

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.

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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 CO₂ 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 ¹⁴CO₂ 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

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_{elim} (μM ⁻¹ hr ⁻¹)	0.33
K_{dec} (hr ⁻¹)	50
K_{elim} (hr ⁻¹)	9000
K_{CO_2} (M ⁻¹ hr ⁻¹)	1.82×10^{-3}
H ₂ O (M)	55

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.

METHODS

PB-PK Model Development

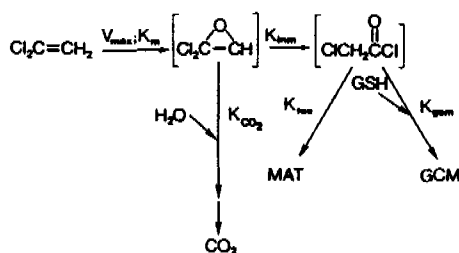
The basic structure of the PB-PK model is similar to that developed by Ramsey and Andersen (1984) for styrene, utilizing a lumped-tissue group approach. Compartments are the "richly perfused" group comprising such tissues as the lung, kidney, and spleen; the "slowly perfused" group, comprising muscle 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

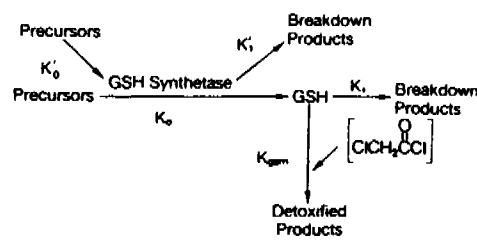
method of Sato and Nakajima (1979). Briefly, tissues were homogenized with normal saline using a Polytron homogenizer. Homogenates were spiked with low levels of VDC and allowed to equilibrate in sealed reaction vessels at 37°C in a water bath for 1–3 hr. A sample of the headspace vapor above the homogenate 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 I) 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_{imm}) was arbitrarily set to 9000 and the second-order reaction rate constant of epoxide with water ($K_{\text{CO}_2} \cdot 55 \text{ M water in liver}$) was therefore 1000, resulting in a value of $1.82 \cdot 10^{-5}$ for K_{CO_2} (Table I).



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_{imm}) and reaction with "everything else" (rate constant K_{de}). Values for K_{imm} and K_{de} (Table I) were obtained by using the PB-PK model for VDC developed until this level and varying K_{de} (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_{imm} and K_{de} 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_{imm} and K_{de} , 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 non-metabolizing 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 ACSL¹ computer program

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

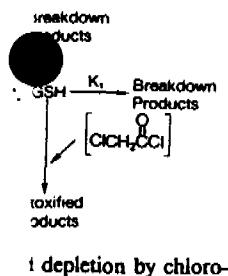
VDC Concentration ($\mu\text{g/L}$)

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that the reaction with VDC epoxide or ion is justified based on where CAC reacted faster than VDC in minor metabolites relative contribution of CAC, compared to the toxicity by binding to available for toxicity, for processes: reaction with K_{GSH} and reaction with K_{GCM} . Values for K_{GSH} and K_{GCM} and varying K_{GSH} (trial model predictions were reported by McKenna et al. 1978a; 350 mg/kg dose, Jones and Hathway, 1978.)

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model. Mass-balance for the various compartments (A). Briefly, for non-steady-state equations were finding, and efflux of is described similarly, exchange between the equations for the liver VDC metabolism and were solved simultaneously, using Gear's algorithm computer program

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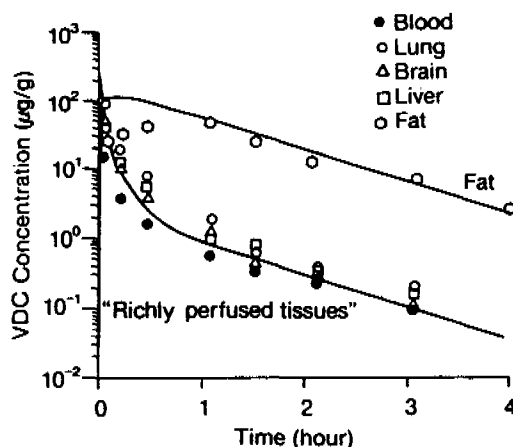


FIG. 1. Model predicted (—) and observed (symbols) VDC concentrations in different tissues of the rat following a single 50 mg/kg iv bolus dose. Predicted VDC concentrations for blood, liver, lung, and brain are shown as a single curve, as tissue:blood partition coefficient for the "richly perfused"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

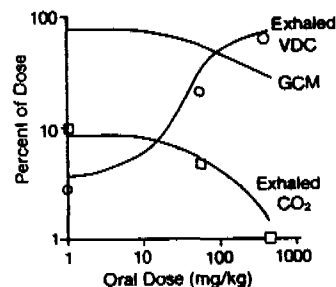


FIG. 2. Model simulations (—) and observed data for the effect of oral dose level on percentage of VDC dose that is exhaled unchanged (○), exhaled as CO_2 (□), or GCM (glutathione-conjugated metabolite (no observed data)). K_1 of 1.0 hr^{-1} used in simulations. (Data source: 1 and 50 mg/kg doses, McKenna et al., 1978a; 350 mg/kg dose, Jones and Hathway, 1978.)

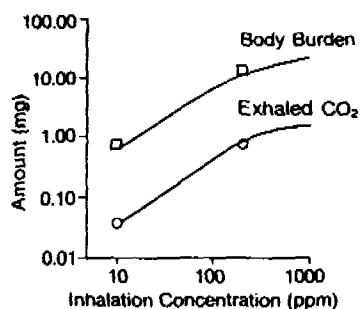


Fig. 3. Model simulations (—) for the effect of inhalation dose level (6-hr exposures) on body burden (□) and amount of dose exhaled as CO₂ (○). (Data source: McKenna *et al.*, 1978b.)

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 CO₂ 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 CO₂ 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).

FIG. 4
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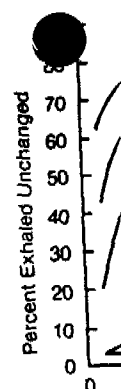


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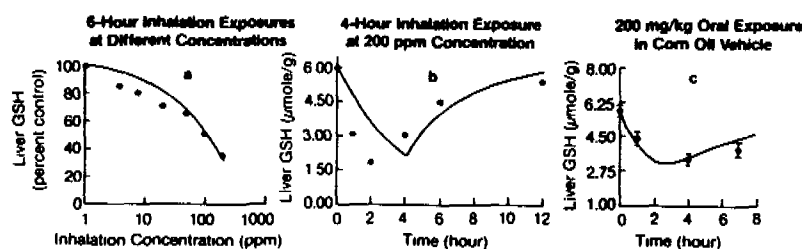


FIG. 4. Predicted (—) and observed (●) liver GSH concentrations following inhalation (a and b) and oral (c) exposure to VDC. (Data source: (a) McKenna *et al.*, 1977; (b) Reynolds *et al.*, 1980.)

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.

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

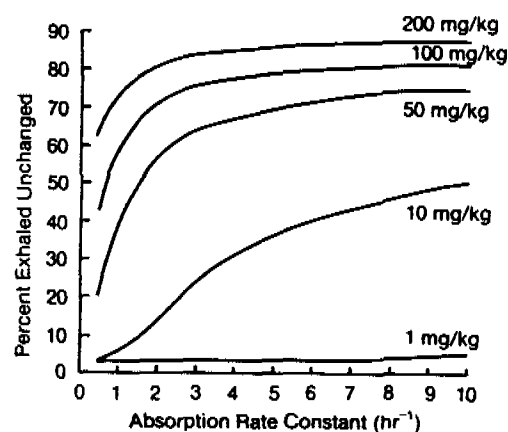


FIG. 5. Simulated effect of oral absorption rate constant on the percentage of VDC dose exhaled unchanged at various dose levels. It can be observed that the effect is negligible at low doses, very dramatic at the intermediate doses, and minor at doses of 200 mg/kg, where virtually all of the dose is 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}).

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ts. While simulating behavior of VDC, sev-made that would not out the PB-PK model. of the low blood:air d saturable metabo-ion is sensitive to the dy. Figure 5 depicts absorption rates on dose that will be ex-rent oral dose levels. performed for doses g/kg. Experience in ion rates of haloge-ta not shown) sug-n rate constant for queous vehicles, like s typically 5.0 hr^{-1} , om corn oil vehicle absorption rate in-her doses, a greater se is exhaled uncal implications of us. That is, greater oil is used as the pared to aqueous by Cheico *et al.* tototoxicity was seen l oil vehicles com-are consistent with etic data in the lit-observation. Mc-l that from a corn 3 and 20% of the g/kg, respectively, redicts similar re-constant of 0.5–1.0 e model predicts Hathway (1978) xhaled after oral d after intraperi-0 mg/kg, respec-ed to simulate intraperitoneal n).

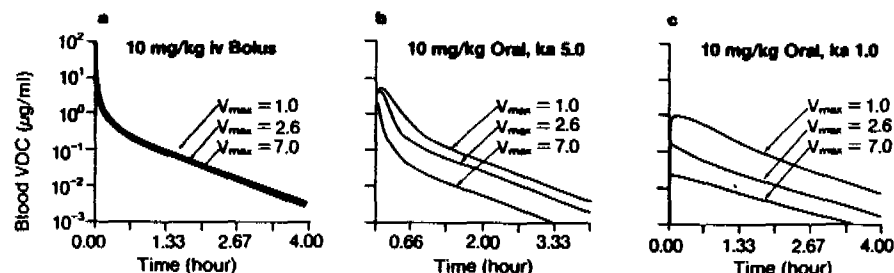


FIG. 6. Simulated blood VDC concentrations following intravenous bolus (a), and oral (b and c) doses. These simulations demonstrate that after intravenous bolus doses effects of enzyme inhibition or induction will not be observed from studying blood VDC levels. These effects will manifest themselves 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,

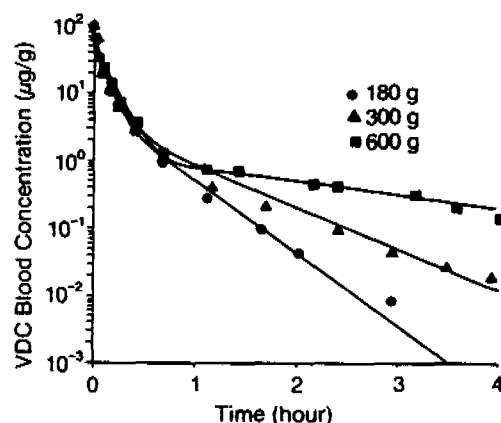


FIG. 7. Predicted (—) and observed (symbols) effects of body weight (body fat) on VDC blood concentrations in rats of different body-weight levels following a single 50 mg/kg iv dose. (Data source: D'Souza 1984).

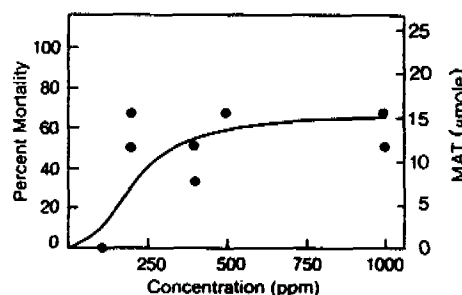
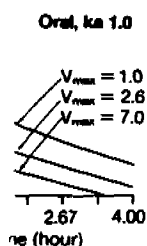


FIG. 8. Predicted (—) metabolite available for toxicity (MAT, chloroacetyl chloride not detoxified by glutathione (see text for more detailed explanation) and mortality dose response for 4-hr VDC inhalation exposures. (Data source: Andersen *et al.*, 1979.)

cardiac output was scaled as a function of body weight to the 0.75 power, and body weight of a 200 g rat to that of a 70 kg human. This type of scaling is an extrapolating halogenated models (Garg 1987; D'Souza 1984). The relationship between the level and the effect in the rat and human is conducted for both (Fig. 9b) extrapolations. In this case, "in this case, it is likely to be the case that would do the curves for VDC dose) and therefore extrapolation to the use of a number of rats to human effects in VDC mg/kg for or exposure for the amount could be lower in the rat at equivalent concentration

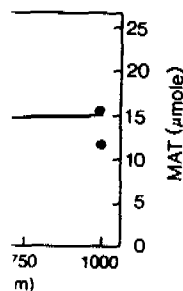
The PB-PK model demonstrates many factors that can affect behavior and compound pharmacokinetics. The other factors are each other with experimental data, however, the chemical, chemical,



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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.

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

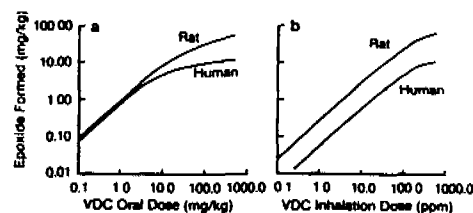


FIG. 9. Simulated amount of VDC epoxide produced at different (a) oral and (b) inhalation dose levels in the rat and human. After oral exposure saturation effects in VDC metabolism are expected in both rat and human at doses of 10 mg/kg, while after inhalation exposure saturation is predicted after about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

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.

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

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

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

$$d_{ACAC}/d_t = K_{inm} \cdot AMM$$

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

$$d_{AMAT}/d_t = K_{fec} \cdot ACACM$$

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

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 ₂ O	Water concentration.
K_{CO_2}	Rate constant for reaction of epoxide with water.

K_{fec}	Fi
K_{gsm}	Fi
K_{inm}	F
K_m	M
K_0	Z
K_{of}	C
K_1	F
K_2	I
MAT	M
$V_{\max}$	I

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K_{fec}	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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