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PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR ETHYLENE DICHLORIDE AND ITS APPLICATION IN RISK ASSESSMENT

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Abbreviated Title: PB-PK and EDC Risk Assessment

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ABSTRACT

Ethylene dichloride (EDC) has been shown to elicit tumors in both rats and mice after lifetime oral dosing. However, no treatment related tumors were observed after inhalation exposure at maximum tolerated doses. In order to quantitate target organ exposure of EDC and its metabolites as a function of dose and exposure route, a physiologically-based pharmacokinetic (PB-PK) model was developed. EDC is metabolized by saturable P-450 oxidation and by conjugation with glutathione (GSH). This perfusion rate-limiting model was developed based on metabolism of EDC by two competing pathways in the liver and lung. Metabolism rates were obtained by *in vivo* Gas Uptake measurements, and partition coefficients were calculated using an *in vitro* approach. Allometric equations were used to scale the model for different species, including humans.

At higher exposures doses, as employed in bioassay studies, tissue GSH is depleted because of conjugation of GSH with EDC and with the oxidation metabolite of EDC. The conjugation product of GSH with parent EDC (GC) is believed to be the carcinogenic moiety. The amount of this metabolite formed in a target organ is dependent on both the concentration of EDC and GSH at that site. In order to compute the amount of this metabolite formed in a target organ, it was necessary for the model to keep track of concentrations of both EDC and GSH with time. A mathematical model for GSH depletion was, therefore, written as part of the PB-PK description of EDC.

The PB-PK model was validated by measuring blood EDC and tissue GSH concentrations in the rat and mouse, and from literature data. The model was used to generate the amount of GC metabolite produced in the liver and lung of the mouse and human. This information was then used to calculate Virtually Safe Doses (VSD's) for the human employing the Multistage Model. VSD's computed using this target organ exposure approach were at least 2 orders of magnitude greater than VSD's calculated using conventional methods.

INTRODUCTION

Ethylene dichloride (EDC; 1,2-dichloroethane) is a large volume chemical extensively used in industry. EDC is presently being used as an alternative to ethylene dibromide as a fumigant for the treatment of food grains to control rodents, insects and soil nematodes. In 1978, a study conducted by the National Cancer Institute demonstrated treatment-related tumors in rats and mice dosed with 75 and 150 mg/kg EDC as a corn oil gavage (NCI, 1978). At about the same time the Bologna Tumor Center in Italy reported that no treatment-related tumors were seen in rats or mice chronically exposed to EDC by inhalation 7 hours daily at the maximum tolerated dose of 150 ppm (Maltoni *et al.*, 1980). These apparently contradictory results of the two bioassays have not been satisfactorily resolved to date.

Pharmacokinetic studies employing compartmental models have been conducted on EDC (Spreafico *et al.*, 1980; Reitz *et al.*, 1982). The results of these studies suggest that the differences in the two bioassays may be because of pharmacokinetic differences, that is, target organ exposure to EDC may have been greater after the gavage dosing then after a 7 hour inhalation exposure. Unfortunately, the compartmental modeling employed in these studies could not quantitate target organ exposures with different doses and routes of exposure, nor extrapolate the relevance of the observations to human exposures situations for assessing human risk.

The objectives of our study were to develop a physiologically-based pharmacokinetic (PB-PK) model for EDC and its reactive metabolites; to demonstrate the use of information derived from this model in cancer risk assessment; and to use the model to help explain the route-of-exposure differences observed in the two EDC bioassays.

METHODS

PB-PK Model Development:

PB-PK models are mathematical models that mimic the way the body handles chemicals. These models are physiologically realistic, and utilize all available antomic, physiologic and physicochemical information.

A schematic representation of the PB-PK model developed for EDC is shown in Fig. 1. For the distribution of EDC within the body, organs were lumped together into three compartments, based on their blood flow and ability to accumulate EDC. For the purposes of this demonstration, the liver and lung were considered to be the only metabolizing organs. All organs were connected to the circulatory system in an anatomically accurate fashion, that is the lungs were modeled as receiving the entire cardiac output, while other organs received a fraction of cardiac output. As EDC is a small, lipophilic, and readily diffusable molecule, the PB-PK model was set up as a perfusion-limited model, that is, there were no diffusion barriers to the distribution of EDC. The parameters used in constructing this model are shown below:

Partition Coefficient:

The steady-state distribution ratio or partition coefficient of EDC was calculated by the method of Sato and Nakajima (1978). These partition coefficients were calculated for the B6C3F1 mouse and Fischer-344 and Sprague Dawley rat. Blood:air partition coefficient was also obtained for human blood. The results are shown in Table 1. The partition coefficients of many tissues were similar, and for modeling purposes these tissues were grouped together. The richly-perfused group included such tissues as the kidney and spleen, the slowly-perfused consisted primarily of muscle, and the fat compartment included body fat depots and such tissues as the skin. It was noted that the human blood:air partition coefficient was somewhat smaller than the rat or mouse. This lower value was, therefore, used in our model when scaling up from the rodent to the human.

Metabolism Rate Constant:

EDC is metabolized by two competing pathways, a saturable pathway that involves P-450 oxidation, and by direct conjugation with glutathione (GSH). A simplified metabolism schematic, as reported by Anders and Livesey (1980) is shown in Scheme 1.

Estimates of metabolism rates for the two pathways were obtained from Gas Uptake measurements (Anderson *et al.*, 1980). The results of the Gas Uptake chamber runs are shown in Fig. 2. As with similar halogenated hydrocarbons, we observed that the oxidative pathway for EDC is saturated at relatively low concentrations and is best described by a Michaelis-Menten type equation. The GSH pathway is first-order till high EDC exposure concentrations, where we observed a shift from first-order kinetics. This change from first-order

shift from first-order kinetics. This change from first-order behavior for the GSH pathway was due to depletion of GSH, so that not enough GSH was available to react with EDC. In the recirculating chamber experiments we (MEA) have conducted with the halogenated hydrocarbons, this behavior is unique for EDC. Metabolism rate constants for the two pathways are shown in Table 1. At exposure concentrations where GSH is not depleted, a first-order rate constant (Kfc) was used in the PB-PK model. At higher exposure concentrations, as used in bioassays, this first-order constant did not provide an adequate description of EDC metabolism and the model, therefore, underestimated EDC concentrations. In order to account for this behavior, a GSH depletion model (D'Souza et al., 1986) had to be incorporated as part of the PB-PK description of EDC. This model kept track of liver and lung GSH levels, which were necessary in order to compute the amount of GC metabolite. The Gas Uptake studies provided rate constants for total metabolism. Metabolism rates between the liver and lung were split using data from the literature on relative enzymatic activities between these two organs, as was accomplished for the methylene chloride model (Anderson et al, 1986). Scale-up of metabolism rates for different species was performed using allometric scaling (Adolph, 1949; Dedrick, 1973; Linstead and Calder, 1981).

Physiologic Parameters:

Physiological parameters that were used to construct the model are well established in the literature (Castor et al., 1956). In most cases allometric equations were utilized to scale these parameters for different species.

Mass-balance differential equations were written for each compartment of the PB-PK model, describing the inflow, outflow, binding and metabolism of EDC, and the formation and detoxification of the intermediate metabolites. These equations were similar to those used by Andersen et al. (1986), except for the GSH model. The ACSL[1] computer program was used to solve this set of simultaneous differential equations by numerical integration, using Gear's algorithm for stiff systems.

RESULTS

THE PB-PK model was validated by measuring EDC and GSH concentrations in the rat and mouse. As the short lived reactive metabolites cannot be measured readily, GSH depletion was used as an indirect validation. Figure 3 depicts PB-PK model predictions and experimentally determined EDC blood concentrations in the rat. Model predictions are in good agreement with observed data at the dose range tested.

Blood concentrations were similarly determined for the mouse and are shown in Fig. 4. Again, experimental data are in close agreement with model predictions. Our predictions were also compared to data in the literature and were found to be in excellent agreement. Spreafico et al. (1980) measured EDC levels in

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[1]Advanced Continuous Simulations Language (Mitchell and Gauthier, Assoc., Inc., Concord, MA).

blood and tissues in the rat at several dose levels after oral, intravenous and inhalation exposures. The results of their studies are very predictable by our model, both for blood and tissue concentrations. EDC blood concentration data of Reitz et al. (1982) after both oral and inhalation exposures in the rat were also in close agreement with model predictions.

GSH concentrations for both liver and lung of the rat and mouse after oral doses of 150 mg/kg were in close agreement with model predictions, as is shown in Figs. 5 and 6, respectively.

The strains of rats used in the two literature studies cited and ours were different, but there were no apparent differences in the pharmacokinetics of EDC because of this difference. Spreafico et al. (1980) used Sprague Dawley rats and Reitz et al. (1982) used Osborne Mendel rats. We employed Fisher-344 rats and B6C3F1 mice in our studies. It appears that strain differences do not significantly affect the biodistribution or metabolism of compounds like EDC.

From the close agreement between model predictions and observed concentrations both from our studies and literature data, it is clear that the PB-PK model developed has strong predictive powers. This has been seen with different dose levels, routes-of-exposure and with two species.

Dose Surrogates:

Several reports in the literature have concluded that the GSH conjugate formed in the metabolism of halogenated hydrocarbons like EDC and ethylene dibromide (EDB) is the carcinogenic moiety, and not the parent compound. For instance, White et al. (1983) compared the amount of DNA single-strand breaks produced by EDB and its tetradeuterated derivative (EDB-d_4), and found that EDB-d_4 was more genotoxic than EDB. As the deuterated compound would preferentially be metabolized via the GSH pathway, these results demonstrated that the GSH pathway and not the oxidative pathway was the source of genotoxic metabolite(s). Similarly, Storer and Conolly (1985) studied hepatic DNA damage by EDC after pretreating mice with either piperonyl butoxide to block microsomal oxidation, or diethyl maleate to deplete GSH, and found that the GSH pathway was responsible for DNA damage and not the oxidative pathway. Andersen et al. (1986) related the number of tumors at each dose level with the PB-PK model predictions of the amount of GSH and oxidative metabolite produced at that dose level, and noted that tumors related quite well with GSH metabolite levels. We have, therefore, used the total amount of the GSH metabolite (GC) produced in a target tissue as a surrogate for dose.

PB-PK Model and Risk Assessment:

Because of the nonlinear metabolism pathways for EDC, the relationship between administered EDC dose, either as inhalation or oral exposure, and the amount of GC metabolite produced in a target tissue is complex. Figure 7 illustrates the relationship between different oral gavage doses of EDC and the resulting GC metabolite that would be produced in the liver for the B6C3F1 mouse. It can be seen that at low doses because of first-order metabolism of EDC, the relationship is 1:1. As the exposure dose is increased, the amount of GC produced is proportionally much greater than administered dose. This non-linearity is because the oxidative pathway for EDC metabolism is saturated, resulting in increasing EDC levels, and, therefore, increasing GC levels. At exaggerated

doses, as employed in bioassay studies, the capacity of the GSH pathway is also overwhelmed, due to depleted GSH concentrations, and the amount of GC produced is now proportionally lower than the administered dose. The dashed line is a direct linear extrapolation of GC metabolite from the 150 mg/kg dose to low doses, ignoring any nonlinearities. It can be readily seen from this type of plot that by not taking into account the nonlinear metabolism of EDC, the risk of liver cancer to the mouse, based on results of a 150 mg/kg dose, can be easily overestimated by an order of magnitude. The PB-PK model was also used to compute this relationship for the human. Liver GC in the human was predicted to be very similar to the mouse, and is, therefore, not shown in Fig. 7.

Figure 8 depicts the relationship between administered dose and lung GC metabolite for the B6C3F1 mouse. A similar relationship is observed for the lung, except that the human GC exposure doses are about 2.5 times smaller. This suggests that by ignoring the nonlinear metabolism of EDC, lung cancer for the mouse may be overestimated by a factor of 10, while for the human it can be overestimated by 25-fold. Administered dose versus GC exposure plots were also constructed for inhalation exposure for the mouse and for oral and inhalation exposures for the rat, with similar findings (plots not shown here).

PB-PK Model and Virtually Safe Doses:

As part of the conventional cancer risk assessment process the number of tumors in each dose group of the bioassay is correlated with the administered dose. Statistical models are used to fit the data in order to extrapolate these findings to low exposure levels. These models estimate the dose required for 1 out of 1 million animals to develop a certain type of tumor during lifetime dosing. This dose is commonly called the Virtually Safe Dose (VSD). The VSD for humans is obtained by dividing the mouse VSD by a factor of 12.7, which is the relative difference in body surface-area between the mouse and man. Additional safety factors are also built into the risk assessments, including the use of confidence intervals, and use of upper limits on estimated exposure of humans. The VSD is then compared with anticipated human exposures to prepare a risk assessment.

The PB-PK model was used to generate GC with different EDC exposure doses in the mouse, rat and the human (as in Plots 7 and 8). The Multistage Model (Crump, 1982) was then used to relate the number of tumors produced in each dose group with GC produced at that tumor site. That is, instead of correlating tumors with administered dose or "external dose," the dose surrogate at the target site or "internal dose" was employed. There was no additional justification for choosing the Multistage Model over several commonly used low dose extrapolation models, than the fact that it is one of the models used most often by the regulatory agencies. The VSD obtained using this "internalized dose" concept was converted to the corresponding EDC exposure concentration both for the mouse as well as the human employing Plots 7 and 8. That is, the extrapolation from mouse to human was made using the PB-PK model and not a surface-area correction as would be done in a conventional risk assessment.

The results of this analysis are shown in Table 2. For comparison purposes, VSD's were calculated both by the conventional method, and by the PB-PK approach. As the regulatory agencies commonly use the Lower Confidence Limit

of dose (LCL), the comparisons employed here are similarly restricted to the LCL's and not to the fitted lines. When comparing the VSD for liver tumors by the two methods, it can be seen that the VSD for liver tumor obtained by using the pharmacokinetic approach is about two orders of magnitude greater than that obtained by the conventional method. VSD for lung tumors, however, shows about a 500-fold difference. These data indicate that by not considering the pharmacokinetics of EDC in the mouse and human, conventional methods may overestimate cancer risk due to low level EDC exposure by 2 or even 3 orders of magnitude.

PB-PK Model and Route-of-Exposure Differences:

In order to address the route-of-exposure differences in the two EDC bioassays, the amount of GC metabolite produced in the liver and lung was computed for oral gavage doses of 75 and 150 mg/kg and compared to those that would be produced by a 7 hour inhalation exposure at 150 ppm. These results are shown in Table 3. It is interesting to note that lower amounts of the dose surrogates are produced at the maximum tolerated inhalation dose of 150 ppm, compared with the other two oral doses. This observation may be a possible reason why treatment related tumors were not seen in the inhalation bioassay, but were observed both at 75 and 150 mg/kg for the oral bioassay.

DISCUSSION

PB-PK models are mechanistic models that account for all known processes that take place from the time a chemical is present at an absorption site for potential inhalation, oral or dermal uptake into the body, to the interaction that takes place between the chemical or its metabolites, and some body tissue. The amount of chemical that is present at an absorption site may, therefore, have a very complex and indirect relationship with the amount of that chemical or its metabolites that is present at a target site and elicits a biological response. The chemical must first get absorbed, it may bind to blood components which may limit its further distribution, it may undergo detoxification or may become more toxic, or it may be eliminated rapidly from the body. An understanding of these processes, as well as the quantitation of the time-course in the way these processes operate and interact may play a very important role in determining the potential risk from exposure to a certain concentration of a chemical.

Once it is known whether the parent chemical or its metabolite(s) is responsible for a toxic or carcinogenic response a PB-PK model can be developed to quantitate the amount of exposure and the time-course of exposure to this moiety at a target site in laboratory animals. Using the model this information can then be scaled up to the human, in order to assess target organ exposure, and, therefore, risk of exposure to humans. The model does not provide insight into the mechanism of cancer nor does it predict sensitivity of one target organ over another or one species over another. The model is simply a tool to quantitate target organ exposure to the relevant chemical species.

One of the major problems in assessing toxicity or cancer risk is route-of-exposure extrapolations. Often, data are only available from one route of intake, while a risk assessment has to be conducted for exposure via another route. Sometimes, like with the EDC and DCM bioassays, the results are positive from one route and negative via another route of exposure, which makes the

results difficult to interpret. Unfortunately, there has been no reliable method so far for any extrapolation between routes of intake. PB-PK models, however, have the potential to quantitate the amount of chemical and its metabolites that are taken in from different exposure sites and reach a target tissue to elicit a response.

Another factor that is not readily resolved without these kinds of models is species differences in biodistribution of chemicals. Although PB-PK models do not predict sensitivity of a biological response of one species over another, they can reliably quantitate target organ doses between different species. Differences in the physiology of different species, like organ blood flow or pulmonary ventilation are taken into to account in these models. Anatomical differences, like organ sizes are appropriately corrected when extrapolating results from one species to another. Also physicochemical parameters of the chemical like blood and tissue binding are taken into consideration. The power and flexibility of these models lies in the fact that they are physiologically realistic and not mathematical "black-boxes" like compartmental models. As more information is gained about the behavior of a chemical it can be added to the model without changing its basic structure, thus improving its predictability.

The PB-PK model developed for EDC is in reality quite basic, as the model only computes gross tissue exposures of the relevant species. Further work in this area should concentrate on cellular distribution, as well as molecular dosimetry aspects like DNA binding and repair processes. All of these processes as well as the processes that take place from the time an unrepaired DNA adduct is formed to the time a tumor is produced is expected to be complex and probably nonlinear with exposure dose. As information is gained about the rates of these processes, they can readily be added on to the PB-PK model.

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GLOSSARY

GC	Metabolite formed by conjugation of EDC and GSH.
K_f	First-order rate constant for EDC metabolism via GSH pathway (hr^{-1}).
K_{fee}	First-order rate constant for reaction of chloroacetaldehyde with everything else besides GSH (hr^{-1}).
K_{gs}	First-order rate constant for reaction of EDC with GSH (hr^{-1}).
K_{gsm}	First-order rate constant for reaction of GSH with chloroacetaldehyde (hr^{-1}).
K_m	Michaelis-Menten constant for oxidation pathway (mg/l).
Q_c	Cardiac output.
Q_f	Fat blood flow.
Q_l	Liver blood flow.
Q_{lu}	Lung blood flow.
Q_p	Alveolar ventilation.
Q_r	Blood flow to richly-perfused tissues.
Q_s	Blood flow to slowly-perfused tissues.
V_{max}	Maximum capacity of oxidation pathway (mg/hr).

LEGENDS

Figure 1. Schematic representation of the pharmacokinetic model developed for EDC. The biodistribution of EDC was modeled by dividing body tissues into three compartments based on their blood flow and relative ability to accumulate EDC. The liver and lung were considered the only metabolizing organs. V_{max} and K_m are the rate constants for the oxidation pathway and K_f is the rate constant for the GSH pathway. GSH↓ represents a glutathione depletion model.

Figure 2. Gas Uptake runs for EDC. Initial chamber concentrations utilized were 300 (■), 500 (●), 1000 (◆) and 2000 (○) ppm. One group of animals exposed to 2000 ppm was treated with 2,3-EP (▲). The lines represent model predictions that were generated by defining a PB-PK model of rats in a closed chamber.

Figure 3. Model predicted (—) and experimentally determined EDC blood concentrations in the rat after corn oil gavage doses of 1.5 (▲), 25 (■) and 150 (●) mg/kg.

Figure 4. Model predicted (—) and experimentally determined (●) EDC blood concentrations in the mouse after a corn oil gavage dose of 150 mg/kg.

Figure 5. GSH concentrations in the liver (□) and lung (●) of the rat following an EDC dose of 150 mg/kg. Symbols represent Mean ± S.D. of 4 rats. The curves are model predictions.

Figure 6. GSH concentrations in the liver (□) and lung (●) of the mouse following an EDC dose of 150 mg/kg. Symbols for the liver represent Mean ± S.D. of 4 mice. The lungs of 4 mice were pooled together and analyzed as a single sample. The curves are model predictions.

Figure 7. Relationship between EDC administered dose and liver GC formed in the mouse. The curves represent the complex relationship predicted by the model. The dashed line is a direct extrapolation from the 150 mg/kg dose, assuming a 1:1 relationship. The curve predicted for the human is virtually superimposable on the mouse curve and is, therefore, not shown in the figure (see text for detailed explanation).

Figure 8. Relationship between EDC administered dose and lung GC metabolite for the mouse and human. The dashed line is a direct extraplation from the 150 mg/kg dose assuming a 1:1 relationship.

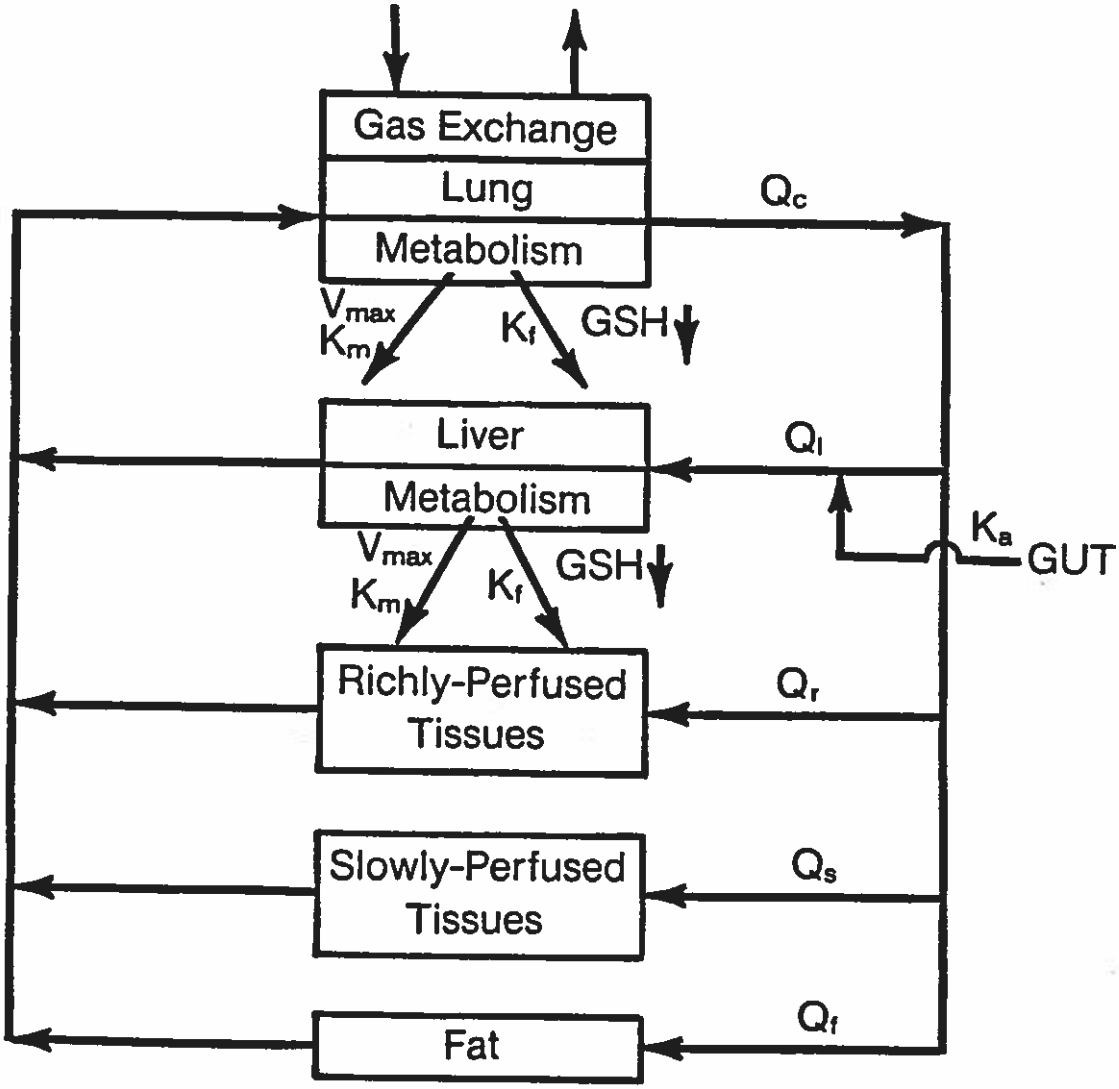
Scheme 1. EDC metabolism pathway. EDC is metabolized by a saturable oxidation pathway and by conjugation with GSH.

Table 1. Partition coefficients and metabolism rate constants obtained for EDC. The letter 'c' at the end of a constant represents the value of the allometrically unscaled constant, that is, per 1 kg of species body-weight.

Table 2. VSD's calculated for EDC employing the conventional approach and the PB-PK approach. The column to the right indicates the ratio of the VSD's using the two approaches.

Table 3. The amount of GC metabolite formed in the liver and lung of the rat following oral and inhalation exposures. This difference may be one explanation for the route-of-exposure differences seen in the two EDC bioassays.

Figure 1


Gas Exchange
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Metabolism
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K_m
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GSH
Liver
Metabolism
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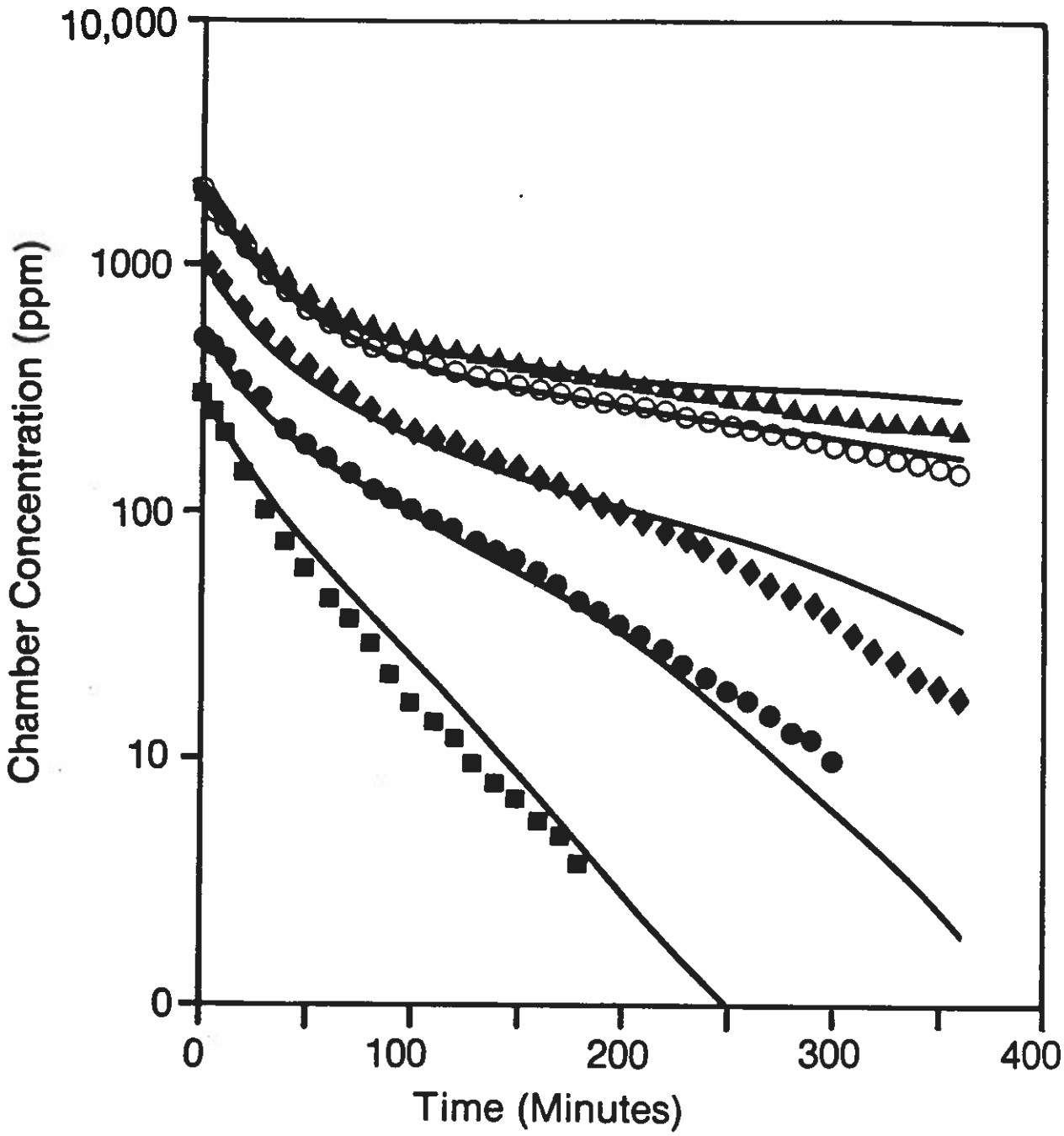
Slowly-Perfused Tissues
Q_s
Fat
Q_f

Figure 2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1000
100
10
0
Chamber Concentration (ppm)
0
100
200
300
400
Time (Minutes)

Figure 3

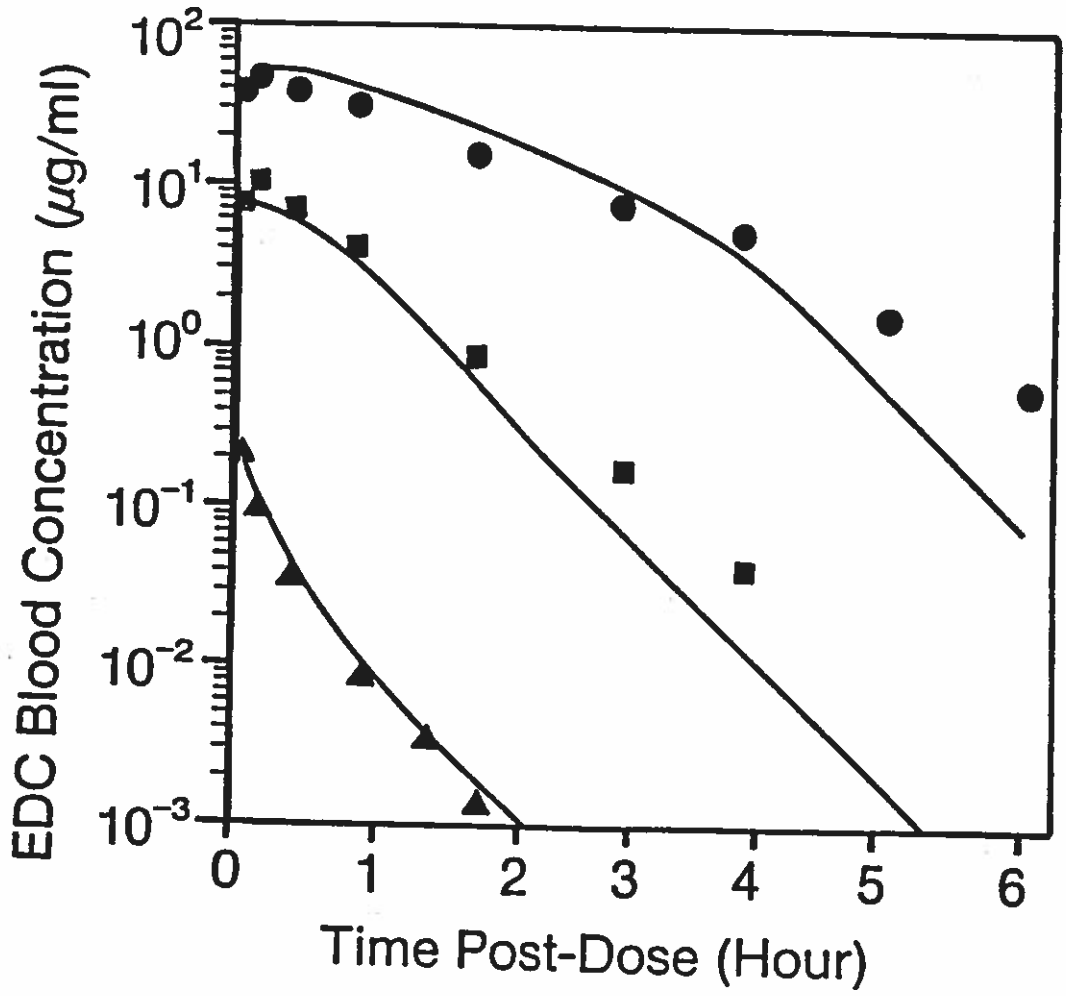
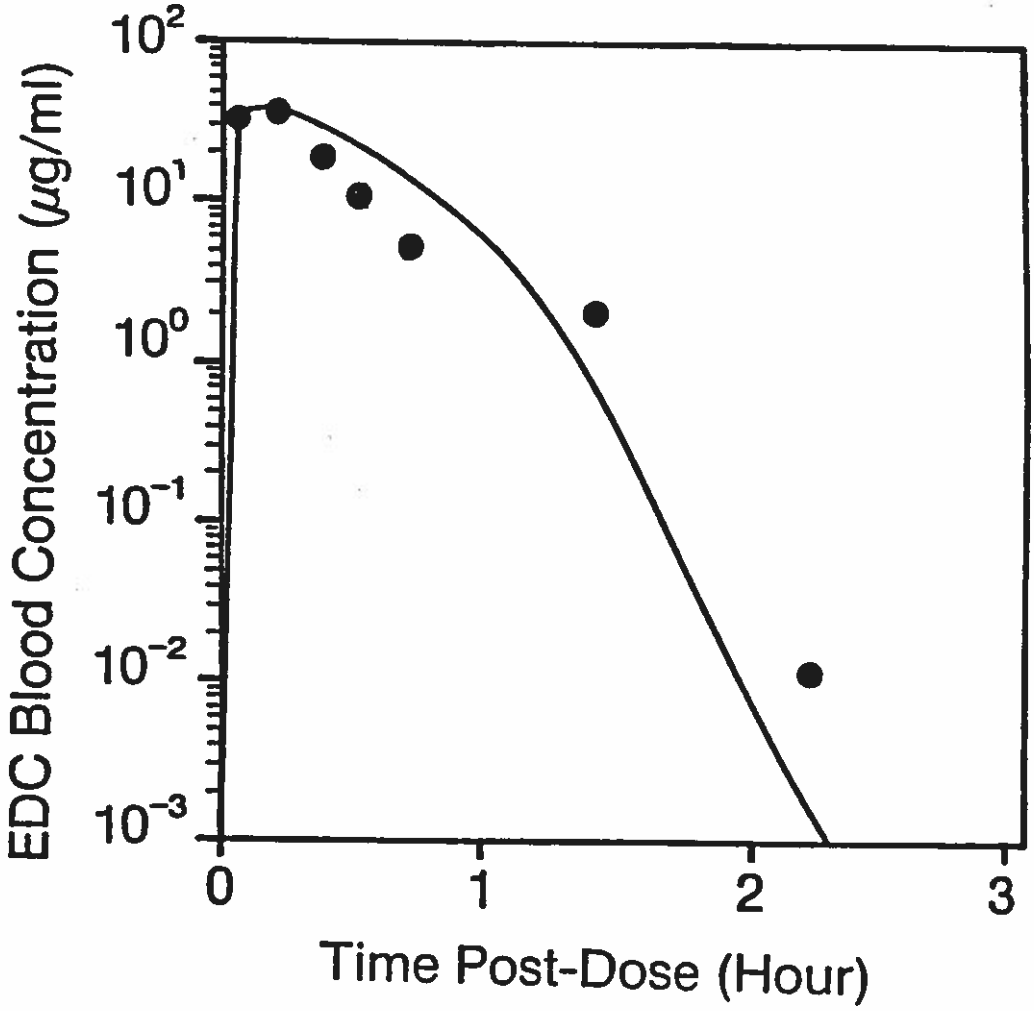

EDC Blood Concentration (μg/ml)
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
0
1
2
3
4
5
6
Time Post-Dose (Hour)

Figure 4


EDC Blood Concentration (μg/ml)
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10¹
10⁰
10⁻¹
10⁻²
10⁻³
0
1
2
3
Time Post-Dose (Hour)

Figure 5

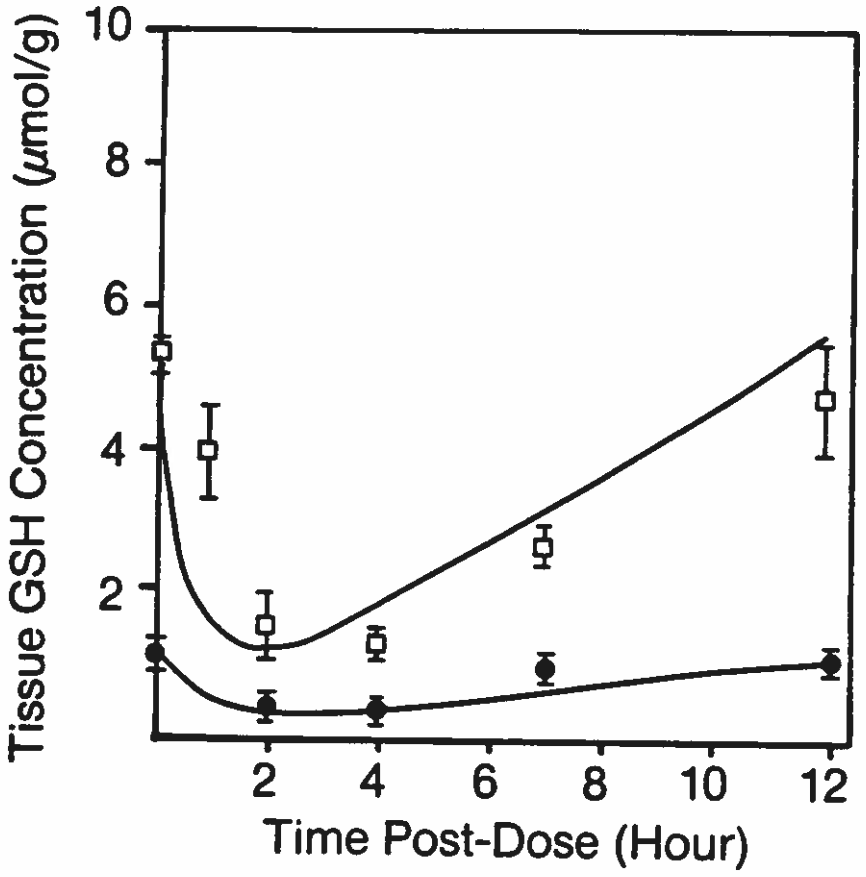
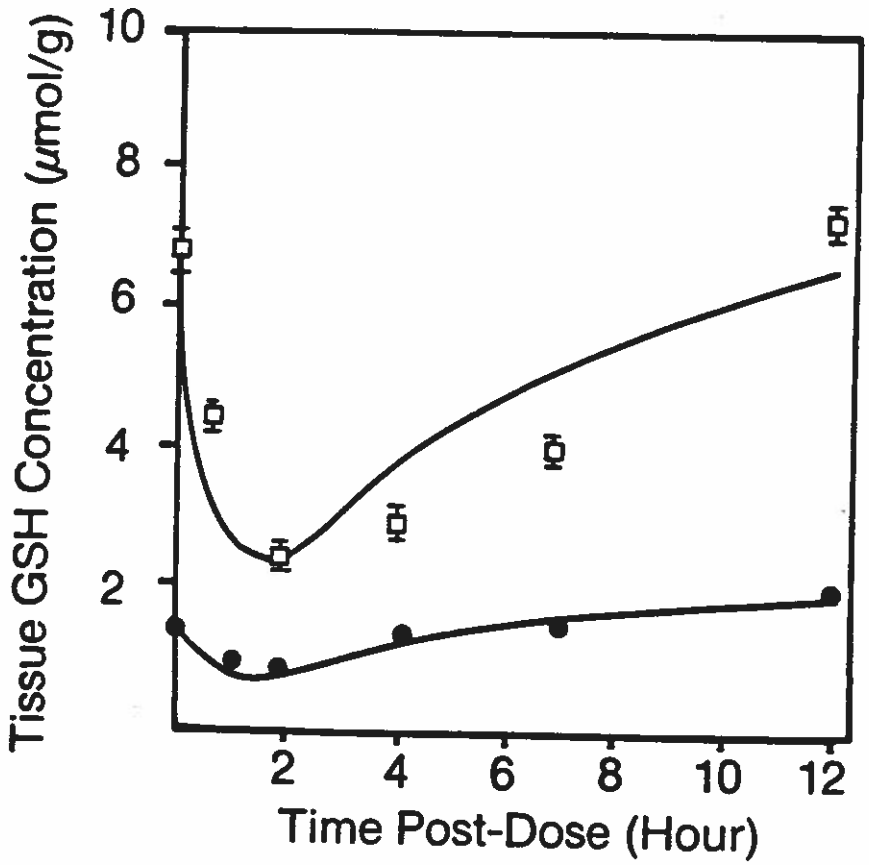

Tissue GSH Concentration (μmol/g)
10
8
6
4
2
2
4
6
8
10
12
Time Post-Dose (Hour)

Figure 6


Tissue GSH Concentration (μmol/g)
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8
6
4
2
2
4
6
8
10
12
Time Post-Dose (Hour)

Figure 7

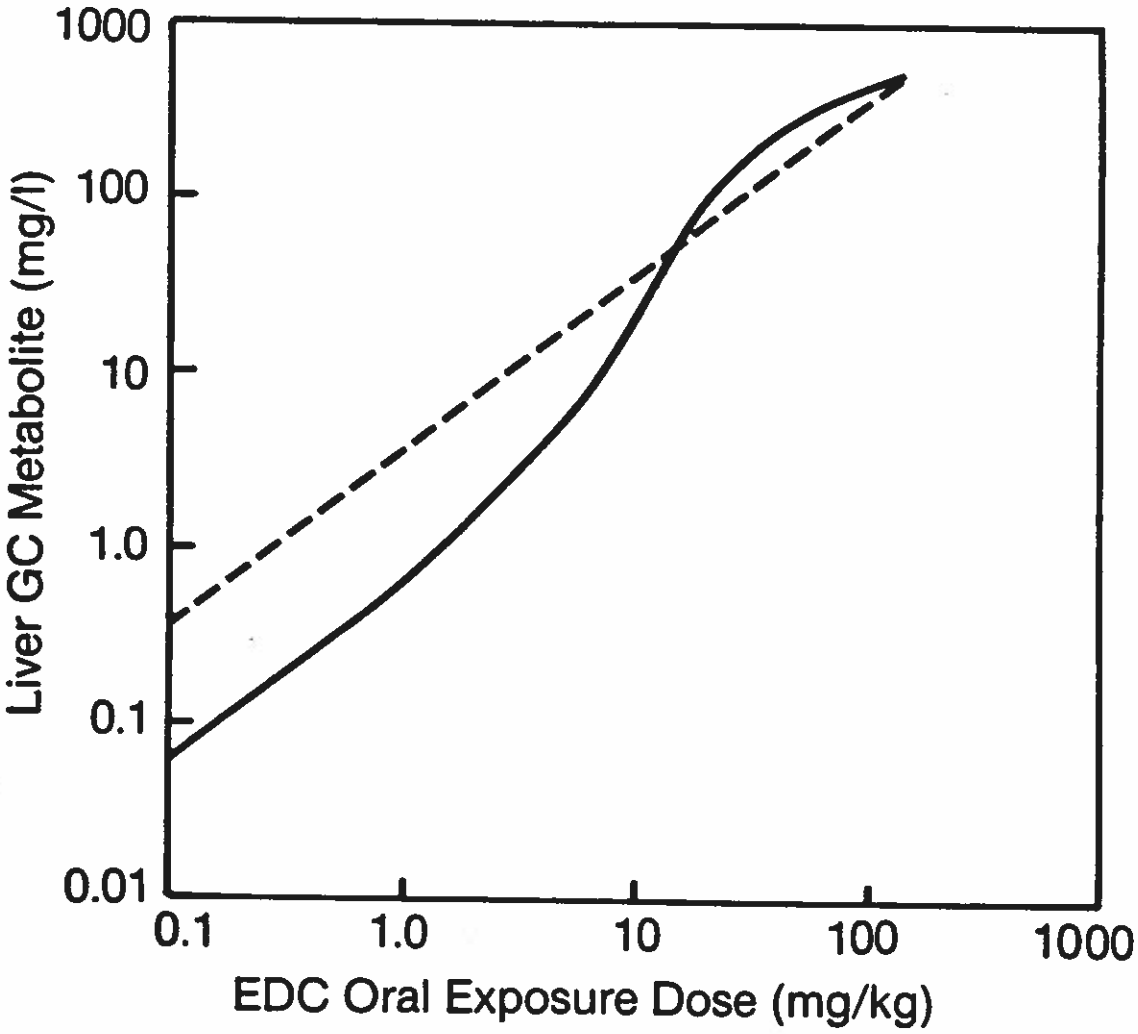
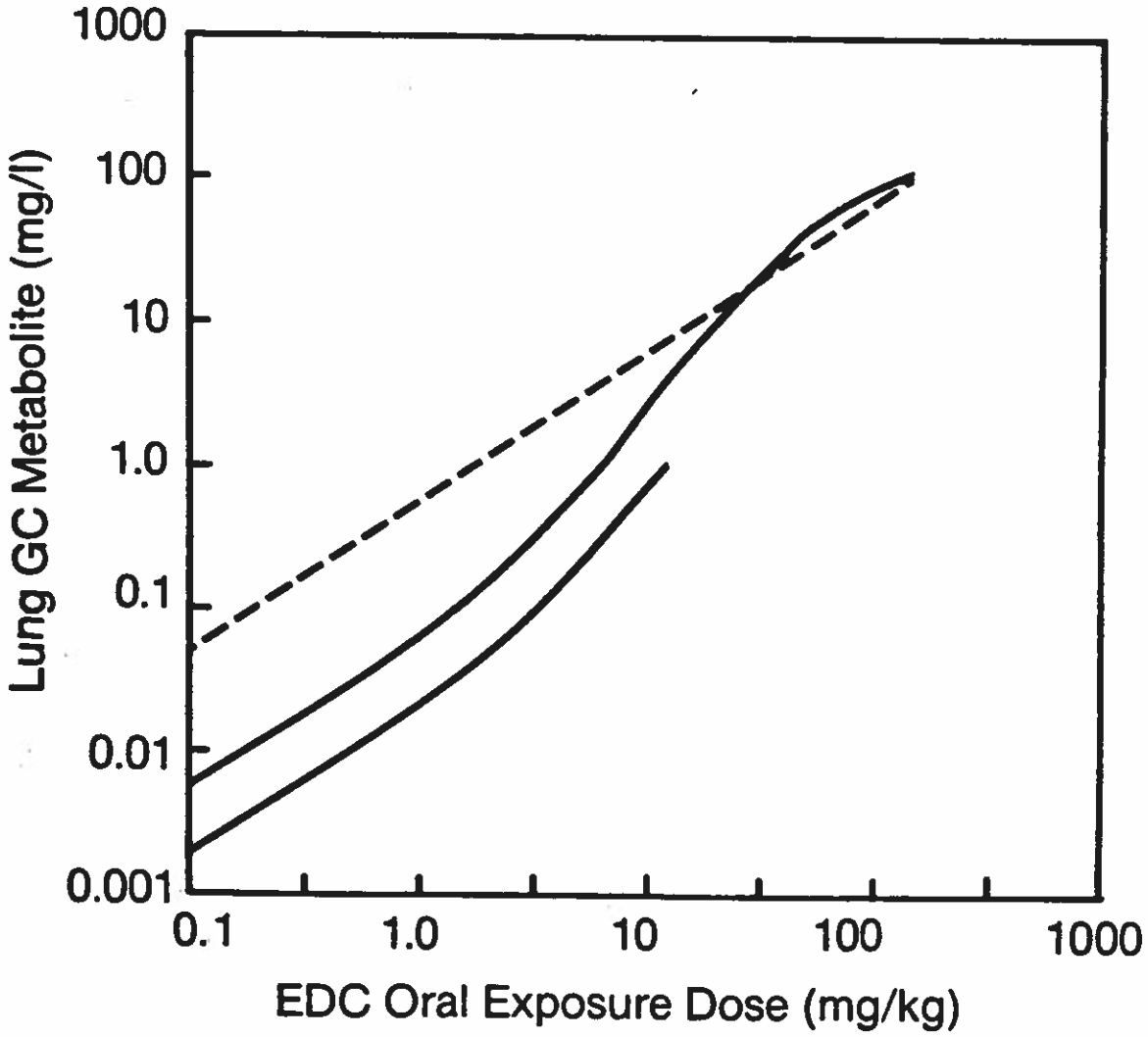

1000
100
10
1.0
0.1
0.01
Liver GC Metabolite (mg/l)
0.1
1.0
10
100
1000
EDC Oral Exposure Dose (mg/kg)

Figure 8


1000
100
10
1.0
0.1
0.01
0.001
Lung GC Metabolite (mg/l)
0.1
1.0
10
100
1000
EDC Oral Exposure Dose (mg/kg)

Scheme 1

ETHYLENE DICHLORIDE METABOLISM

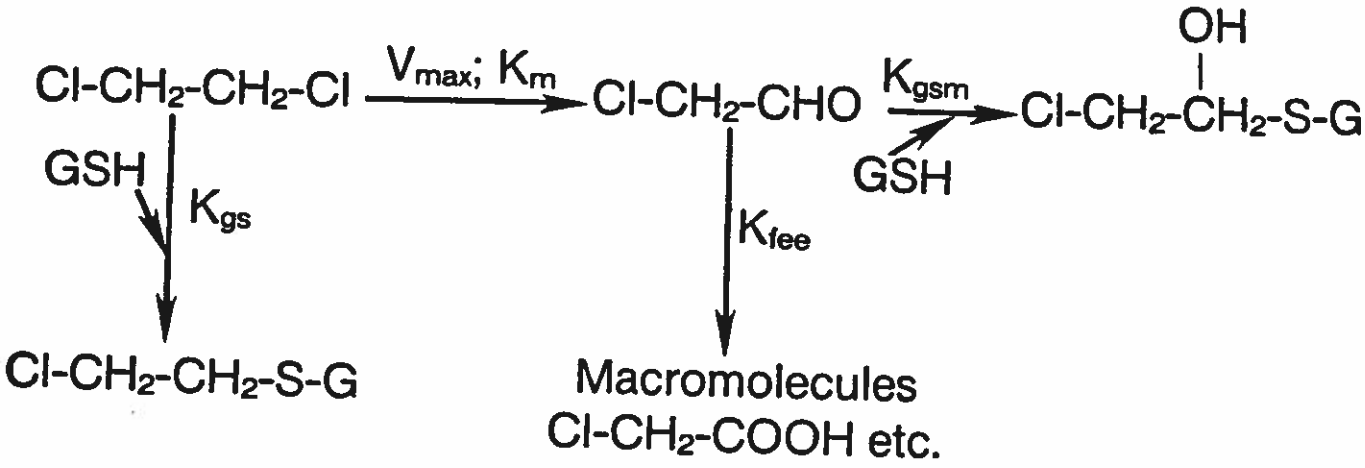


Table 1

PARTITION COEFFICIENTS

Ratio	Sprague Dawley Rat	Fisher 344 Rat	B6C3F1 Mouse	Human
Blood:Air	27.6	30.4	29.7	21.1
Richly-Perfused:Blood	1.1	1.2	1.0	—
Slowly-Perfused:Blood	0.8	0.8	0.8	—
Fat:Blood	12.2	11.4	12.1	—

METABOLISM RATE CONSTANTS

EDC:	$V_{maxc} = 3.25$	$K_m = 0.25$	$K_{fc} = 9.0$
GSH Model:	$K_{gsc} = 0.0014$	$K_{feec} = 4500$	$K_{gsmc} = 0.14$

EDC VIRTUALLY SAFE DOSES - 1/MILLION RISK

		Conventional			PB-PK		
	Estimate Type	Mouse EDC µg/kg/d	Human EDC µg/kg/d	GC Metabolite µg/l/d	Mouse EDC µg/kg/d	Human EDC µg/kg/d	Ratio, PB-PK/ Conventional
Organ	Best Est.	3.1	0.24	150	240	230	960
Liver	95% LCL	0.46	0.036	2.3	3.8	3.6	100
Lung	Best Est.	270	21	245	4100	7500	360
	95% LCL	0.99	0.078	0.91	15	39	500

Table 2

Dose and Route	GC Metabolite	
	Liver	Lung
Corn Oil Gavage		
150 mg/kg	630	131
75 mg/kg	372	71
Inhalation		
150 ppm * 7 hours	230	64