr and then water and food were withdrawn for 8 hr (from 4 PM to 12 midnight). Food and a saturated solution of [^{14}C]MC were presented at 12 midnight. At 8 AM the following morning, fresh water was substituted for the solution of [^{14}C]MC and the animals had free access to food and water until they were killed 48 hr later (56 hr after beginning the exposure).

The solution of [^{14}C]MC was administered with a glass water bottle which contained two stainless-steel balls in the sipper tube in order to eliminate leakage or evaporation of MC during periods when the animal was not drinking. Air was drawn through the cages at approximately 500 ml/min. Activated charcoal traps served to absorb [^{14}C]MC and ethanolamine traps (3 parts ethanolamine to 7 parts propylene glycol monomethyl ether) collected [^{14}C]CO_2. The activity of the [^{14}C]MC drinking water (dpm/ml) was determined at the beginning and end of the experiment and remained relatively constant ($2.70 \pm 0.23 \times 10^{+5}$ dpm/ml at the start; $2.62 \pm 0.24 \times 10^{+5}$ dpm/ml at the end; $n = 4$ in both cases).

Samples of urine and feces were collected during exposure, and samples of liver, kidney, fat, skin, and carcass homogenate were collected at the end of exposure for analysis of radioactivity.

Physiological modeling. A four-compartment physiological model similar to that developed by Ramsey and Andersen (1984) was used to describe the behavior of MC in rats, mice, and humans. Tissue volumes and blood and airflow rates employed in these simulations are listed in Table 1. The three species used in these studies differ widely in the percentage of fat in their carcass (Mouse = 4%, Rat = 7%, Human = 23%). Consequently, the percentage of cardiac output directed to the fat compartments was adjusted between species to reflect the different amounts of fat: Mouse = 2%, Rat = 5%, Human = 9%. To maintain mass balance in the model, the flows to the richly perfused compartment were adjusted appropriately (Table 1).

To adapt this model for drinking-water exposures, uptake of MC from the gastrointestinal tract was simulated as a zero-order process depositing MC directly into the liver compartment. This mathematical form of the model assumes rapid absorption of MC from the gastrointestinal tract. This assumption is consistent with the data of Withey *et al.* (1982) who studied the pharmacokinetics of four similar solvents (methylene chloride, chloroform, 1,2-dichloroethane, and trichloroethylene) and reported that following gavage with aqueous solutions of these materials, peak blood levels were reached in about 5 min.

This form of the model also assumes that zero-order input is a good approximation of the drinking process (i.e., that the animals consume a large number of small "sips" of the dosing solution throughout the exposure period). Pharmacokinetic analyses conducted by others suggest that this is a reasonable assumption (NAS, 1986).

Simulation of bolus gavage results was conducted as described by Ramsey and Andersen (1984). Absorption from the gastrointestinal tract was assumed to be a first-order process, with a rate constant of 1.25 hr^{-1}, and all material absorbed from the gastrointestinal tract was deposited directly into the liver compartment.

Partition coefficients. Tissue/air partition coefficients were determined for rat blood, liver, fat, and muscle using the vial equilibration technique of Sato and Nakajima (1979). Measurements were made at Wright Patterson AFB and were generously supplied by M. L. Gargas (personal communication). Tissue/blood partition coefficients for rat fat, muscle, and liver were then calculated by dividing the measured tissue/air partition coefficients by the blood/air coefficient for rat blood. Tissue/blood partition coefficients for the slowly perfused and rapidly perfused groups of tissues were assumed to be equal to the tissue/blood partition coefficients for muscle and liver respectively.

Blood/air partition coefficients for human and mouse blood were also determined by M. L. Gargas at Wright Patterson AFB (personal communication). Tissue/blood partition coefficients for human and mouse tissues were calculated by dividing the measured or estimated rat tissue/air partition coefficient by the blood/air partition coefficient for humans and mice respectively. All partition coefficients used in these simulations are listed in Table 1.

Metabolic rate constants (rat). Schumann *et al.* (1982a) exposed rats to 150 and 1500 ppm of [^{14}C]MC for 6 hr. Samples of expired air and excreta were collected for 66 hr postexposure in an apparatus which separated [^{14}C]MC from radioactive metabolites. After 66 hr the rats were killed and the levels of radioactivity remaining in the carcass (assumed to be all metabolites) were determined by combustion analysis. This procedure provides a quantitative measure of the total amounts of MC metabolites formed from [^{14}C]MC and eliminated postexposure. However, since samples of urine and expired air were not collected during the inhalation exposure, any radioactive metabolites eliminated during this period would have been lost. This would lead to an underestimation of the overall rate of metabolism. In order to compensate for this underestimation, data from the drinking water studies were used to derive a correction factor.

In the drinking-water study, water containing [^{14}C]MC was provided to the animals for 8 hr and samples were collected during this period and for an additional 48 hr postexposure. Animals were observed to drink regularly throughout this period. Forty-one percent of the total metabolites were collected during the exposure period, and 59% of the total metabolites were collected after exposure. Since the experimental conditions were similar to those used in the inhalation exposures, it was assumed that this procedure would give a reasonable approximation of the percentage of total metabolites recovered

postexposure in the inhalation experiments. Consequently, the postexposure levels of metabolism reported by Schumann *et al.* (1982a) in inhalation experiments were divided by 0.59 to give an estimate of total metabolism in the inhalation studies.

At this point, the PB-PK model was fully defined except for the metabolic rate constants V_{max} and K_m. Thus it was possible to estimate V_{max} and K_m by a computerized least-squares optimization procedure which varied the rate constants until the model correctly predicted the corrected amounts of metabolites formed during the exposures to 150 and 1500 ppm MC. Final values for the enzymatic rate constants were $V_{max}C = 0.419 \pm 0.095$; $K_m = 5.75 \pm 2.49$ (mean and standard deviation of estimates). Details of this type of computerized optimization have been reported elsewhere (Agin and Blau, 1982).

Computer simulation. Simultaneous differential and algebraic equations describing the movement of MC through the body were formulated as a computer program. Simulations were conducted with the SimuSolv software package which contains routines for numerical integration, graphics display, and derivation of model parameters from experimental data. SimuSolv was developed by Agin and Blau (1982) and is commercially available from Mitchell & Gauthier Associates.[2]

TABLE 1

PARAMETERS USED IN THE PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR METHYLCHLOROFORM[a]

	Human	Rat	Mouse
Weights			
Body wt (kg)	83.0	0.215	0.029
Liver	3.1%	4.0%	4.0%
Rapidly perfused	3.7%	5.0%	5.0%
Slowly perfused	61.1%	75.0%	78.0%
Fat	23.1%	7.0%	4.0%
Flows (liters/hr)			
Alveolar vent.	348.0	5.11	1.26
Cardiac output	348.0	5.11	1.26
	Percentage of cardiac output		
Liver	24.0	24.0	24.0
Rapidly perfused	49.0	53.0	56.0
Slowly perfused	18.0	18.0	18.0
Fat	9.0	5.0	2.0
Partition coefficients			
Blood/air	2.53	5.76	10.8
Liver/air	8.6	8.6	8.6
Rapidly perfused/air	8.6	8.6	8.6
Slowly perfused/air	3.15	3.15	3.15
Fat/air	263.	263.	263.
Biochemical constants			
$V_{max}C$ (allometric)	0.419	0.419	0.419
K_m (mg/liter)	5.75	5.75	5.75
K_e (hr^{-1})	—	1.25	—

[a] Metabolic parameters for the rat ($V_{max}C$, K_m) were obtained from the data of Schumann *et al.* (1982a) by computer optimization. $V_{max}C$ is an allometric measure of the maximum velocity of metabolism such that the maximum enzyme rate (V_{max}) may be calculated for any size animal according to the equation $V_{max} = V_{max}C \times (\text{body wt})^{+0.7}$.

RESULTS

Rat Simulations

Inhalation simulations. After incorporation of the appropriate physiological and physical chemical parameters (Table 1), the PB-PK model was used to predict the time course of the venous blood concentrations of MC in rats after exposure to 150 or 1500 ppm MC. Experimental data previously collected by Schumann *et al.* (1982a) were used to check the predictions, and the results are shown in Fig. 1. The data showed the best agreement with model predictions for blood level measurements made postexposure. Blood levels measured during the exposure were somewhat lower than model predictions at 4, 5, and 6 hr, but the predictions were still within a factor of 2 of the experimental data (Fig. 1).

Several other types of data were available from the studies of Schumann *et al.* (1982a) to check the accuracy of the PB-PK model. These were (1) the total body burden of MC and metabolites after 6 hr of exposure, (2) the concentration of radioactivity in fat tissue at the end of the exposure, and (3) the concentration of radioactivity in liver tissue at the end of the exposure.

Comparisons between the model predictions and these additional data are summa-

[2] Mitchell & Gauthier Associates, 73 Junction Square Drive, Concord, MA 01742.

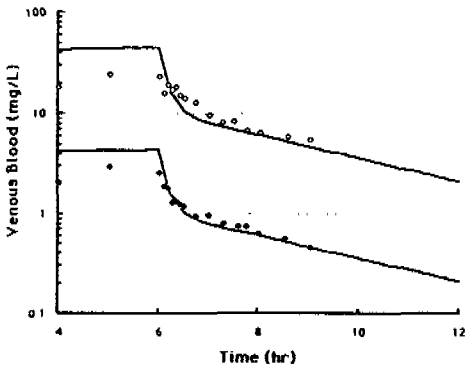

FIG 1. Blood levels of MC in rats during and following a 6-hr inhalation exposure to 150 ppm (closed circles) or 1500 ppm (open circles) of MC. Values predicted by computer simulations are shown as a solid line(s).

rized in Table 2. Overall, the ratio of predicted to actual data for these two exposures ranged from a low of 0.72 to a maximum of 1.92 and the mean ratio was 1.28 ± 0.48 (standard deviation; Table 2).

Intravenous administration. The PB-PK model was then used to attempt a dose route extrapolation: inhalation exposure to intravenous injection. Venous blood concentrations were measured in groups of animals (six per group) injected with MC dissolved in heparinized plasma. Intravenous injection was simulated in the model as a rapid infusion into pooled venous blood (about 1 min in duration). Three doses were studied: 8.8, 26, and 47 mg/kg. The only changes made to the PB-PK model for this simulation were in the equations describing entry of MC into the body. Experimental data and model predictions for 4 hr postinjection are shown in Fig. 2.

Bolus gavage (MC in water). A third route of administration (bolus gavage) was investigated. Six rats were administered a dose of 14.2 mg/kg MC dissolved in water, and serial blood samples were taken for analysis at various times afterwards. The equations in the PB-PK model were modified to reflect the new route of exposure. Uptake of MC was simulated as a first-order process with a rate constant (K_a) equal to 1.25 hr^{-1}. The model predictions and experimental data for this route are shown in Fig. 3.

Drinking-water exposure. A variant of the bolus gavage route was consumption of MC in drinking water over an extended period of time. Animals were presented with water containing radioactive MC for 8 hr and samples of urine, expired air, and selected tissues were analyzed for radioactivity at various times thereafter. The average water consumption of the rats (mean wt = 250 g) during the 8 hr in which they had access to the treated water was 8.1 ± 3.8 ml, corresponding to an average dose of 116 mg/kg. Overall recovery of radioactivity in these experiments (based on water consumption) was 95.2 ± 4.33% (standard deviation). Entry of MC into the animal was simulated as a zero-order process depositing MC in the liver compartment at a constant rate for 8 hr. Other than the changes to the input equations, no parameters were changed in the PB-PK model for drinking-water simulation. Simulated rates of MC elimination in exhaled air and experimental data (radioactivity recovered from charcoal traps) are shown in Fig. 4.

In addition, the use of [^{14}C]MC made it possible to predict the extent of MC metabolism for this route of metabolism. The model predicted that 5.49 μmol of MC (about 2% the ingested MC) would be metabolized, 8.19 μmol (about 3% of the ingested MC) was actually recovered in urine and CO_2 (Table 2).

Mouse Simulations

Inhalation simulations. The PB-PK model was then used for interspecies extrapolation by predicting the pharmacokinetic behavior of MC in male B6C3F1 mice after inhalation exposure. Model predictions were compared to experimental data gathered by Schumann *et al.* (1982a). Other than adjusting the appropriate physiological and biochemical parameters, no changes were made in the PB-PK model for simulation of the mouse exposures.

TABLE 2

COMPARISON OF PREDICTED AND OBSERVED VALUES FOR SELECTED PARAMETERS FROM INHALATION EXPOSURES AND DRINKING WATER EXPOSURES IN *YOUNG* RATS AND *YOUNG* MICE (2–3 months OF AGE) AND HUMAN VOLUNTEERS[a]

	Observed	Predicted	Ratio (pred/obs)
Young rat—150 ppm			
End exposure blood level (mg/liter)	2.64	4.37	1.66
Body burden at 6 hr (μmol)	33.0	25.5	0.77
Conc in fat (μmol/liter)	724.0	1264.0	1.75
Conc in liver (μmol/liter)	68.2	48.9	0.72
Young rat—1500 ppm			
End exposure blood level (mg/liter)	23.1	44.4	1.92
Body burden at 6 hr (μmol)	263	237	0.90
Conc in fat (μmol/liter)	8400	12800	1.52
Conc in liver (μmol/liter)	503	500	0.99
Young rat—drinking water (116 mg/kg)			
Amount metabolized (μmol)	8.19	5.49	0.67
Young mouse—150 ppm			
End exposure blood level (mg/liter)	9.27	8.54	0.92
Body burden at 6 hr (μmol)	4.97	3.30	0.66
Amount metabolized (μmol)	1.10	1.02	0.93
Conc in fat (μmol/liter)	1330	1570	1.19
Conc in liver (μmol/liter)	75.7	50.6	0.67
Young mouse—1500 ppm			
End exposure blood level (mg/liter)	111.0	88.1	0.79
Body burden at 6 hr (μmol)	39.5	25.8	0.65
Amount metabolized (μmol)	2.01	1.90	0.94
Conc in fat (μmol/liter)	16200	16100	0.99
Conc in liver (μmol/liter)	631	525	0.83
Human 35 and 350 ppm			
Amount metab. 35 ppm (μmol)	32.4	32.2	0.99
Amount metab. 350 ppm (μmol)	246	236	0.96

[a] Inhalation data are from Schumann *et al.* (1982a) and Nolan *et al.* (1984).

The predicted and observed venous blood concentrations (collected at the orbital sinus) in mice exposed to either 150 or 1500 ppm of MC are shown in Fig. 5. The PB-PK model predicted that MC would be eliminated from the mouse much more rapidly than the rat, and this is consistent with the observed data (Figs. 1, 5). This is also consistent with the observation of Schumann that elimination half-lives for MC in mice were 5- to 10-fold less than the corresponding half-lives in rats (Schumann *et al.* 1982a).

Several additional sources of experimental data were available for assessing the consis-

HALATION EXPO-
GE) AND HUMAN

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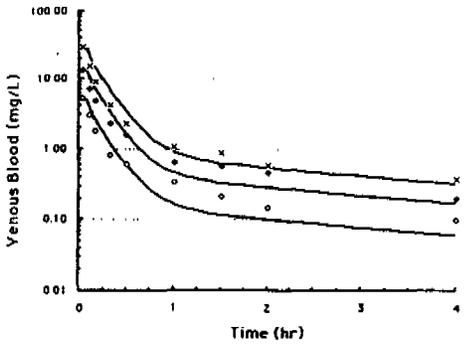

FIG. 2. Blood levels of MC in rats following intravenous injection of 8.8 mg/kg (open circles), 26 mg/kg (closed circles), or 47 mg/kg (crosses) of MC dissolved in rat plasma. MC was dissolved in heparinized plasma at concentrations calculated to give the indicated doses while maintaining a constant vehicle volume of 1 ml plasma/kg of body wt. Values predicted by computer simulations are shown as a solid line(s).

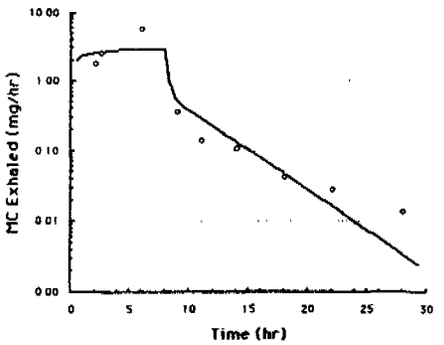

FIG. 4. Rate of elimination of ^{14}C[MC] in exhaled air (mg equivalents of MC/hr) during and following *ad libitum* exposure of rats to a solution of [^{14}C]MC in drinking water. The average water consumption of the rats (mean wt = 250 g) during the 8 hr in which they had access to the treated water was 8.1 ± 3.8 ml, corresponding to an average dose of 116 mg/kg. Values predicted by computer simulations are shown as a solid line(s).

tency of the PB-PK model for mice. Observed and predicted values of the end exposure body burden, the end exposure liver concentration, the end exposure fat concentration, and the total amount of metabolites recovered are summarized in Table 2. The ratio of predicted to actual data ranged from a low of 0.65 to a high of 1.19 and the mean ratio was 0.86 ± 0.16 (standard deviation; Table 2).

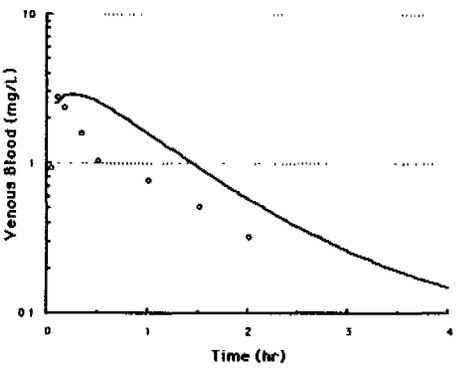

FIG. 3. Blood levels of MC in rats following oral gavage with a solution of MC in water. The dose of MC was 14.2 ± 1.9 mg/kg (standard deviation), administered in approximately 4.0 ml water/kg body wt. Values predicted by computer simulations are shown as a solid line(s). Absorption from the gastrointestinal tract was simulated as a first-order process with a rate constant of 1.25 hr^{-1}.

Human Exposures

The PB-PK model was used to predict the disposition of inhaled MC in humans. Model predictions were compared to data gathered by Nolan *et al.* (1984) at 35 and 350 ppm MC. Experimentally observed and simulated concentrations of MC in venous blood are shown in Fig. 6a. Concentrations of MC into blood was somewhat lower than predicted

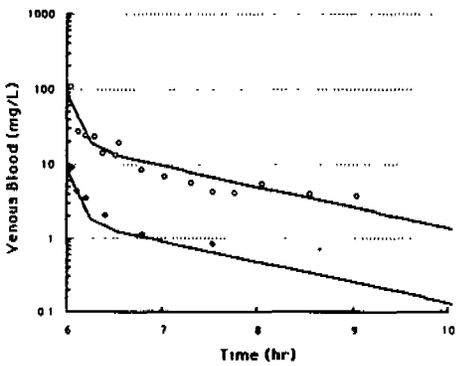

FIG. 5. Blood levels of MC in mice during and following a 6-hr inhalation exposure to 150 ppm (closed circles) or 1500 ppm (open circles) of MC. Values predicted by computer simulations are shown as a solid line(s).

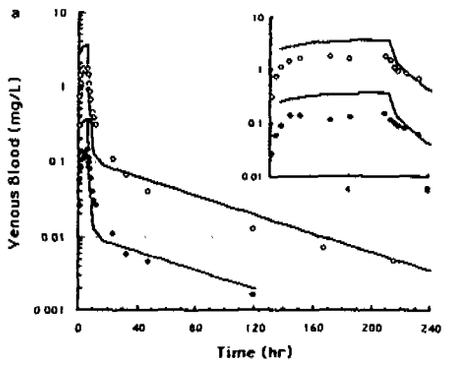

FIG. 6a. Concentration of MC in exhaled air (mg/liter) during and following a 6-hr inhalation exposure of human volunteers to 35 ppm (closed circles) or 350 ppm (open circles) of MC. Values predicted by computer simulations are shown as a solid line(s).

during exposure (insert) but was well described for the post exposure period (main figure).

Predicted and observed concentrations of MC in expired air are shown in Fig. 6b. Concentrations of MC in expired air were well simulated by the model during exposure (insert). The model predicted a slightly more rapid decline in the concentration of MC in expired air than was observed during the period of 10–30 hr, but the final slow elimination phase (30–240 hr) was well simulated by the model (main figure). Predicted and observed values of expired air and venous blood concentrations were both proportional to exposure concentration throughout the range of 35–350 ppm MC.

The PB-PK model predicted that 4.30 mg equivalents of MC would be metabolized during the 240 hr following a 6-hr exposure to 35 ppm MC, and 4.32 mg equivalents of MC metabolites were actually recovered by Nolan *et al.* (1984). Similarly, the model predicted that 31.5 mg equivalents of MC tabolites would be formed during the 240 hr following a 6-hr exposure to 350 ppm MC, and 32.9 mg were actually recovered (Table 2).

Older Animals

Old rats, inhalation simulations. Schumann *et al.* (1982b) also studied the pharmacokinetics of MC in control and exposed (1500 ppm, 6 hr/day, 5 days/week) male rats obtained from a study of the chronic inhalation toxicity of MC. The age of these animals was approximately 18.5 months, and the average body weight was 481 g. They reported that the disposition of a single dose of [^{14}C]MC in the treated and control animals was nearly identical (i.e., that chronic preexposure to unlabeled MC had not altered pharmacokinetic behavior through induction of enzymes, alteration of renal function, etc.). However, the disposition of MC in old animals differed significantly from the disposition previously observed in younger animals (Schumann *et al.*, 1982a).

The PB-PK model was used to predict the rate of elimination of MC in exhaled air in untreated (control) 18.5-month-old male rats following a 6-hr exposure to 1500 ppm MC. Experimental data and the initial simulation are shown in Fig. 7a. The PB-PK model predicted a much more rapid decline in the rate of MC exhalation when the physiological parameters chosen for young rats were used (heavy line, Fig. 7a).

Lutz *et al.* (1977) improved the accuracy of their simulation of polybrominated biphenyl compounds in rats by increasing the relative

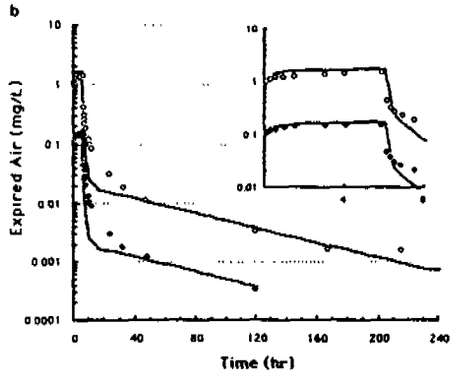

FIG. 6b. Concentration of MC in venous blood (mg/liter) during and following a 6-hr inhalation exposure of human volunteers to 35 ppm (closed circles) or 350 ppm (open circles) of MC. Values predicted by computer simulations are shown as a solid line(s).

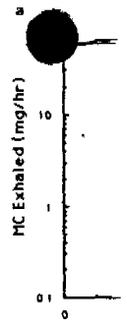

FIG. 7a. Rate
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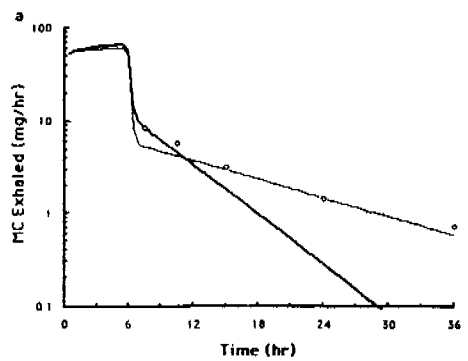

FIG. 7a. Rate of elimination of [^{14}C]MC in exhaled air (mg equivalents of MC/hr) following exposure of old (18 months) rats to 1500 ppm of [^{14}C]MC for 6 hr. Actual experimental data are shown as open circles on the plot. Simulated values with a fat compartment equal to 7% of body weight (i.e., simulation based on the parameters for young rats) are shown by the heavy solid line, while the light solid line shows the model predictions with a fat compartment equal to 18% of body weight.

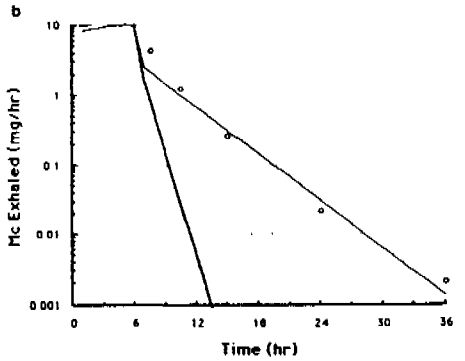

FIG. 7b. Rate of elimination of [^{14}C]MC in exhaled air (mg equivalents of MC/hr) following exposure of old (18 months) mice to 1500 ppm of [^{14}C]MC for 6 hr. Actual experimental data are shown as open circles on the plot. Simulated values with a fat compartment equal to 4% of body weight (i.e., simulation based on the parameters for young mice) are shown by the heavy solid line, while the light solid line shows the model predictions with a fat compartment equal to 18% of body weight.

proportion of body weight allocated to the fat compartment. Consequently, we conducted a second computer optimization to determine whether a similar modification would give a better simulation of the data from the 18.5-month-old rats. The computer was allowed to vary the volume of the fat compartment (VF) from 0 to 30% of body weight during simulations. Corresponding decreases in the fraction of body weight assigned to the slowly perfused compartment maintained a constant percentage of the carcass as perfused tissue (91% of body weight perfused). Greatly improved fits to the exhaled air data were obtained after the second optimization (light line, Fig. 7a). This optimization indicated the PB-PK model was most consistent with the exhaled air data when the VF was increased from 7% of body weight to 18% of body weight.

The model with the larger fat compartment was then used to predict several other types of experimental data available from the studies of Schumann *et al.* (1982b). These included end exposure body burden, end exposure concentration of MC-derived radioactivity in the liver, end exposure concentration of MC-derived radioactivity in the fat, and total amount of radioactive metabolites collected in 66 hr subsequent to the 6-hr exposure. The comparisons between the PB-PK model prediction and the actual data are summarized in Table 3.

The predictions of the PB-PK model for old rats with the original fat compartment (7% of body weight) are summarized in column 2 of Table 3. The model underpredicted the end exposure body burden and amount metabolized, but overpredicted the concentration of MC in fat tissue. After increasing the size of the fat compartment to 18% of body weight, the PB-PK model predicted lower concentrations of MC in fat tissue, and increased body burdens and amounts metabolized, giving a better simulation of the rat data.

Old mice inhalation simulations. Schumann *et al.* (1982b) also collected data in old male B6C3F1 mice (18.5 months of age) obtained from a study of the chronic inhalation toxicity of MC in mice. As was observed with the rats, the pharmacokinetics of MC in old

TABLE 3

COMPARISON OF OBSERVED AND PREDICTED VALUES FOR SELECTED PARAMETERS FROM INHALATION EXPOSURES IN *OLD* RATS AND *OLD* MICE (18.5 months OF AGE)[a]

	Actual	Before reoptimization	After reoptimization
	Old rat—1500 ppm		
Body burden at 6 hr (μmol)	744	490	638
(Ratio)		(0.66)	(0.86)
Amount metabolized (μmol)	31.8	17.5	22.1
(Ratio)		(0.55)	(0.69)
Conc in fat (μmol/liter)	5,685	11,700	6200
(Ratio)		(2.06)	(1.09)
Conc in liver (μmol/liter)	606	494	454
(Ratio)		(0.82)	(0.75)
	Old mouse—1500 ppm		
Body burden at 6 hr (μmol)	149	35.1	71.4
(Ratio)		(0.24)	(0.48)
Amount metabolized (μmol)	13.6	2.61	4.11
(Ratio)		(0.19)	(0.30)
Conc in fat (μmol/liter)	10,600	15,900	8790
(Ratio)		(1.50)	(0.83)
Conc in liver (μmol/liter)	1,790	532	491
(Ratio)		(0.30)	(0.27)

[a] Experimental data are from Schum[illegible] *et al.* (1982b). Physiological parameters for rats and mice before computer optimization are listed in Table 1, ex[illegible] body weight which was 481 g for the rats and 39.8 g for the old mice. After optimization, the size of the fat c[illegible]ment was 18.6 and 18.1% of body weight in rats and mice, respectively.

unexposed (control) mice differed significantly from the pharmacokinetics of MC in young control mice.

When the PB-PK model utilized to describe MC disposition in young mice was used to predict the rate of elimination of MC in exhaled air, a poor description of the experimental data was obtained (heavy line, Fig. 7b). However, when the size of the fat compartment was increased from 4% of body weight to 18% of body weight, a much better description of the experimental data was obtained (light line, Fig. 7b).

When the model with 4% fat was used to simulate additional parameters measured in older mice, it underestimated the body burden, the amount metabolized, and the concentration of radioactivity in liver tissue and overestimated the concentration of radioactivity in fat (Table 3). When the size of the fat compartment was increased to 18% of body weight, the description of the experimental data was improved, but the model still underpredicted the amount metabolized and the concentration of radioactivity in liver tissue.

DISCUSSION

Versatility of the PB-PK Model

The first objective of these studies was to demonstrate the extent to which incorporation of physiological and biochemical principles into pharmacokinetic modeling would allow extrapolation between different routes of administration and different species. Accordingly, we have not conducted extensive "curve fitting" procedures to try to improve

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22.1
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4.11
(0.30)
8790
(0.83)
491
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the appearance of the data presented here, and it is obvious that the extrapolations were not perfect in every case. However, since we are proposing that these techniques may be useful in the area of risk assessment, it is important to keep in mind the level of precision which currently exists in this process.

Cancer risk estimations produced from the same sets of data often differ by several orders of magnitude, depending upon the assumptions used about the shape of the dose–response curve at low concentrations. Furthermore, it is not uncommon for bioassays of the same material in the same species to differ by as much as an order of magnitude in their endpoints (tumor incidences). Consequently, we felt that if our integrated PB-PK model could predict relevant pharmacokinetic parameters for MC in different species and dose routes within a factor of 2 or 3, we would have demonstrated its potential for increasing the accuracy of current risk estimation procedures.

The construction of the PB-PK model used here was relatively straightforward. Physiological parameters for the various species have been published in the scientific literature (Davis and Mapleson, 1981; Caster *et al.*, 1956; ICRP, 1975), and partition coefficients were measured according to published procedures (Sato and Nakajima, 1979). The most difficult task was the estimation of the rate constants for *in vivo* metabolism.

MC is not extensively metabolized in any of the species studied (Schumann *et al.*, 1982a,b; Nolan *et al.*, 1984). Consequently the gas uptake technique used by Gargas *et al.* (1988) for estimation of *in vivo* metabolism with other compounds was not suitable for this material, since uptake depends upon metabolism once tissue loading has occurred. Furthermore, a variety of metabolites are formed from biotransformation of MC (trichloroethanol, the glucuronide of trichloroethanol, and trichloroacetic acid). The production of multiple metabolites, each of which is present at very low levels, makes it difficult to estimate total metabolism by chemical analysis.

However, radioactive metabolites of MC are easily separated from [^{14}C]MC and may be readily quantified. Consequently, we have used available data on the total levels of radioactive metabolites obtained in balance studies with rats exposed to [^{14}C]MC to estimate the rates of metabolism in this species. Levels of metabolites produced during exposure and postexposure were calculated with the PB-PK model, which contains the structure necessary to deal with the complexities of postexposure metabolism. We then employed allometric scaling procedures to estimate metabolic rate constants in the other species. Since the metabolic rate constants were obtained from a limited data set (two experiments in rats with four animals/experiment), there is obviously room for error in these parameters. The magnitude of the possible error may be roughly estimated from the standard deviations for the estimates of $V_{max}C$ and K_m, which were 23 and 43% of the mean, respectively. Nevertheless, it is encouraging that once the metabolic rate constants were obtained in this manner, they were able to accurately predict metabolism in two other species exposed to MC vapor (mice and humans) as well as in rats exposed to MC in drinking water (Table 2).

It must be emphasized that since metabolism plays a minor role in the elimination of MC in the various species, the behavior of MC itself (as distinct from MC metabolites) is almost entirely dependent upon the solubility in various tissues (partition coefficients) and the physiology of the various species. Small errors in the values chosen for the metabolic rate constants will have little effect on the overall disposition of MC.

Schumann *et al.* (1982a) utilized a conventional, data-based pharmacokinetic model to describe the time course of MC in rats. In this approach, the venous blood time/concentration curve for rats was actually described better by a simple two-compartment open model with elimination from the central compartment (Schumann *et al.*, 1982a) than the present PB-PK model. However, when

we attempted to use this model to describe the time course of MC in the venous blood of mice, a very poor fit was obtained (data not shown), pointing out the difficulties of using conventional models for extrapolating to humans. In contrast, once a PB-PK model had been developed for rats, it immediately provided a good description of the blood level data obtained in mice (Fig. 5) as well as humans (Fig. 6a).

Furthermore, the development of a PB-PK model allowed us to predict other types of data of potential toxicological significance. For example, the concentration of MC in liver tissue at the end of inhalation exposures to MC was well described in both rats and mice (Table 2). Since the liver has been identified as the target organ in both rats and mice during long-term animal bioassays (Rampy *et al.*, 1978; Quast *et al.*, 1984), this finding has obvious significance.

Another advantage of PB-PK models is their ability to provide quantitative descriptions of material administered by a variety of dose routes. In this particular case, the pharmacokinetics of MC in rats after inhalation (Fig. 1), intravenous injection (Fig. 2), bolus gavage (Fig. 3), and drinking-water administration (Fig. 4) could be described with a single, integrated model. Conventional data-based pharmacokinetic models lack the capacity for this type of route to route extrapolation.

Pharmacokinetics in Older Animals

A variety of physiological and biochemical changes may occur in test animals during the course of a lifetime bioassay, where animals are started on test a few weeks after weaning and continue on test until nearly the end of their natural lives. Development of a PB-PK model offers a mechanism whereby the consequences of these changes may be quantitatively evaluated.

For example, the PB-PK developed for MC indicates that the concentrations of MC in fat tissue are 20–100 times higher than in other tissues. Consequently, the increased size of the fat compartment in older, sedentary animals would be expected to increase the amount of MC taken up by older animals exposed to a given concentration of MC, and the elimination of MC from these older animals should occur more slowly. These predictions of the PB-PK have been experimentally verified in the data of Schumann *et al.* for both rats and mice (Figs. 7a, 7b; Tables 2, 3).

Most of the changes in the pharmacokinetics of MC in older rats were correctly predicted when the size of the body fat compartment was increased (Table 3). However, the situation appears to be somewhat more complex with B6C3F1 mice. The increased half-life of MC and lower concentration of radioactivity in the fat compartment of older mice were correctly predicted by a PB-PK model with a larger fat compartment. However, the predicted body burden and amount of MC metabolized in these mice were still much lower than actually observed by Schumann *et al.* (1982b) (Table 3). It appears that additional factors, such as increased metabolic capacity, may have to be considered in evaluating the kinetics of MC in older mice. The nature of these factors remains to be elucidated.

Relating Animals Studies to Human Exposures

MC has been studied in two long-term inhalation bioassays at the Toxicology Research Laboratory of the Dow Chemical Co. Animals were exposed to MC for 6 hr/day, 5 days/week for up to 2 years. MC exposure was not associated with increases in the incidences of either benign or malignant tumors in these studies. Reversible microscopic alterations in liver tissue were noted in both rats and mice, but cytotoxicity and necrosis were not seen. The no observed adverse effect levels (NOAEL) for the cellular changes established in these studies were 875 ppm in rats and 1500 ppm in mice (Rampy *et al.*, 1978;

TABLE 4

COMPARISON OF THE LIFETIME AVERAGE CONCENTRATION OF MC IN THE LIVER (ACL, DOSE SURROGATE FOR MC EFFECTS ON LIVER) FOR RATS AND MICE AT THE NO OBSERVED ADVERSE EFFECT LEVELS (NOAEL) WITH VALUES OF THE SAME DOSE SURROGATE IN HUMANS CONSUMING 2 liters/day OF WATER CONTAINING SMALL AMOUNTS OF MC[a]

	Conc (ppm)	Route	Average ACL (μmol/liter)	Safety factor relative To mouse	Safety factor relative To rat
Rat	875	Inhalation	28	—	—
Mouse	1500	Inhalation	95	—	—
Human	0.001	Water	3.4×10^{-5}	$2.8 \times 10^{+6}$	$8.2 \times 10^{+5}$
	0.003	Water	1.0×10^{-4}	$9.5 \times 10^{+5}$	$2.8 \times 10^{+5}$
	0.010	Water	3.4×10^{-4}	$2.8 \times 10^{+5}$	$8.2 \times 10^{+4}$
	0.030	Water	1.0×10^{-3}	$9.5 \times 10^{+4}$	$2.8 \times 10^{+4}$
	0.100	Water	3.4×10^{-3}	$2.8 \times 10^{+4}$	$8.2 \times 10^{+3}$
	0.300	Water	1.0×10^{-2}	$9.5 \times 10^{+3}$	$2.8 \times 10^{+3}$

[a] The NOAEL for the rat was 875 ppm, 6 hr/day, 5 days/week for 1 year (Rampy *et al.*, 1978) and the NOAEL for mice exposed 6 hr/day, 5 days/week for 2 years was 1500 ppm (Quast *et al.*, 1984). All dose surrogates are reported in units of μmol/liter. "Safety factors" are calculated by dividing the calculated animal dose surrogate by the predicted human dose surrogate.

Quast *et al.*, 1984). McNutt *et al.* (1975) reported similar effects in the liver of mice exposed to 250 ppm continuously for several weeks.

Since the liver is the target organ in both animal species, it is assumed that the liver would be the site where any adverse effects would occur in humans. Because the effects of MC on liver tissue were mild and reversible (i.e., without necrosis), it is not possible to determine whether they were caused by parent chemical or more reactive metabolites. Consequently we have chosen to use the average concentration of MC in the liver over the lifetime of the animal (ACL) as a "dose surrogate" for MC. Rationale for selection of dose surrogates with PB-PK models has been discussed in more detail by Andersen in the proceedings of a workshop conducted at the NAS (1987).

Selection of this type of dose surrogate for MC is probably a health protective assumption. Reactive metabolites, rather than parent chemical, are responsible for the toxicity of many other halogenated solvents, and levels of the enzyme which produces metabolites of MC appear to be lower in humans than in the small rodent species. For example, we can use the allometric equation to calculate that the concentration of MC-metabolizing enzyme (units of enzyme per liter of tissue) is about 13-fold lower in human liver than it is in mouse liver. Consequently, if we had chosen to use production of reactive metabolites as the dose surrogate rather than ACL, the estimated risk to humans would be considerably lower than with the present procedure.

Once the dose surrogate was chosen, the PB-PK model was used to calculate the ACL for rats and mice at the NOAEL in the long-term inhalation studies at Dow Chemical. The ACL for rats was about 55.2 μmol/liter during the 1-year exposure period, or about 27.6 μmol/liter averaged over the 2-year lifespan. The corresponding value for B6C3F1 mice was 95.1 μmol/liter (Table 4).

Small quantities of MC (typically 1–10 ppb, highest observed 300 ppb) have been detected in some finished drinking-water supplies. However, there are no long-term studies in animals in which MC has been administered by this route, and even if there were,

the limited solubility of MC would limit the dose that could be given to the animals. Consequently, there is a need for a mathematical procedure to quantitatively relate the "internal dose" in humans drinking such water to the "internal dose" in animal inhalation studies. This was readily accomplished with the PB-PK model developed in these studies.

ACLs associated with human consumption of water containing the indicated concentrations of MC are summarized in Table 4. It is obvious from this table that the ACLs in humans consuming this water are much lower than the ACLs in animals during the chronic inhalation studies. For example, the ACL in humans exposed to 10 ppb of MC in drinking water are 81,000-fold lower than the ACL at the NOAEL in the rat, and 270,000-fold lower than the ACL in the mouse (Table 4, columns 4 and 5).

Simulations with the PB-PK model revealed that "steady-state" levels of MC in the human liver are achieved within a relatively short period. The ACL after 100 days of drinking water containing 10 ppb MC was only 2.7 times higher than the ACL after a single day of consuming such water. This indicates that the risk to humans will not increase disproportionately after long periods of consumption, as might be the case with compounds such as lead or mercury.

There are bound to be many uncertainties in human risk estimations derived from animal studies. Although they cannot address all of the uncertainties inherent in this process, PB-PK models offer promise for improving the basis for high dose/low dose, interspecies, and dose route extrapolations. It should be noted, however, that PB-PK models are most useful when they are combined with independent studies elucidating the mechanism of toxicity so that appropriate dose surrogates may be chosen.

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Physiologically Based Pharmacokinetic Model for Vinylidene Chloride

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Physiologically Based Pharmacokinetic Model for Vinylidene Chloride. D'SOUZA, R. W., AND ANDERSEN, M. E. (1988). *Toxicol. Appl. Pharmacol.* 95, 230–240. Vinylidene chloride (VDC), a potent hepatotoxin and suspected carcinogen, is metabolized by mixed-function oxidases into a reactive metabolite(s) which is responsible for its toxicity. The metabolite is detoxified by glutathione (GSH), and liver GSH status is an important factor in the expression of VDC toxicity. A physiologically based pharmacokinetic (PB-PK) model has been developed for VDC in the rat based on oxidative metabolism of VDC and subsequent GSH detoxification of metabolite. The model offers insight into the complex interrelationship between the processes of absorption, metabolism, and GSH conjugation, and simulates the manner in which these factors operate in regulating VDC toxicity. The PB-PK model successfully predicts blood, tissue, and exhaled air concentrations of VDC, and liver GSH levels as a function of dose and route of administration. The model also explains the complex dose–response mortality curves seen with VDC. Because of the low blood:air partition coefficient of VDC and its saturable metabolism, the amount of VDC dose that is metabolized is sensitive to the rate of absorption. After an intravenous bolus dose, most of the administered VDC is exhaled unchanged within a few minutes. Blood VDC half-life is not representative of metabolism rates but to reequilibration of VDC from fat. Rats with greater fat content, therefore, display longer VDC blood half-lives. Simulations are shown to demonstrate the strength of PB-PK modeling techniques in understanding the kinetic behavior of VDC in the rat under a variety of experimental conditions.

Mathematical modeling of physiologic systems to determine the kinetic behavior of compounds, commonly known as physiologically based pharmacokinetic (PB-PK) modeling, has recently been introduced in the area of cancer risk assessment. The utility of this type of modeling has been demonstrated in explaining differences in cancer bioassay data after oral and inhalation exposure to methylene chloride (Andersen *et al.*, 1987) and in improving the process of estimating cancer risk for low level exposure to methylene chloride (Andersen *et al.*, 1987) and ethylene dichloride (D'Souza *et al.*, 1987). This modeling can also be used to explain or predict potential for toxicities other than cancer. To illustrate this utility a PB-PK model has been developed for vinylidene chloride (VDC; 1,1-dichloroethylene) in the rat. The model demonstrates the manner in which the interaction of various, often nonlinear processes, can affect both the pharmacokinetics and toxic potential of VDC.

VDC is a large volume chemical that is an air and drinking-water contaminant (U.S. EPA, 1982, 1986). VDC has been shown to be a potent hepatotoxin (Jenkins *et al.*, 1972; Reynolds *et al.*, 1975; Andersen *et al.*, 1979) and is also a suspected carcinogen (Maltoni *et al.*, 1977). The metabolism of VDC has been well studied (Henschler, 1977; Leibman and Ortiz, 1977). VDC is metabolized by mixed-

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function oxidases into an epoxide. This epoxide is unstable and can decompose to highly reactive intermediates, primarily chloroacetyl chloride (CAC). The intermediates are either detoxified by glutathione (GSH), bind to macromolecules, or are further metabolized to a dihydrodiol, resulting in CO_2 elimination in expired air. A portion of unchanged VDC dose is also eliminated in exhaled air. It is the reactive intermediates like CAC, and not parent VDC, that is responsible for the acute hepatotoxic effects of VDC (Andersen *et al.*, 1977, 1978; Henschler, 1977). The metabolism, and hence the toxicity, of VDC is affected by several factors. These include dose level, route of exposure, GSH status, and oral dosing vehicle employed.

VDC metabolism is saturable and dose–response curves for mortality in rats are complex, increasing sharply between 100 and 200 ppm inhalation concentration, but remaining virtually flat between 200 and 1000 ppm for a 4-hr inhalation exposures (Andersen *et al.*, 1979). Large differences in the pharmacokinetics and metabolic fate of VDC have been observed with an increase in exposure dose after oral (Jones and Hathway, 1978; McKenna *et al.*, 1978a), inhalation (McKenna *et al.*, 1978b; Andersen *et al.*, 1979), intravenous (Putcha *et al.*, 1986), and intraperitoneal (Jones and Hathway, 1978) routes of exposure. Furthermore, McKenna *et al.* (1977, 1978a,b) have shown that the amount of covalent binding, VDC exhalation, and VDC-related $^{14}CO_2$ exhalation is different after oral and inhalation exposures, and an increase in dose has different effects for oral and inhalation exposures. Since VDC metabolites are detoxified by GSH, VDC toxicity is sensitive to liver GSH concentration. Toxicity is, therefore, affected by such factors as fasting (Jaeger *et al.*, 1974; Andersen and Jenkins, 1977) and diurnal variation in GSH concentrations (Jaeger *et al.*, 1973). Effects of delivery vehicle on the pharmacokinetics and hepatotoxicity have also been observed after oral dosing. Cheico *et al.* (1981) dosed rats orally with VDC in corn oil, mineral oil, and an aqueous Tween vehicle and observed that hepatotoxicity was greater with vehicles like corn oil and mineral oil from which absorption is expected to be slow compared with Tween from which absorption of VDC would be more rapid. They also observed that the half-life of exhaled VDC was more prolonged after corn oil and mineral oil, compared to Tween vehicle.

Our purpose was to develop a physiologically based pharmacokinetic model for VDC in the rat that could be used to explain the changes in VDC behavior seen with the above factors and could also predict the pharmacokinetic behavior and toxic potential of VDC in untested conditions.

TABLE 1

PARAMETERS USED IN THE VDC PB-PK MODEL

Partition coefficients	
Liver:blood	1.1
Richly perfused:blood	1.1
Slowly perfused:blood	0.6
Fat:blood	18.4
Blood:air	5.0
Kinetic constants	
V_{max} (mg hr^{-1})	2.6
K_m (mg liter^{-1})	0.25
K_{gsm} (μM^{-1} hr^{-1})	0.33
K_{fec} (hr^{-1})	50
K_{inm} (hr^{-1})	9000
K_{CO_2} (M^{-1} hr^{-1})	1.82×10^{-5}
H_2O (M)	55

METHODS

PB-PK Model Development

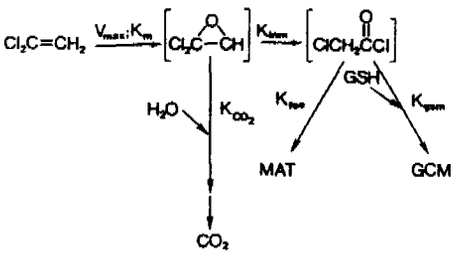
The basic structure of the PB-PK model is similar to that developed by Ramsey and Andersen (1984) for styrene, utilizing a lumped-tissue group approach. Compartments are the "richly perfused" group comprising such tissues as the lung, kidney, and spleen; the "slowly perfused" group, comprising muscle and skin; and a fat compartment. Metabolism was assumed to take place solely in the liver. The various parameters that were used in developing the PB-PK model are shown below.

Partition coefficients. Partition coefficients for blood:air and tissue:blood (Table 1) were determined by the

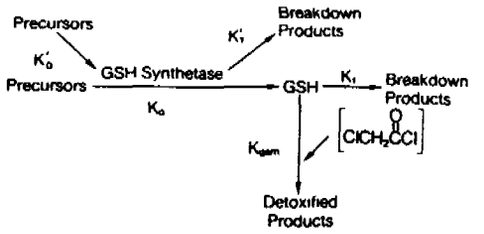
method of Sato and Nakajima (1979). Briefly, tissues were homogenized with normal saline using a Polytron homogenizer. Homogenates were spiked with low levels of VDC and allowed to equilibrate in sealed reaction vessels at 37°C in a water bath for 1–3 hr. A sample of the headspace vapor above the homogenate was then analyzed for VDC content. By appropriate mathematical corrections, the tissue:air partition coefficient was computed. Tissue:blood partition coefficient was obtained by dividing the tissue:air partition coefficient by the blood:air partition coefficient. Experience with partition coefficient measurements of over 50 chemicals in one of our (M.E.A.) laboratories has indicated that reaction of chemical with tissue homogenate does not typically take place. This may be due to the fact that cofactors, oxygen, etc., are not supplied, and also because the tissues are not handled in a particularly gentle manner. As a rule, two to three measurements are made a few hours apart. If the results are identical, this indicates to us that metabolism is not occurring.

Physiologic parameters. Values for organ volume and blood flow were taken from Gargas *et al.* (1986).

Modeling VDC metabolism. The pathway of VDC metabolism (Scheme I) contained a single activation step. Metabolism rate constants for conversion of VDC to the epoxide (V_{max} and K_m, Table 1) were determined by gas uptake studies (Gargas *et al.*, 1986). Metabolism was found to be saturable, as previously reported in the literature (Andersen *et al.*, 1979). It was assumed that VDC epoxide converted almost instantaneously to CAC or reacted with water to form CO_2 as the end product. The reaction of epoxide with water resulting in CO_2 was modeled similar to that reported by Dekant *et al.* (1984) for trichloroethylene oxide. Based on the data of McKenna *et al.* (1978), where 10% of radiolabel was recovered as CO_2 in expired air after a 1 mg/kg oral dose of radiolabel VDC, these two reactions were split in a 9:1 ratio (epoxide to CAC or CO_2, respectively). In order to simulate virtually instantaneous reactions while maintaining the 9:1 ratio, the first-order rate constant for conversion of epoxide to CAC (K_{inm}) was arbitrarily set to 9000 and the second-order reaction rate constant of epoxide with water (K_{CO_2} * 55 M water in liver) was therefore 1000, resulting in a value of $1.82 * 10^{-5}$ for K_{CO_2} (Table 1).



SCHEME I. Model for VDC metabolism pathways.



SCHEME II. GSH synthesis and depletion by chloroacetyl chloride.

For simplicity, it was assumed that the reaction with GSH was only with CAC and not with VDC epoxide or other intermediates. This assumption is justified based on the data of Liebler *et al.* (1985) where CAC reacted with model thiols (*in vitro*) 10^3 times faster than VDC epoxide and 10^5 times faster than minor metabolites formed from the epoxide. The relative contribution of the GSH pathway in detoxifying CAC, compared to the ability of this intermediate to elicit toxicity by binding to macromolecules (metabolite available for toxicity, MAT), was modeled as two first-order processes: reaction of CAC with GSH (rate constant K_{gsm}) and reaction with "everything else" (rate constant K_{foe}). Values for K_{gsm} and K_{foe} (Table 1) were obtained by using the PB-PK model for VDC developed until this level and varying K_{foe} (trial and error) until GSH depletion model predictions were a visual fit to GSH depletion data reported by McKenna *et al.* (1977), for inhalation exposures to increasing VDC concentrations. Starting values for K_{gsm} and K_{foe} were approximated based on previous experience with modeling GSH conjugation with reactive metabolites (Andersen *et al.*, 1986; D'Souza *et al.*, 1987, 1988).

Modeling GSH turnover. The model for GSH turnover and reaction with CAC (Scheme II) is similar to that reported for GSH depletion by ethylene dichloride (D'Souza *et al.*, 1988). It is somewhat simpler in that, unlike ethylene dichloride, parent VDC does not conjugate with GSH. Except for K_{gsm} and K_{foe}, the other rate constants are similar to those utilized by D'Souza *et al.* (1988).

Mathematical construction of model. Mass-balance differential equations were written for the various compartments in the model (Appendix A). Briefly, for nonmetabolizing compartments differential equations were written to describe the influx, binding, and efflux of VDC. The lung compartment was described similarly, but included a description of gas exchange between the lung and the ambient air. The equations for the liver compartment also accounted for VDC metabolism and GSH turnover. These equations were solved simultaneously by numerical integration, using Gear's algorithm for stiff systems. The ACSL[1] computer program

[1] Advanced Continuous Simulations Language, Mitchell and Gauthier, Concord, MA.

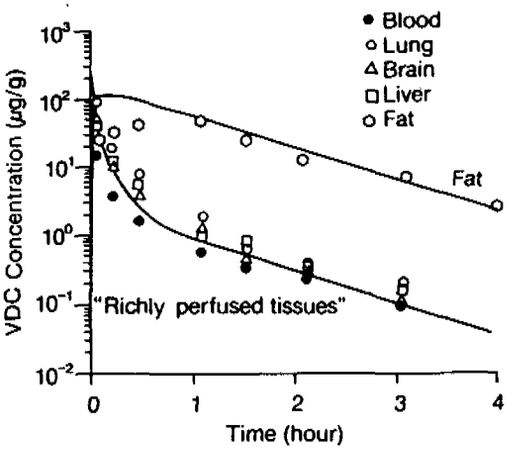


FIG. 1. Model predicted (—) and observed (symbols) VDC concentrations in different tissues of the rat following a single 50 mg/kg iv bolus dose. Predicted VDC concentrations for blood, liver, lung, and brain are shown as a single curve, as tissue:blood partition coefficient for the "richly perfused" tissue group was approximately one. (Data source: D'Souza, 1984).

was employed on an IBM PC-AT computer to solve these equations.

Animal studies. Although some experimental studies were performed to validate various portions of the model and to demonstrate predictability of the model, much of the data utilized to confirm model predictions were taken from the literature. Because the literature for VDC metabolism and acute toxicity is quite extensive (U.S. EPA, 1986) it was unnecessary to repeat many of the experiments. In all the figures, curves are PB-PK model predictions and symbols are experimental data. When the data source is acknowledged and referenced, the data were taken from the literature. When no reference is made, the data were collected by the authors for this study.

RESULTS

Once the PB-PK model was constructed, simulations could be generated to predict the disposition of VDC, its metabolic products, or liver GSH. In order to validate the model, predictions were generated for blood and tissue VDC concentration–time profiles after an iv bolus dose. These predictions were confirmed with experimental data (Fig. 1). Since the tissue:blood partition coefficient for the richly perfused group of tissues was 1.1, no difference in VDC concentrations was expected between this group of tissues and blood. Therefore, model prediction is shown as a single curve for the richly perfused tissues. Simulated and experimentally determined fat concentrations are also shown. As predicted, VDC concentrations in fat are considerably higher than concentrations in other tissues. The PB-PK model appears to predict VDC biodistribution in these iv dosing studies.

Dose level and route-of-exposure effects. The model was used to gain insight into the disposition of VDC as a function of dose level and route of exposure (Fig. 2). Because of saturation of VDC metabolism, there is a decrease in the percentage of dose that is converted to the epoxide as dose is increased. The percentage of VDC dose that reacts with GSH (glutathione-conjugated metabolite, GCM), or is eventually exhaled as CO_2, decreases with increasing dose (Fig. 2). This decrease begins at about a 50 mg/kg dose of VDC, indicating the dose where saturation of the oxidative pathway occurs. Although a decrease is seen in the parameters noted above, because of the complex interaction between the processes of absorption, metabolism and GSH conjugation, the relative decreases in these parameters do not run parallel. At the

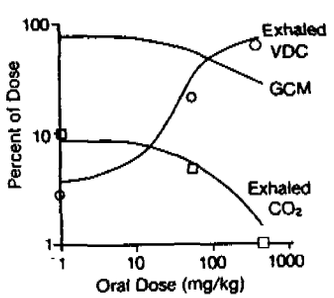


FIG. 2. Model simulations (—) and observed data for the effect of oral dose level on percentage of VDC dose that is exhaled unchanged (○), exhaled as CO_2 (□), or GCM (glutathione-conjugated metabolite (no observed data)). K_a of 1.0 hr^{-1} used in simulations. (Data source: 1 and 50 mg/kg doses, McKenna *et al.*, 1978a; 350 mg/kg dose, Jones and Hathway, 1978.)

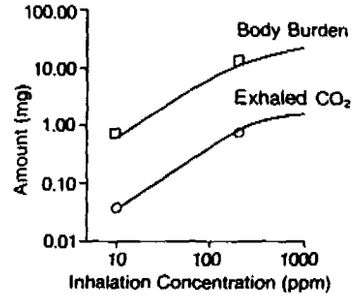


FIG. 3. Model simulations (—) for the effect of inhalation dose level (6-hr exposures) on body burden (□) and amount of dose exhaled as CO_2 (○). (Data source: McKenna *et al.*, 1978b.)

point where metabolism is saturated, an increasing percentage of absorbed VDC dose is also exhaled in the breath (Fig. 2). Experimental data for exhaled VDC and CO_2 are also shown to confirm model predictions. Once again, model predictions agree quite well with reported data.

A series of simulations were performed to study the effects of inhalation exposures (6 hr) on the disposition of VDC (Fig. 3). After inhalation exposures an apparent linear relationship exists between exposure concentration and the parameters shown, up to about 200 ppm concentration. At concentrations exceeding 200 ppm, VDC metabolism is saturated and the model predicts a less than proportional increase in the parameters shown with an increase in exposure concentration. Data from the literature (McKenna *et al.*, 1978) for body burden and CO_2 exhalation (Fig. 3) are shown along with the simulations. Saturation of VDC metabolism at about 200 ppm concentration has also been confirmed by Dallas *et al.* (1983).

GSH depletion effects. Figure 4 illustrates the effects of inhalation (Figs. 4a and 4b) and oral (Fig. 4c) VDC exposures on liver GSH levels. Experimentally measured liver GSH concentrations for inhalation (McKenna *et al.*, 1977; Reynolds *et al.*, 1980) and oral exposures agree quite well with model predicted values. As GSH levels are inversely related to toxicity, prediction of the time course of GSH depletion indirectly allows for the estimation of toxic potential.

Absorption rate effects. While simulating various aspects of the behavior of VDC, several observations were made that would not be readily noticed without the PB-PK model. For instance, because of the low blood:air partition coefficient and saturable metabolism of VDC, its disposition is sensitive to the rate of entry into the body. Figure 5 depicts the effects of increasing absorption rates on the percentage of VDC dose that will be exhaled unchanged at different oral dose levels. These simulations were performed for doses ranging from 1 to 200 mg/kg. Experience in studying the oral absorption rates of halogenated hydrocarbons (data not shown) suggests that the absorption rate constant for these compounds from aqueous vehicles, like Tween/water mixtures, is typically 5.0 hr^{-1}, while the rate constant from corn oil vehicle is typically 1.0 hr^{-1}. As absorption rate increases, particularly at higher doses, a greater percentage of VDC dose is exhaled unchanged. The toxicological implications of this observation are obvious. That is, greater toxicity will be seen if corn oil is used as the oral delivery vehicle compared to aqueous vehicles. Data collected by Cheico *et al.* (1981), where greater hepatotoxicity was seen with corn oil and mineral oil vehicles compared to a Tween vehicle, are consistent with this finding. Pharmacokinetic data in the literature also confirm this observation. McKenna *et al.* (1978a) noted that from a corn oil vehicle approximately 3 and 20% of the oral doses of 1 and 50 mg/kg, respectively, were exhaled. Our model predicts similar results if an absorption rate constant of 0.5–1.0 hr^{-1} is used. Similarly, the model predicts data reported by Jones and Hathway (1978) where 0.6 and 62% were exhaled after oral and 11 and 90% were exhaled after intraperitoneal doses of 0.5 and 350 mg/kg, respectively, (k_a of 10 hr^{-1} was used to simulate rapid absorption for the intraperitoneal doses; simulations not shown).

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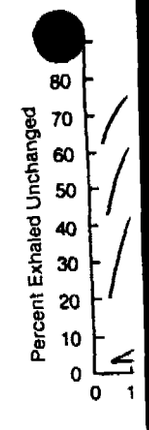

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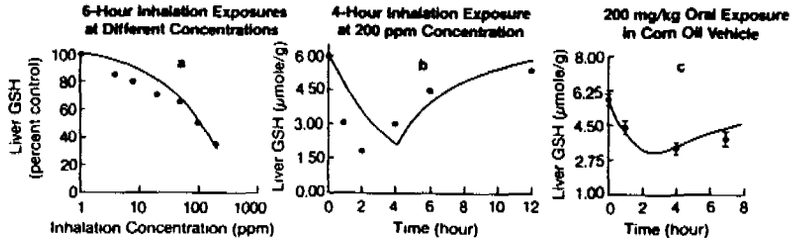


FIG. 4. Predicted (—) and observed (●) liver GSH concentrations following inhalation (a and b) and oral (c) exposure to VDC. (Data source: (a) McKenna *et al.*, 1977; (b) Reynolds *et al.*, 1980.)

Intravenous dosing studies. The model also predicts that after a bolus intravenous dose of VDC, because of the rapid input of VDC into the body, most of the dose will be exhaled unchanged within the first few minutes (simulations not shown). This prediction can be confirmed by studying the data reported by Jones and Hathway (1978), where 80% of an intravenous dose was exhaled after a low 0.5 mg/kg intravenous dose. Furthermore, if VDC metabolism was partially blocked or induced (Fig. 6a) no change in blood VDC half-life would be seen after an iv bolus dose. A similar simulation was performed for oral doses with absorption rate constants of 1 and 5 hr^{-1} to illustrate the effect of absorption rate on blood VDC kinetics. As absorption rate decreases, metabolism differences due to changes in V_{max} become more apparent. These observations suggest that unlike studies with nonvolatile compounds, intravenous bolus dosing has limited utility when studying the pharmacokinetic behavior or metabolism of volatile materials for which exposure from the environment is not intravenous.

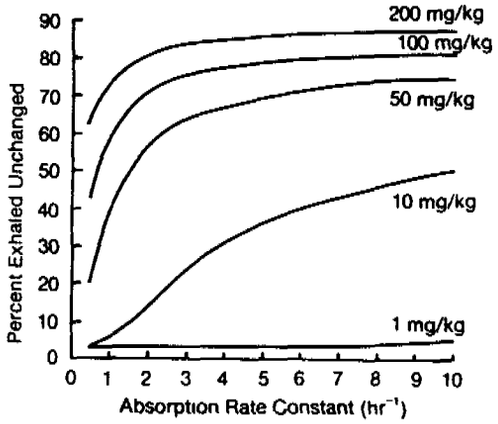


FIG. 5. Simulated effect of oral absorption rate constant on the percentage of VDC dose exhaled unchanged at various dose levels. It can be observed that the effect is negligible at low doses, very dramatic at the intermediate doses, and minor at doses of 200 mg/kg, where virtually all of the dose is exhaled, regardless of the absorption rate. This prediction suggests that VDC toxicity at say 50 mg/kg dose will be much greater with corn oil vehicle (absorption rate constant 0.5–2 hr^{-1}) compared to an aqueous vehicle (absorption rate constant 3–6 hr^{-1}).

Body-fat effects. PB-PK model simulations suggested that VDC blood half-life was due to reequilibration of VDC from fat depots back into the blood. Therefore, an increase in body fat would be expected to cause an increase in blood VDC half-life. Simulations were performed to determine blood VDC half-life in rats of three body-weight levels, 180, 300, and 600 g, and the simulations compared to literature data (D'Souza, 1984) to confirm predictions (Fig. 7). The only parameter that was changed when performing these simulations was percentage of body fat. For the three body-weight groups, body fat was set at 7% for the 180-g group, 12% for the 300-g group, and 22% for the 600-g group (Zucker and Zucker, 1963).

Effect of dose level on mortality. Andersen *et al.* (1979) showed an interesting dose–response relationship between inhaled VDC dose level and mortality in rats. They noted a very sharp increase in mortality, from 0 to

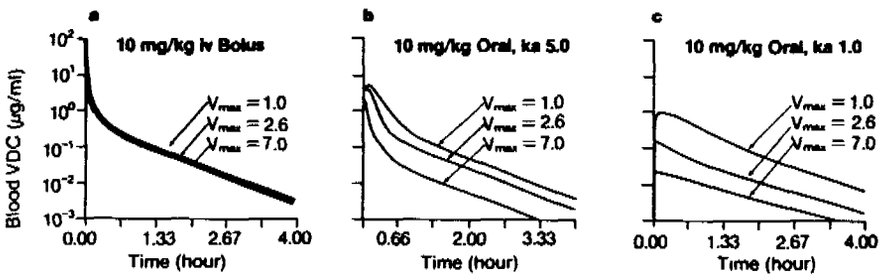


FIG. 6. Simulated blood VDC concentrations following intravenous bolus (a), and oral (b and c) doses. These simulations demonstrate that after intravenous bolus doses effects of enzyme inhibition or induction will not be observed from studying blood VDC levels. These effects will manifest themselves in blood VDC levels after oral dosing. The slower the absorption rate constant, the more pronounced the effect.

50%, when inhalation exposure concentration for a 4-hr exposure was increased from 100 to 200 ppm. However, from 200 to 1000 ppm there was virtually no increase in percentage mortality. The PB-PK model was used to study the relationship between inhalation exposure concentration and MAT. This relationship was then plotted on the dose–response plot of Andersen *et al.* (1979). The result is shown in Fig. 8. It is clear from this plot that the dose–response relationship for VDC is similar to the model predicted dose–MAT relationship. It can be seen that 50% mortality is achieved when about 12 μmol MAT is produced. Using this kind of information, mortality can be predicted for, say, other routes of intake. That is, regardless of the VDC dose and exposure route, 50% mortality is predicted when 12 μmol MAT is formed.

Preliminary extrapolation to humans. Although none of the parameters for constructing a PB-PK model were obtained in humans, a preliminary attempt was made to extrapolate the model to humans. The PB-PK model for the rat was scaled to the human by correcting for known physiologic differences between the rat and human, and by estimating VDC metabolism rates in humans based on allometric scaling principles (Dedrick *et al.*, 1973). In extrapolation to the human,

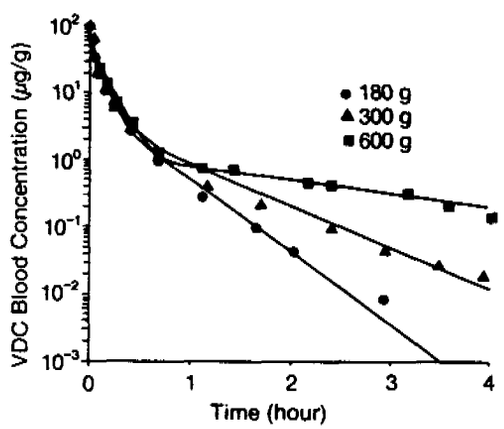


FIG. 7. Predicted (—) and observed (symbols) effects of body weight (body fat) on VDC blood concentrations in rats of different body-weight levels following a single 50 mg/kg iv dose. (Data source: D'Souza 1984).

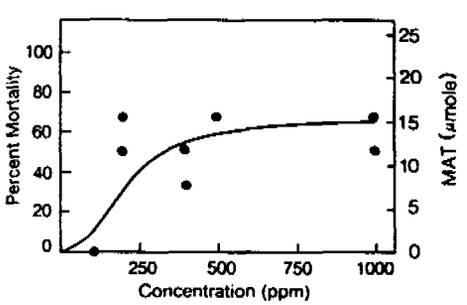
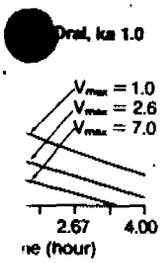


FIG. 8. Predicted (—) metabolite available for toxicity (MAT, chloroacetyl chloride not detoxified by glutathione (see text for more detailed explanation) and mortality dose response for 4-hr VDC inhalation exposures. (Data source: Andersen *et al.*, 1979.)



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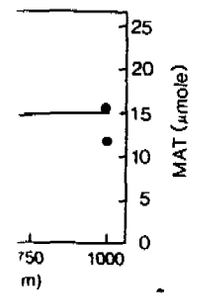

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cardiac output and pulmonary ventilation was scaled as a function of body weight (BW) to the 0.7 power, V_{max} as BW to the 0.74 power, and body fat was changed from 7% for a 200 g rat to 20% for a 70-kg human. This type of scaling has been successfully used in extrapolating animal data to the human for halogenated hydrocarbons using PB-PK models (Gargas *et al.*, 1986; Andersen *et al.*, 1987; D'Souza *et al.*, 1987). Figure 9 displays the relationship between VDC exposure dose level and the amount of epoxide formed for the rat and human. This simulation was conducted for both oral (Fig. 9a) and inhalation (Fig. 9b) exposures. Although in previous simulations MAT was used as the "internal dose," in this case, because humans are unlikely to be exposed to VDC concentrations that would decrease liver GSH, the shape of the curves for MAT and epoxide (versus VDC dose) are expected to be similar, and therefore epoxide is being used. Additionally, the use of epoxide serves to minimize the number of rate constants to be scaled from rat to human. It can be seen that saturation effects in VDC metabolism begin at about 10 mg/kg for oral, and 200 ppm for inhalation exposures for both the rat and human. Also, the amount of epoxide formed is predicted to be lower in the human compared to the rat at equivalent mg/kg oral or ppm inhalation concentrations.

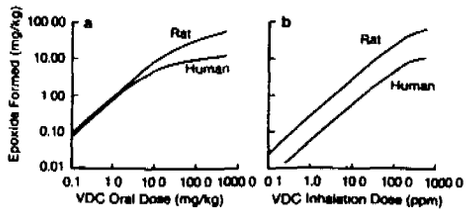

FIG. 9. Simulated amount of VDC epoxide produced at different (a) oral and (b) inhalation dose levels in the rat and human. After oral exposure saturation effects in VDC metabolism are expected in both rat and human at doses of 10 mg/kg, while after inhalation exposure saturation is predicted after about 200 ppm (6-hr) exposures.

DISCUSSION

The PB-PK model developed for VDC demonstrates the complex interplay of the many factors that contribute to the kinetic behavior and subsequently the toxicity of the compound. In terms of metabolism and pharmacokinetics at least, data from the literature appear to be different from one study to the other and at times appear to contradict each other when only minor differences in experimental design exist. The PB-PK model, however, demonstrates that given the physical, chemical, and biochemical properties of VDC, such differences are expected and can be predicted. For example, VDC metabolism profile, in terms of percentage VDC exhaled, percentage metabolized and percentage conjugated with glutathione is different for different routes of exposure and dose levels as reported by McKenna *et al.* (1978a,b) and Jones and Hathway (1978). It appears that slightly different experimental conditions can have large effects on the kinetic behavior of VDC.

For example, Putcha *et al.* (1986) have shown that although ether anesthetic used in their experiments may have had potential to inhibit VDC metabolism, no differences were seen in the kinetic behavior of VDC when ether-treated and untreated rats were compared after intravenous bolus injections of 10 mg/kg. The PB-PK model was used to study such a situation and simulations indicate that with intravenous bolus dosing no differences will be seen in VDC blood kinetics even if metabolism is blocked. Also, Putcha *et al.* (1986) noted a slight increase in the blood clearance of VDC with an increase in intravenous dose, while most of the other studies suggest a decrease in clearance because of saturation of metabolism. Putcha *et al.* (1986) calculated clearance from the area under the blood VDC time plot from the first time point (2–3 min postdose) to infinity. The PB-PK model demonstrates that for an intravenous dose most of the injected dose will be exhaled within the first few minutes, and, therefore,

under these experimental conditions VDC blood clearance will not show a decrease in clearance with increasing dose, but may actually show an increase, depending on when the first sample was taken (simulations not shown). Similarly, the effect of corn oil vehicle on hepatotoxicity (Cheico *et al.*, 1981) can also be readily explained on the basis of different rates of absorption of VDC from various dosing vehicles.

The purpose of obtaining animal kinetic data is to help in predicting the risk of chemicals to humans under realistic exposure situations. Because of the extreme sensitivity of VDC kinetics to these various factors, any model that is used to predict risk of VDC exposure to humans must be sensitive to these factors in order to meaningfully extrapolate the animal data to humans. A model that is restricted to extrapolation within a narrow data base cannot offer insight in untested conditions and, therefore, would have limited utility in extrapolation of animal data to the human. Scaling of the PB-PK model from the rat to the human presented here serves mainly the purpose of illustrating the manner in which these models can be used to extrapolate dose and toxicity information from animal to human for risk assessment. The PB-PK model for the human has parameters (V_{max} and K_m, for example) that were not obtained or verified in the human, nor have the final blood VDC concentrations in the human been validated by experimental data.

In summary, the metabolism and pharmacokinetics of VDC is complex and is affected by many factors. A composite model, physiologically realistic in design, has been developed to explain the kinetic behavior of VDC, predict untested situations, and help assess human risk to VDC exposure.

APPENDIX A

Mass Balance Equations for VDC Model

Mass balance differential equations for lung uptake of compound and for influx and efflux in noneliminating compartments were similar to those for methylene chloride (Andersen *et al.*, 1987), while equations for glutathione synthesis and depletion can be found in D'Souza *et al.* (1988). Differential equations describing VDC metabolism, which are unique to this model, are shown below.

$$d_{AM}/d_t = (V_{max} * CVL)/K_m + CVL)$$

$$d_{AMM}/d_t = d_{AM}/d_t - d_{ACO_2}/d_t - d_{ACAC}/d_t$$

$$d_{ACO_2}/d_t = K_{CO_2} * CMM * H_2O * VL$$

$$d_{ACAC}/d_t = K_{inm} * AMM$$

$$d_{ACACM}/d_t = d_{ACAC} - d_{AMAT}/d_t - d_{GCM}/d_t$$

$$d_{AMAT}/d_t = K_{fee} * ACACM$$

$$d_{GCM}/d_t = K_{gsm} * GSH * CCACM * VL$$

APPENDIX B

Nomenclature

ACAC	Amount of chloroacetyl chloride formed.
ACACM	Amount of chloroacetyl chloride present at time, *t*.
ACO_2	Amount of CO_2 formed.
AM	Amount of epoxide formed.
AMAT	Amount of metabolite available for toxicity.
AMM	Amount of epoxide present at time, *t*.
AGCM	Amount of glutathione-conjugated metabolite.
CCACM	Concentration of chloroacetyl chloride at time, *t*.
CMM	Concentration of epoxide at time, *t*.
d/d_t	Differential.
GCM	Glutathione-conjugated metabolite.
GSH	Glutathione concentration.
H_2O	Water concentration.
K_{CO_2}	Rate constant for reaction of epoxide with water.

K_{fee} First-order rate constant for formation of MAT.

K_{gsm} First-order rate constant for formation of GCM.

K_{inm} First-order rate constant for chloroacetyl chloride formation.

K_m Michaelis constant for oxidative pathway.

K_0 Zero-order glutathione synthesis, time and GSH dependent.

$K_{0'}$ Glutathione synthetase formation.

K_1 First-order rate constant for glutathione breakdown.

$K_{1'}$ First-order rate constant for glutathione synthetase breakdown.

MAT Metabolite available for toxicity.

V_{max} Maximum velocity of oxidative pathway.

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Physiologically Based Pharmacokinetic Modeling with Methylchloroform: Implications for Interspecies, High Dose/Low Dose, and Dose Route Extrapolations

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Physiologically Based Pharmacokinetic Modeling with Methylchloroform: Implications for Interspecies, High Dose/Low Dose, and Dose Route Extrapolations. REITZ, R. H., MCDOUGAL, J. N., HIMMELSTEIN, M. W., NOLAN, R. J., AND SCHUMANN, A. M. (1988). *Toxicol. Appl. Pharmacol.* **95**, 185–199. A unified physiologically based pharmacokinetic (PB-PK) model was developed and used to describe the disposition of methylchloroform (1,1,1-trichloroethane, MC) in three different species (rats, mice, and humans) after four different routes of exposure (inhalation, intravenous injection, bolus gavage, and drinking water administration). Metabolism of MC followed Michaelis–Menten kinetics in each species. V_{max}'s were calculated from the allometric equation: $V_{max} = 0.419\ BW^{0.7}$, and K_m appeared to be identical in each species (5.75 mg equivalents/liter). Once the PB-PK model had been developed for young adult animals (1–3 months of age), it was used to study the disposition of MC in older rats and mice (approximately 18.5 months of age). Most of the changes in the pharmacokinetic behavior of MC in older rats could be simulated by increasing the size of the fat compartment in the PB-PK model from 7 to 18% of body weight. However, the pharmacokinetic behavior in older mice was more complex; increasing the size of the fat compartment in this species from 4 to 18% only accounted for part of the observed differences between old and young animals. An appropriate dose surrogate (average area under the liver concentration/time curve) was selected and the PB-PK model was used to make quantitative comparisons between "internal doses" of MC in long term animal studies and "internal doses" associated with human exposures to MC. Values of the dose surrogate in humans consuming 2 liters/day of water with typical levels of MC contamination (1–10 ppb) were four to six orders of magnitude lower than the dose surrogates in the rodent studies at levels of MC exposure which failed to produce adverse effects on the liver (875–1500 ppm, 6 hr/day, 5 days/week).

Lifetime bioassays in rodents are conducted to assess the potential of various substances to produce chronic toxicity, including carcinogenicity. When these studies are completed, they are used to estimate the risk that similar toxicity will be manifested in human populations exposed to the same agents. However, there are significant differences between the conditions in the lifetime rodent bioassay and conditions existing in the human environment, and these differences need to be considered in hazard evaluations.

Physiologically based pharmacokinetic

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(PB-PK) models provide a technique for eliminating some of the uncertainty in risk estimations (NAS, 1986; Andersen *et al.*, 1987). These models calculate the "internal dose" of relevant chemical species in target organs during specific exposure scenarios, and are useful for high dose/low dose extrapolations, dose route extrapolations, and interspecies extrapolations.

The utility of the PB-PK models stems from the fact that they realistically describe the sizes of organ compartments, partitioning of test material between blood and tissues, flow of blood and gases through the organs, and rates of metabolic transformation of the test chemical. The concept of incorporating this type of information into pharmacokinetic models was proposed 50 years ago by Teorell (1937), but computer hardware and software to implement his ideas were not available at that time. Examples of PB-PK models implemented with modern technology may be found in the works of Himmelstein and Lutz (1979), Dedrick (1973), and Fiserova-Bergerova (1975).

In order to demonstrate the versatility of this technique with a specific compound, we have developed a PB-PK model for 1,1,1-trichloroethane (methylchloroform, MC). This model was based on the model used by Ramsey and Andersen (1984) to describe the disposition of styrene, and details of the mathematical formulation of such models may be found in that publication.

Our objectives in this project were threefold:

(1) To demonstrate the ability of the PB-PK model to perform high dose/low dose, dose route, and interspecies extrapolations.

(2) To determine whether PB-PK models could be used to predict previously reported changes in MC disposition in older rats and mice (Schumann *et al.*, 1982b).

(3) To develop a mechanism for making quantitative comparisons between chronic animal inhalation studies and human exposures.

MATERIALS AND METHODS

Test materials. A sample of 1,1,1-trichloroethane (MC) was obtained from the Inorganic Chemicals Department of the Dow Chemical Co. This material was analyzed by gas chromatography (7.5% Oronite NIW plus 2.5% Carbowax 20 M TPA on 80/100 mesh Chromosorb W.H.P.) using a programmed temperature gradient of 4°C/min, starting at 70°C and finishing at 130°C. The test material was found to be more than 99.8% MC.

Radioactive MC [2-^{14}C], Lot No. 1119-293, (sp act 2.51 mCi/mmol) was obtained from New England Nuclear (Boston, MA) This material was repurified before use by preparative gas chromatography (10% SP 1000 on Chromosorb WAW, isothermal 100°C). The radiochemical purity after purification was found to be 97.9%. For administration to the animals, a saturated solution was prepared by stirring [^{14}C]MC in water for 8 hr (excess MC present). The sp act of the MC used in these experiments was 4.53×10^{-3} mCi/mmol and the concentration was 3.57 mg/ml ($2.66 \times 10^{+5}$ dpm/ml).

Animals. Male Fischer 344 rats (weight range 240–260 g) were obtained from Charles River Breeding Laboratories (Kingston, NY) and acclimated to the laboratory for at least 7 days before being placed on test. The animals were housed in stainless-steel cages in rooms designed to maintain 22°C, with relative humidity controlled between 40 and 60%, and a 12 hr/day lighting cycle from 7 AM to 7 PM. Animals were fed Certified Purina Chow (Ralston Purina, St. Louis, MO) and municipal water *ad libitum*.

Intravenous injections. Male F344 rats were anesthetized with ketamine/xylene and cannulas were implanted the day before the exposures (minimum 18 hr recovery time). MC was dissolved in heparinized rat plasma at a concentration sufficient to give doses of 8.84, 25.6, and 47.0 mg/kg when volumes of 1.0 ml plasma/kg were injected into an indwelling *femoral* cannula of rats. Blood samples (0.1 ml) were collected from an indwelling *jugular* cannula and immediately transferred to a sealed vial containing 1.0 ml hexane. After 45 min extraction, aliquots of the hexane layer were analyzed for MC by gas chromatography with an electron capture detector.

Oral gavage (MC in water). Solutions of MC in water were prepared by stirring overnight in a closed glass vessel. The concentration of MC in the water was determined by gas chromatography before administration to each rat, and was in the range of 0.8–1.0 mg/ml. MC was administered to rats (mean weight 250 g) in a volume of 4.0 ml to give a dose of 14.2 ± 1.9 mg/kg ($n = 6$). Blood samples were collected from indwelling *jugular* cannulas and analyzed for MC by gas chromatography as outlined above.

Drinking-water exposure. This exposure was carried out essentially as described by Frantz and Watanabe (1983). Animals were placed in all-glass metabolism

D METHODS

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cages designed to allow separate collection of urine, feces, expired air, and CO_2. Animals were allowed to acclimate to the cages for 32 hr and then water and food were withdrawn for 8 hr (from 4 PM to 12 midnight). Food and a saturated solution of [^{14}C]MC were presented at 12 midnight. At 8 AM the following morning, fresh water was substituted for the solution of [^{14}C]MC and the animals had free access to food and water until they were killed 48 hr later (56 hr after beginning the exposure).

The solution of [^{14}C]MC was administered with a glass water bottle which contained two stainless-steel balls in the sipper tube in order to eliminate leakage or evaporation of MC during periods when the animal was not drinking. Air was drawn through the cages at approximately 500 ml/min. Activated charcoal traps served to absorb [^{14}C]MC and ethanolamine traps (3 parts ethanolamine to 7 parts propylene glycol monomethyl ether) collected [^{14}C]CO_2. The activity of the [^{14}C]MC drinking water (dpm/ml) was determined at the beginning and end of the experiment and remained relatively constant ($2.70 \pm 0.23 \times 10^{+5}$ dpm/ml at the start; $2.62 \pm 0.24 \times 10^{+5}$ dpm/ml at the end; $n = 4$ in both cases).

Samples of urine and feces were collected during exposure, and samples of liver, kidney, fat, skin, and carcass homogenate were collected at the end of exposure for analysis of radioactivity.

Physiological modeling. A four-compartment physiological model similar to that developed by Ramsey and Andersen (1984) was used to describe the behavior of MC in rats, mice, and humans. Tissue volumes and blood and airflow rates employed in these simulations are listed in Table 1. The three species used in these studies differ widely in the percentage of fat in their carcass (Mouse = 4%, Rat = 7%, Human = 23%). Consequently, the percentage of cardiac output directed to the fat compartments was adjusted between species to reflect the different amounts of fat: Mouse = 2%, Rat = 5%, Human = 9%. To maintain mass balance in the model, the flows to the richly perfused compartment were adjusted appropriately (Table 1).

To adapt this model for drinking-water exposures, uptake of MC from the gastrointestinal tract was simulated as a zero-order process depositing MC directly into the liver compartment. This mathematical form of the model assumes rapid absorption of MC from the gastrointestinal tract. This assumption is consistent with the data of Withey *et al.* (1982) who studied the pharmacokinetics of four similar solvents (methylene chloride, chloroform, 1,2-dichloroethane, and trichloroethylene) and reported that following gavage with aqueous solutions of these materials, peak blood levels were reached in about 5 min.

This form of the model also assumes that zero-order input is a good approximation of the drinking process (i.e., that the animals consume a large number of small "sips" of the dosing solution throughout the exposure period). Pharmacokinetic analyses conducted by others suggest that this is a reasonable assumption (NAS, 1986).

Simulation of bolus gavage results was conducted as described by Ramsey and Andersen (1984). Absorption from the gastrointestinal tract was assumed to be a first-order process, with a rate constant of 1.25 hr^{-1}, and all material absorbed from the gastrointestinal tract was deposited directly into the liver compartment.

Partition coefficients. Tissue/air partition coefficients were determined for rat blood, liver, fat, and muscle using the vial equilibration technique of Sato and Nakajima (1979). Measurements were made at Wright Patterson AFB and were generously supplied by M. L. Gargas (personal communication). Tissue/blood partition coefficients for rat fat, muscle, and liver were then calculated by dividing the measured tissue/air partition coefficients by the blood/air coefficient for rat blood. Tissue/blood partition coefficients for the slowly perfused and rapidly perfused groups of tissues were assumed to be equal to the tissue/blood partition coefficients for muscle and liver respectively.

Blood/air partition coefficients for human and mouse blood were also determined by M. L. Gargas at Wright Patterson AFB (personal communication). Tissue/blood partition coefficients for human and mouse tissues were calculated by dividing the measured or estimated rat tissue/air partition coefficient by the blood/air partition coefficient for humans and mice respectively. All partition coefficients used in these simulations are listed in Table 1.

Metabolic rate constants (rat). Schumann *et al.* (1982a) exposed rats to 150 and 1500 ppm of [^{14}C]MC for 6 hr. Samples of expired air and excreta were collected for 66 hr postexposure in an apparatus which separated [^{14}C]MC from radioactive metabolites. After 66 hr the rats were killed and the levels of radioactivity remaining in the carcass (assumed to be all metabolites) were determined by combustion analysis. This procedure provides a quantitative measure of the total amounts of MC metabolites formed from [^{14}C]MC and eliminated postexposure. However, since samples of urine and expired air were not collected during the inhalation exposure, any radioactive metabolites eliminated during this period would have been lost. This would lead to an underestimation of the overall rate of metabolism. In order to compensate for this underestimation, data from the drinking water studies were used to derive a correction factor.

In the drinking-water study, water containing [^{14}C]MC was provided to the animals for 8 hr and samples were collected during this period and for an additional 48 hr postexposure. Animals were observed to drink regularly throughout this period. Forty-one percent of the total metabolites were collected during the exposure period, and 59% of the total metabolites were collected after exposure. Since the experimental conditions were similar to those used in the inhalation exposures, it was assumed that this procedure would give a reasonable approximation of the percentage of total metabolites recovered

postexposure in the inhalation experiments. Consequently, the postexposure levels of metabolism reported by Schumann *et al.* (1982a) in inhalation experiments were divided by 0.59 to give an estimate of total metabolism in the inhalation studies.

At this point, the PB-PK model was fully defined except for the metabolic rate constants V_{max} and K_m. Thus it was possible to estimate V_{max} and K_m by a computerized least-squares optimization procedure which varied the rate constants until the model correctly predicted the corrected amounts of metabolites formed during the exposures to 150 and 1500 ppm MC. Final values for the enzymatic rate constants were $V_{max}C = 0.419 \pm 0.095$; $K_m = 5.75 \pm 2.49$ (mean and standard deviation of estimates). Details of this type of computerized optimization have been reported elsewhere (Agin and Blau, 1982).

Computer simulation. Simultaneous differential and algebraic equations describing the movement of MC through the body were formulated as a computer program. Simulations were conducted with the SimuSolv software package which contains routines for numerical integration, graphics display, and derivation of model parameters from experimental data. SimuSolv was developed by Agin and Blau (1982) and is commercially available from Mitchell & Gauthier Associates.[2]

TABLE 1

PARAMETERS USED IN THE PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR METHYLCHLOROFORM[a]

	Human	Rat	Mouse
Weights			
Body wt (kg)	83.0	0.215	0.029
Liver	3.1%	4.0%	4.0%
Rapidly perfused	3.7%	5.0%	5.0%
Slowly perfused	61.1%	75.0%	78.0%
Fat	23.1%	7.0%	4.0%
Flows (liters/hr)			
Alveolar vent.	348.0	5.11	1.26
Cardiac output	348.0	5.11	1.26
	Percentage of cardiac output		
Liver	24.0	24.0	24.0
Rapidly perfused	49.0	53.0	56.0
Slowly perfused	18.0	18.0	18.0
Fat	9.0	5.0	2.0
Partition coefficients			
Blood/air	2.53	5.76	10.8
Liver/air	8.6	8.6	8.6
Rapidly perfused/air	8.6	8.6	8.6
Slowly perfused/air	3.15	3.15	3.15
Fat/air	263.	263.	263.
Biochemical constants			
$V_{max}C$ (allometric)	0.419	0.419	0.419
K_m (mg/liter)	5.75	5.75	5.75
K_a (hr^{-1})	—	1.25	—

[a] Metabolic parameters for the rat ($V_{max}C$, K_m) were obtained from the data of Schumann *et al.* (1982a) by computer optimization. $V_{max}C$ is an allometric measure of the maximum velocity of metabolism such that the maximum enzyme rate (V_{max}) may be calculated for any size animal according to the equation $V_{max} = V_{max}C \times (\text{body wt})^{+0.7}$.

RESULTS

Rat Simulations

Inhalation simulations. After incorporation of the appropriate physiological and physical chemical parameters (Table 1), the PB-PK model was used to predict the time course of the venous blood concentrations of MC in rats after exposure to 150 or 1500 ppm MC. Experimental data previously collected by Schumann *et al.* (1982a) were used to check the predictions, and the results are shown in Fig. 1. The data showed the best agreement with model predictions for blood level measurements made postexposure. Blood levels measured during the exposure were somewhat lower than model predictions at 4, 5, and 6 hr, but the predictions were still within a factor of 2 of the experimental data (Fig. 1).

Several other types of data were available from the studies of Schumann *et al.* (1982a) to check the accuracy of the PB-PK model. These were (1) the total body burden of MC and metabolites after 6 hr of exposure, (2) the concentration of radioactivity in fat tissue at the end of the exposure, and (3) the concentration of radioactivity in liver tissue at the end of the exposure.

Comparisons between the model predictions and these additional data are summa-

[2] Mitchell & Gauthier Associates, 73 Junction Square Drive, Concord, MA 01742.

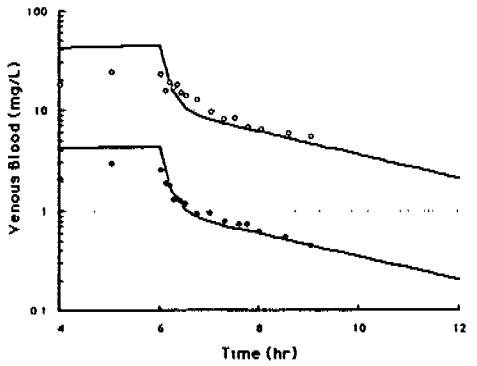

FIG 1. Blood levels of MC in rats during and following a 6-hr inhalation exposure to 150 ppm (closed circles) or 1500 ppm (open circles) of MC. Values predicted by computer simulations are shown as a solid line(s).

rized in Table 2. Overall, the ratio of predicted to actual data for these two exposures ranged from a low of 0.72 to a maximum of 1.92 and the mean ratio was 1.28 ± 0.48 (standard deviation; Table 2).

Intravenous administration. The PB-PK model was then used to attempt a dose route extrapolation: inhalation exposure to intravenous injection. Venous blood concentrations were measured in groups of animals (six per group) injected with MC dissolved in heparinized plasma. Intravenous injection was simulated in the model as a rapid infusion into pooled venous blood (about 1 min in duration). Three doses were studied: 8.8, 26, and 47 mg/kg. The only changes made to the PB-PK model for this simulation were in the equations describing entry of MC into the body. Experimental data and model predictions for 4 hr postinjection are shown in Fig. 2.

Bolus gavage (MC in water). A third route of administration (bolus gavage) was investigated. Six rats were administered a dose of 14.2 mg/kg MC dissolved in water, and serial blood samples were taken for analysis at various times afterwards. The equations in the PB-PK model were modified to reflect the new route of exposure. Uptake of MC was simulated as a first-order process with a rate constant (K_a) equal to 1.25 hr^{-1}. The model predictions and experimental data for this route are shown in Fig. 3.

Drinking-water exposure. A variant of the bolus gavage route was consumption of MC in drinking water over an extended period of time. Animals were presented with water containing radioactive MC for 8 hr and samples of urine, expired air, and selected tissues were analyzed for radioactivity at various times thereafter. The average water consumption of the rats (mean wt = 250 g) during the 8 hr in which they had access to the treated water was 8.1 ± 3.8 ml, corresponding to an average dose of 116 mg/kg. Overall recovery of radioactivity in these experiments (based on water consumption) was 95.2 ± 4.33% (standard deviation). Entry of MC into the animal was simulated as a zero-order process depositing MC in the liver compartment at a constant rate for 8 hr. Other than the changes to the input equations, no parameters were changed in the PB-PK model for drinking-water simulation. Simulated rates of MC elimination in exhaled air and experimental data (radioactivity recovered from charcoal traps) are shown in Fig. 4.

In addition, the use of [^{14}C]MC made it possible to predict the extent of MC metabolism for this route of metabolism. The model predicted that 5.49 μmol of MC (about 2% the ingested MC) would be metabolized, 8.19 μmol (about 3% of the ingested MC) was actually recovered in urine and CO_2 (Table 2).

Mouse Simulations

Inhalation simulations. The PB-PK model was then used for interspecies extrapolation by predicting the pharmacokinetic behavior of MC in male B6C3F1 mice after inhalation exposure. Model predictions were compared to experimental data gathered by Schumann *et al.* (1982a). Other than adjusting the appropriate physiological and biochemical parameters, no changes were made in the PB-PK model for simulation of the mouse exposures.

TABLE 2

COMPARISON OF PREDICTED AND OBSERVED VALUES FOR SELECTED PARAMETERS FROM INHALATION EXPOSURES AND DRINKING WATER EXPOSURES IN *YOUNG* RATS AND *YOUNG* MICE (2–3 months OF AGE) AND HUMAN VOLUNTEERS[a]

	Observed	Predicted	Ratio (pred/obs)
Young rat—150 ppm			
End exposure blood level (mg/liter)	2.64	4.37	1.66
Body burden at 6 hr (μmol)	33.0	25.5	0.77
Conc in fat (μmol/liter)	724.0	1264.0	1.75
Conc in liver (μmol/liter)	68.2	48.9	0.72
Young rat—1500 ppm			
End exposure blood level (mg/liter)	23.1	44.4	1.92
Body burden at 6 hr (μmol)	263	237	0.90
Conc in fat (μmol/liter)	8400	12800	1.52
Conc in liver (μmol/liter)	503	500	0.99
Young rat—drinking water (116 mg/kg)			
Amount metabolized (μmol)	8.19	5.49	0.67
Young mouse—150 ppm			
End exposure blood level (mg/liter)	9.27	8.54	0.92
Body burden at 6 hr (μmol)	4.97	3.30	0.66
Amount metabolized (μmol)	1.10	1.02	0.93
Conc in fat (μmol/liter)	1330	1570	1.19
Conc in liver (μmol/liter)	75.7	50.6	0.67
Young mouse—1500 ppm			
End exposure blood level (mg/liter)	111.0	88.1	0.79
Body burden at 6 hr (μmol)	39.5	25.8	0.65
Amount metabolized (μmol)	2.01	1.90	0.94
Conc in fat (μmol/liter)	16200	16100	0.99
Conc in liver (μmol/liter)	631	525	0.83
Human 35 and 350 ppm			
Amount metab. 35 ppm (μmol)	32.4	32.2	0.99
Amount metab. 350 ppm (μmol)	246	236	0.96

[a] Inhalation data are from Schumann *et al.* (1982a) and Nolan *et al.* (1984).

The predicted and observed venous blood concentrations (collected at the orbital sinus) in mice exposed to either 150 or 1500 ppm of MC are shown in Fig. 5. The PB-PK model predicted that MC would be eliminated from the mouse much more rapidly than the rat, and this is consistent with the observed data (Figs. 1, 5). This is also consistent with the observation of Schumann that elimination half-lives for MC in mice were 5- to 10-fold less than the corresponding half-lives in rats (Schumann *et al.* 1982a).

Several additional sources of experimental data were available for assessing the consis-

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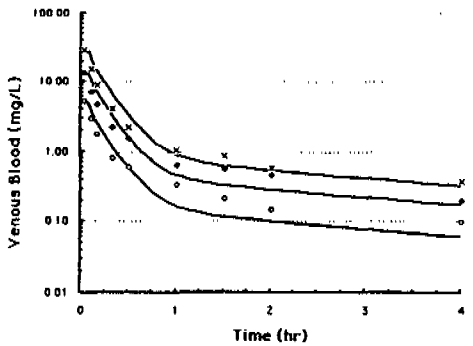


FIG. 2. Blood levels of MC in rats following intravenous injection of 8.8 mg/kg (open circles), 26 mg/kg (closed circles), or 47 mg/kg (crosses) of MC dissolved in rat plasma. MC was dissolved in heparinized plasma at concentrations calculated to give the indicated doses while maintaining a constant vehicle volume of 1 ml plasma/kg of body wt. Values predicted by computer simulations are shown as a solid line(s).

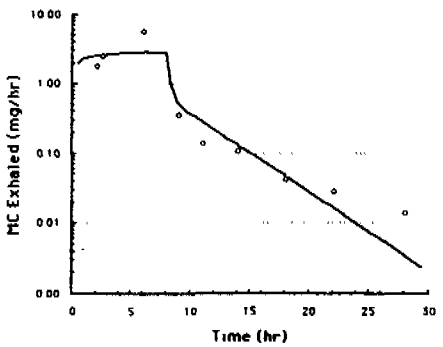


FIG. 4. Rate of elimination of ^{14}C[MC] in exhaled air (mg equivalents of MC/hr) during and following *ad libitum* exposure of rats to a solution of [^{14}C]MC in drinking water. The average water consumption of the rats (mean wt = 250 g) during the 8 hr in which they had access to the treated water was 8.1 ± 3.8 ml, corresponding to an average dose of 116 mg/kg. Values predicted by computer simulations are shown as a solid line(s).

tency of the PB-PK model for mice. Observed and predicted values of the end exposure body burden, the end exposure liver concentration, the end exposure fat concentration, and the total amount of metabolites recovered are summarized in Table 2. The ratio of predicted to actual data ranged from a low of 0.65 to a high of 1.19 and the mean ratio was 0.86 ± 0.16 (standard deviation; Table 2).

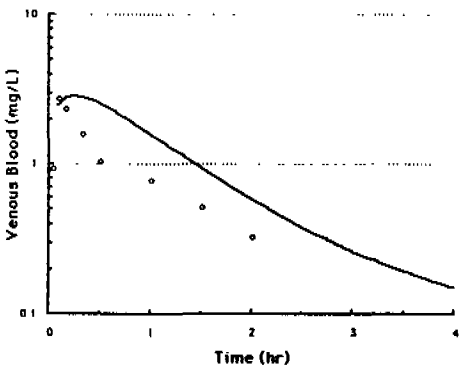


FIG. 3. Blood levels of MC in rats following oral gavage with a solution of MC in water. The dose of MC was 14.2 ± 1.9 mg/kg (standard deviation), administered in approximately 4.0 ml water/kg body wt. Values predicted by computer simulations are shown as a solid line(s). Absorption from the gastrointestinal tract was simulated as a first-order process with a rate constant of 1.25 hr^{-1}.

Human Exposures

The PB-PK model was used to predict the disposition of inhaled MC in humans. Model predictions were compared to data gathered by Nolan *et al.* (1984) at 35 and 350 ppm MC. Experimentally observed and simulated concentrations of MC in venous blood are shown in Fig. 6a. Concentrations of MC into blood was somewhat lower than predicted

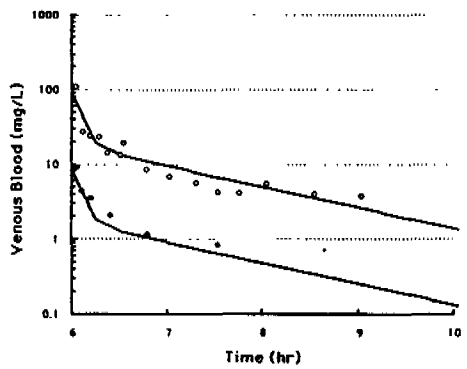


FIG. 5. Blood levels of MC in mice during and following a 6-hr inhalation exposure to 150 ppm (closed circles) or 1500 ppm (open circles) of MC. Values predicted by computer simulations are shown as a solid line(s).

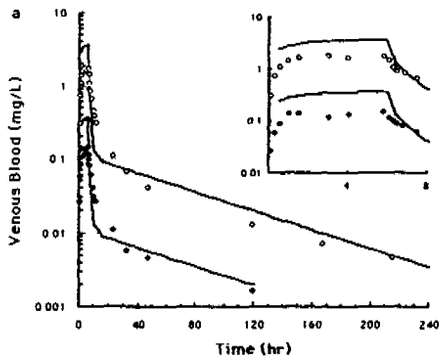


FIG. 6a. Concentration of MC in exhaled air (mg/liter) during and following a 6-hr inhalation exposure of human volunteers to 35 ppm (closed circles) or 350 ppm (open circles) of MC. Values predicted by computer simulations are shown as a solid line(s).

during exposure (insert) but was well described for the post exposure period (main figure).

Predicted and observed concentrations of MC in expired air are shown in Fig. 6b. Concentrations of MC in expired air were well simulated by the model during exposure (insert). The model predicted a slightly more rapid decline in the concentration of MC in expired air than was observed during the period of 10–30 hr, but the final slow elimination phase (30–240 hr) was well simulated by the model (main figure). Predicted and observed values of expired air and venous blood concentrations were both proportional to exposure concentration throughout the range of 35–350 ppm MC.

The PB-PK model predicted that 4.30 mg equivalents of MC would be metabolized during the 240 hr following a 6-hr exposure to 35 ppm MC, and 4.32 mg equivalents of MC metabolites were actually recovered by Nolan *et al.* (1984). Similarly, the model predicted that 31.5 mg equivalents of MC tabolites would be formed during the 240 hr following a 6-hr exposure to 350 ppm MC, and 32.9 mg were actually recovered (Table 2).

Older Animals

Old rats, inhalation simulations. Schumann *et al.* (1982b) also studied the pharmacokinetics of MC in control and exposed (1500 ppm, 6 hr/day, 5 days/week) male rats obtained from a study of the chronic inhalation toxicity of MC. The age of these animals was approximately 18.5 months, and the average body weight was 481 g. They reported that the disposition of a single dose of [^{14}C]MC in the treated and control animals was nearly identical (i.e., that chronic preexposure to unlabeled MC had not altered pharmacokinetic behavior through induction of enzymes, alteration of renal function, etc.). However, the disposition of MC in old animals differed significantly from the disposition previously observed in younger animals (Schumann *et al.*, 1982a).

The PB-PK model was used to predict the rate of elimination of MC in exhaled air in untreated (control) 18.5-month-old male rats following a 6-hr exposure to 1500 ppm MC. Experimental data and the initial simulation are shown in Fig. 7a. The PB-PK model predicted a much more rapid decline in the rate of MC exhalation when the physiological parameters chosen for young rats were used (heavy line, Fig. 7a).

Lutz *et al.* (1977) improved the accuracy of their simulation of polybrominated biphenyl compounds in rats by increasing the relative

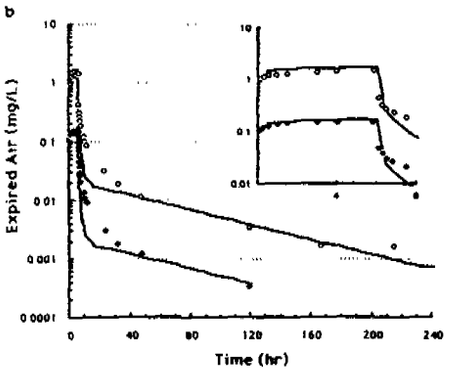
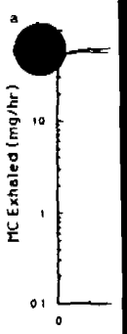


FIG. 6b. Concentration of MC in venous blood (mg/liter) during and following a 6-hr inhalation exposure of human volunteers to 35 ppm (closed circles) or 350 ppm (open circles) of MC. Values predicted by computer simulations are shown as a solid line(s).



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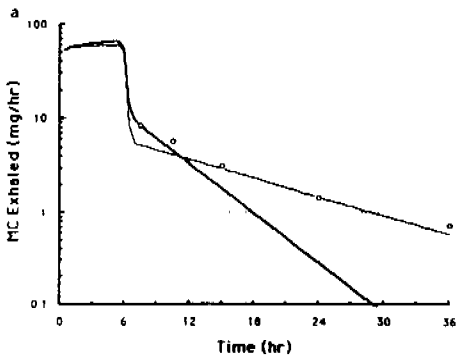

FIG. 7a. Rate of elimination of [^{14}C]MC in exhaled air (mg equivalents of MC/hr) following exposure of old (18 months) rats to 1500 ppm of [^{14}C]MC for 6 hr. Actual experimental data are shown as open circles on the plot. Simulated values with a fat compartment equal to 7% of body weight (i.e., simulation based on the parameters for young rats) are shown by the heavy solid line, while the light solid line shows the model predictions with a fat compartment equal to 18% of body weight.

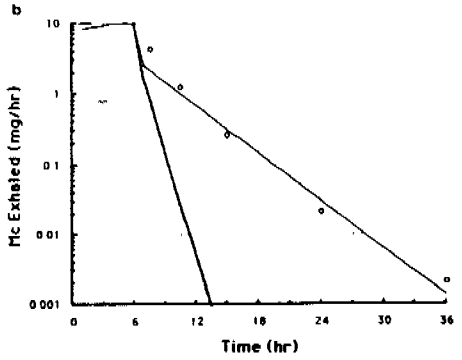

FIG. 7b. Rate of elimination of [^{14}C]MC in exhaled air (mg equivalents of MC/hr) following exposure of old (18 months) mice to 1500 ppm of [^{14}C]MC for 6 hr. Actual experimental data are shown as open circles on the plot. Simulated values with a fat compartment equal to 4% of body weight (i.e., simulation based on the parameters for young mice) are shown by the heavy solid line, while the light solid line shows the model predictions with a fat compartment equal to 18% of body weight.

proportion of body weight allocated to the fat compartment. Consequently, we conducted a second computer optimization to determine whether a similar modification would give a better simulation of the data from the 18.5-month-old rats. The computer was allowed to vary the volume of the fat compartment (VF) from 0 to 30% of body weight during simulations. Corresponding decreases in the fraction of body weight assigned to the slowly perfused compartment maintained a constant percentage of the carcass as perfused tissue (91% of body weight perfused). Greatly improved fits to the exhaled air data were obtained after the second optimization (light line, Fig. 7a). This optimization indicated the PB-PK model was most consistent with the exhaled air data when the VF was increased from 7% of body weight to 18% of body weight.

The model with the larger fat compartment was then used to predict several other types of experimental data available from the studies of Schumann *et al.* (1982b). These included end exposure body burden, end exposure concentration of MC-derived radioactivity in the liver, end exposure concentration of MC-derived radioactivity in the fat, and total amount of radioactive metabolites collected in 66 hr subsequent to the 6-hr exposure. The comparisons between the PB-PK model prediction and the actual data are summarized in Table 3.

The predictions of the PB-PK model for old rats with the original fat compartment (7% of body weight) are summarized in column 2 of Table 3. The model underpredicted the end exposure body burden and amount metabolized, but overpredicted the concentration of MC in fat tissue. After increasing the size of the fat compartment to 18% of body weight, the PB-PK model predicted lower concentrations of MC in fat tissue, and increased body burdens and amounts metabolized, giving a better simulation of the rat data.

Old mice inhalation simulations. Schumann *et al.* (1982b) also collected data in old male B6C3F1 mice (18.5 months of age) obtained from a study of the chronic inhalation toxicity of MC in mice. As was observed with the rats, the pharmacokinetics of MC in old

TABLE 3

COMPARISON OF OBSERVED AND PREDICTED VALUES FOR SELECTED PARAMETERS FROM INHALATION EXPOSURES IN *OLD* RATS AND *OLD* MICE (18.5 months OF AGE)[a]

	Actual	Before reoptimization	After reoptimization
	Old rat—1500 ppm		
Body burden at 6 hr (μmol)	744	490	638
(Ratio)		(0.66)	(0.86)
Amount metabolized (μmol)	31.8	17.5	22.1
(Ratio)		(0.55)	(0.69)
Conc in fat (μmol/liter)	5,685	11,700	6200
(Ratio)		(2.06)	(1.09)
Conc in liver (μmol/liter)	606	494	454
(Ratio)		(0.82)	(0.75)
	Old mouse—1500 ppm		
Body burden at 6 hr (μmol)	149	35.1	71.4
(Ratio)		(0.24)	(0.48)
Amount metabolized (μmol)	13.6	2.61	4.11
(Ratio)		(0.19)	(0.30)
Conc in fat (μmol/liter)	10,600	15,900	8790
(Ratio)		(1.50)	(0.83)
Conc in liver (μmol/liter)	1,790	532	491
(Ratio)		(0.30)	(0.27)

[a] Experimental data are from Schumann *et al.* (1982b). Physiological parameters for rats and mice before computer optimization are listed in Table 1, except for body weight which was 481 g for the rats and 39.8 g for the old mice. After optimization, the size of the fat compartment was 18.6 and 18.1% of body weight in rats and mice, respectively.

unexposed (control) mice differed significantly from the pharmacokinetics of MC in young control mice.

When the PB-PK model utilized to describe MC disposition in young mice was used to predict the rate of elimination of MC in exhaled air, a poor description of the experimental data was obtained (heavy line, Fig. 7b). However, when the size of the fat compartment was increased from 4% of body weight to 18% of body weight, a much better description of the experimental data was obtained (light line, Fig. 7b).

When the model with 4% fat was used to simulate additional parameters measured in older mice, it underestimated the body burden, the amount metabolized, and the concentration of radioactivity in liver tissue and overestimated the concentration of radioactivity in fat (Table 3). When the size of the fat compartment was increased to 18% of body weight, the description of the experimental data was improved, but the model still underpredicted the amount metabolized and the concentration of radioactivity in liver tissue.

DISCUSSION

Versatility of the PB-PK Model

The first objective of these studies was to demonstrate the extent to which incorporation of physiological and biochemical principles into pharmacokinetic modeling would allow extrapolation between different routes of administration and different species. Accordingly, we have not conducted extensive "curve fitting" procedures to try to improve

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the appearance of the data presented here, and it is obvious that the extrapolations were not perfect in every case. However, since we are proposing that these techniques may be useful in the area of risk assessment, it is important to keep in mind the level of precision which currently exists in this process.

Cancer risk estimations produced from the same sets of data often differ by several orders of magnitude, depending upon the assumptions used about the shape of the dose–response curve at low concentrations. Furthermore, it is not uncommon for bioassays of the same material in the same species to differ by as much as an order of magnitude in their endpoints (tumor incidences). Consequently, we felt that if our integrated PB-PK model could predict relevant pharmacokinetic parameters for MC in different species and dose routes within a factor of 2 or 3, we would have demonstrated its potential for increasing the accuracy of current risk estimation procedures.

The construction of the PB-PK model used here was relatively straightforward. Physiological parameters for the various species have been published in the scientific literature (Davis and Mapleson, 1981; Caster *et al.*, 1956; ICRP, 1975), and partition coefficients were measured according to published procedures (Sato and Nakajima, 1979). The most difficult task was the estimation of the rate constants for *in vivo* metabolism.

MC is not extensively metabolized in any of the species studied (Schumann *et al.*, 1982a,b; Nolan *et al.*, 1984). Consequently the gas uptake technique used by Gargas *et al.* (1988) for estimation of *in vivo* metabolism with other compounds was not suitable for this material, since uptake depends upon metabolism once tissue loading has occurred. Furthermore, a variety of metabolites are formed from biotransformation of MC (trichloroethanol, the glucuronide of trichloroethanol, and trichloroacetic acid). The production of multiple metabolites, each of which is present at very low levels, makes it difficult to estimate total metabolism by chemical analysis.

However, radioactive metabolites of MC are easily separated from [^{14}C]MC and may be readily quantified. Consequently, we have used available data on the total levels of radioactive metabolites obtained in balance studies with rats exposed to [^{14}C]MC to estimate the rates of metabolism in this species. Levels of metabolites produced during exposure and postexposure were calculated with the PB-PK model, which contains the structure necessary to deal with the complexities of postexposure metabolism. We then employed allometric scaling procedures to estimate metabolic rate constants in the other species. Since the metabolic rate constants were obtained from a limited data set (two experiments in rats with four animals/experiment), there is obviously room for error in these parameters. The magnitude of the possible error may be roughly estimated from the standard deviations for the estimates of $V_{max}C$ and K_m, which were 23 and 43% of the mean, respectively. Nevertheless, it is encouraging that once the metabolic rate constants were obtained in this manner, they were able to accurately predict metabolism in two other species exposed to MC vapor (mice and humans) as well as in rats exposed to MC in drinking water (Table 2).

It must be emphasized that since metabolism plays a minor role in the elimination of MC in the various species, the behavior of MC itself (as distinct from MC metabolites) is almost entirely dependent upon the solubility in various tissues (partition coefficients) and the physiology of the various species. Small errors in the values chosen for the metabolic rate constants will have little effect on the overall disposition of MC.

Schumann *et al.* (1982a) utilized a conventional, data-based pharmacokinetic model to describe the time course of MC in rats. In this approach, the venous blood time/concentration curve for rats was actually described better by a simple two-compartment open model with elimination from the central compartment (Schumann *et al.*, 1982a) than the present PB-PK model. However, when

we attempted to use this model to describe the time course of MC in the venous blood of mice, a very poor fit was obtained (data not shown), pointing out the difficulties of using conventional models for extrapolating to humans. In contrast, once a PB-PK model had been developed for rats, it immediately provided a good description of the blood level data obtained in mice (Fig. 5) as well as humans (Fig. 6a).

Furthermore, the development of a PB-PK model allowed us to predict other types of data of potential toxicological significance. For example, the concentration of MC in liver tissue at the end of inhalation exposures to MC was well described in both rats and mice (Table 2). Since the liver has been identified as the target organ in both rats and mice during long-term animal bioassays (Rampy *et al.*, 1978; Quast *et al.*, 1984), this finding has obvious significance.

Another advantage of PB-PK models is their ability to provide quantitative descriptions of material administered by a variety of dose routes. In this particular case, the pharmacokinetics of MC in rats after inhalation (Fig. 1), intravenous injection (Fig. 2), bolus gavage (Fig. 3), and drinking-water administration (Fig. 4) could be described with a single, integrated model. Conventional data-based pharmacokinetic models lack the capacity for this type of route to route extrapolation.

Pharmacokinetics in Older Animals

A variety of physiological and biochemical changes may occur in test animals during the course of a lifetime bioassay, where animals are started on test a few weeks after weaning and continue on test until nearly the end of their natural lives. Development of a PB-PK model offers a mechanism whereby the consequences of these changes may be quantitatively evaluated.

For example, the PB-PK developed for MC indicates that the concentrations of MC in fat tissue are 20–100 times higher than in other tissues. Consequently, the increased size of the fat compartment in older, sedentary animals would be expected to increase the amount of MC taken up by older animals exposed to a given concentration of MC, and the elimination of MC from these older animals should occur more slowly. These predictions of the PB-PK have been experimentally verified in the data of Schumann *et al.* for both rats and mice (Figs. 7a, 7b; Tables 2, 3).

Most of the changes in the pharmacokinetics of MC in older rats were correctly predicted when the size of the body fat compartment was increased (Table 3). However, the situation appears to be somewhat more complex with B6C3F1 mice. The increased half-life of MC and lower concentration of radioactivity in the fat compartment of older mice were correctly predicted by a PB-PK model with a larger fat compartment. However, the predicted body burden and amount of MC metabolized in these mice were still much lower than actually observed by Schumann *et al.* (1982b) (Table 3). It appears that additional factors, such as increased metabolic capacity, may have to be considered in evaluating the kinetics of MC in older mice. The nature of these factors remains to be elucidated.

Relating Animals Studies to Human Exposures

MC has been studied in two long-term inhalation bioassays at the Toxicology Research Laboratory of the Dow Chemical Co. Animals were exposed to MC for 6 hr/day, 5 days/week for up to 2 years. MC exposure was not associated with increases in the incidences of either benign or malignant tumors in these studies. Reversible microscopic alterations in liver tissue were noted in both rats and mice, but cytotoxicity and necrosis were not seen. The no observed adverse effect levels (NOAEL) for the cellular changes established in these studies were 875 ppm in rats and 1500 ppm in mice (Rampy *et al.*, 1978;

TABLE 4

COMPARISON OF THE LIFETIME AVERAGE CONCENTRATION OF MC IN THE LIVER (ACL, DOSE SURROGATE FOR MC EFFECTS ON LIVER) FOR RATS AND MICE AT THE NO OBSERVED ADVERSE EFFECT LEVELS (NOAEL) WITH VALUES OF THE SAME DOSE SURROGATE IN HUMANS CONSUMING 2 liters/day OF WATER CONTAINING SMALL AMOUNTS OF MC[a]

	Conc (ppm)	Route	Average ACL (μmol/liter)	Safety factor relative	
				To mouse	To rat
Rat	875	Inhalation	28	—	—
Mouse	1500	Inhalation	95	—	—
Human	0.001	Water	3.4×10^{-5}	$2.8 \times 10^{+6}$	$8.2 \times 10^{+5}$
	0.003	Water	1.0×10^{-4}	$9.5 \times 10^{+5}$	$2.8 \times 10^{+5}$
	0.010	Water	3.4×10^{-4}	$2.8 \times 10^{+5}$	$8.2 \times 10^{+4}$
	0.030	Water	1.0×10^{-3}	$9.5 \times 10^{+4}$	$2.8 \times 10^{+4}$
	0.100	Water	3.4×10^{-3}	$2.8 \times 10^{+4}$	$8.2 \times 10^{+3}$
	0.300	Water	1.0×10^{-2}	$9.5 \times 10^{+3}$	$2.8 \times 10^{+3}$

[a] The NOAEL for the rat was 875 ppm, 6 hr/day, 5 days/week for 1 year (Rampy *et al.*, 1978) and the NOAEL for mice exposed 6 hr/day, 5 days/week for 2 years was 1500 ppm (Quast *et al.*, 1984). All dose surrogates are reported in units of μmol/liter. "Safety factors" are calculated by dividing the calculated animal dose surrogate by the predicted human dose surrogate.

Quast *et al.*, 1984). McNutt *et al.* (1975) reported similar effects in the liver of mice exposed to 250 ppm continuously for several weeks.

Since the liver is the target organ in both animal species, it is assumed that the liver would be the site where any adverse effects would occur in humans. Because the effects of MC on liver tissue were mild and reversible (i.e., without necrosis), it is not possible to determine whether they were caused by parent chemical or more reactive metabolites. Consequently we have chosen to use the average concentration of MC in the liver over the lifetime of the animal (ACL) as a "dose surrogate" for MC. Rationale for selection of dose surrogates with PB-PK models has been discussed in more detail by Andersen in the proceedings of a workshop conducted at the NAS (1987).

Selection of this type of dose surrogate for MC is probably a health protective assumption. Reactive metabolites, rather than parent chemical, are responsible for the toxicity of many other halogenated solvents, and levels of the enzyme which produces metabolites of MC appear to be lower in humans than in the small rodent species. For example, we can use the allometric equation to calculate that the concentration of MC-metabolizing enzyme (units of enzyme per liter of tissue) is about 13-fold lower in human liver than it is in mouse liver. Consequently, if we had chosen to use production of reactive metabolites as the dose surrogate rather than ACL, the estimated risk to humans would be considerably lower than with the present procedure.

Once the dose surrogate was chosen, the PB-PK model was used to calculate the ACL for rats and mice at the NOAEL in the long-term inhalation studies at Dow Chemical. The ACL for rats was about 55.2 μmol/liter during the 1-year exposure period, or about 27.6 μmol/liter averaged over the 2-year lifespan. The corresponding value for B6C3F1 mice was 95.1 μmol/liter (Table 4).

Small quantities of MC (typically 1–10 ppb, highest observed 300 ppb) have been detected in some finished drinking-water supplies. However, there are no long-term studies in animals in which MC has been administered by this route, and even if there were,

the limited solubility of MC would limit the dose that could be given to the animals. Consequently, there is a need for a mathematical procedure to quantitatively relate the "internal dose" in humans drinking such water to the "internal dose" in animal inhalation studies. This was readily accomplished with the PB-PK model developed in these studies.

ACLs associated with human consumption of water containing the indicated concentrations of MC are summarized in Table 4. It is obvious from this table that the ACLs in humans consuming this water are much lower than the ACLs in animals during the chronic inhalation studies. For example, the ACL in humans exposed to 10 ppb of MC in drinking water are 81,000-fold lower than the ACL at the NOAEL in the rat, and 270,000-fold lower than the ACL in the mouse (Table 4, columns 4 and 5).

Simulations with the PB-PK model revealed that "steady-state" levels of MC in the human liver are achieved within a relatively short period. The ACL after 100 days of drinking water containing 10 ppb MC was only 2.7 times higher than the ACL after a single day of consuming such water. This indicates that the risk to humans will not increase disproportionately after long periods of consumption, as might be the case with compounds such as lead or mercury.

There are bound to be many uncertainties in human risk estimations derived from animal studies. Although they cannot address all of the uncertainties inherent in this process, PB-PK models offer promise for improving the basis for high dose/low dose, interspecies, and dose route extrapolations. It should be noted, however, that PB-PK models are most useful when they are combined with independent studies elucidating the mechanism of toxicity so that appropriate dose surrogates may be chosen.

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