



CHEMICAL MANUFACTURERS ASSOCIATION

January 3, 1995

Jim Cogliano, Ph.D.
Chief, Cancer Statistics and Epidemiology
U.S. EPA (8602)
401 M Street, SW
Washington, D.C. 20460

Dear Dr. Cogliano:

As a follow-up to our meeting of October 6, 1994, five copies of a draft manuscript entitled, "Predicting Cancer Risk from Vinyl Chloride Exposure with a Physiologically-Based Pharmacokinetic Model," are enclosed. The manuscript describes the development and validation of the model in both humans and rodents. The manuscript also addresses an unique situation: the availability of human cancer incidence data to check the validity of the model for estimating human cancer risk.

The draft manuscript has been submitted to *Toxicology and Applied Pharmacology* for peer-review and publication. The draft, therefore, will be revised based on the peer-review comments. ChemRisk retains ownership and copyright privileges for the manuscript.

I will contact you in the next week to discuss with you the possibility of modifying the vinyl chloride unit risk estimate in the Heast Table based on the PB-PK data described in the enclosed draft manuscript.

Thank you, and I wish you a happy new year!

Sincerely,

Hasmukh C. Shah, Ph.D.
Manager, Vinyl Chloride Panel

cc: Richard Reitz, ChemRisk
Michael Gargas, ChemRisk
Vinyl Chloride Health Committee, CMA

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

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ABSTRACT

Predicting Cancer Risk from Vinyl Chloride Exposure with a Physiologically-Based Pharmacokinetic Model. Reitz, R.H., Gargas, M.L., Andersen, M.E., Provan, W.M., and Green, T.L. (1994) *Toxicol. Appl. Pharmacol.*, 000, 000-000.

A Physiologically-Based Pharmacokinetic (PB-PK) model capable of describing the metabolism of vinyl chloride (VC) in rats, mice, and humans has been developed and validated by comparison with experimental data from experiments not used in model development. This PB-PK model has been used to predict measures of delivered dose (reactive VC metabolites produced in the livers of the affected species) hypothesized to be involved in the induction of liver angiosarcoma in rats, mice, and human populations exposed to VC. Measures of delivered dose in rats were fit to an empirical dose response model (the linearized multi stage model of Crump et al.) and used to make predictions of liver angiosarcoma incidence in mice and human populations exposed to VC. This procedure gave a good prediction of angiosarcoma incidence in mice. Predictions of angiosarcoma incidence in humans were more than two orders of magnitude lower than risk estimations which did not utilize pharmacokinetic data (HEAST, 1994), but were still almost an order of magnitude higher than actually observed in exposed human populations.

INTRODUCTION

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

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

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

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

Objectives:

Physiologically-based pharmacokinetic (PBPK) models of chemical disposition have been developed for a variety of chemicals, including the chlorinated ethylenes (NAS, 1987). These models are particularly well suited for risk extrapolations because they are based on specific physiological and biochemical properties of the different species and dose routes as well as physical chemical information about the solubilities and vapor pressures of the different compounds (Andersen et al., 1987). Our objectives in this project were:

1. To develop a PB-PK model capable of predicting the metabolism of VC in both rodents and humans.
2. To validate this model with existing data sets for rodents and humans.
3. To develop a quantitative risk assessment procedure based on the predictions of the validated PB-PK model for VC.
4. To compare the PB-PK based risk assessment procedure with the existing EPA risk assessment procedure (HEAST, 1994)
5. And finally, to compare the results from this PB-PK based risk assessment with the actual incidence of liver angiosarcomas in human populations exposed to VC in the workplace. (Simonato et al., 1991).

METHODS

Construction of the PB-PK Model:

The PB-PK model for VC was based on a PB-PK model developed by Ramsey & Andersen (1984) to describe the kinetics of inhaled styrene in rats and humans. In this model a series of simultaneous differential equations describing the distribution, elimination, and metabolism of chemical was incorporated into a computer program using an integrated software package containing routines for numerical integration, optimization, sensitivity analysis, and graphical display. This software package (SimuSolv⁵) is commercially available from Mitchell & Gauthier Associates, 200 Baker Ave, Concord MA 01742-0013, USA.

The VC model contains four tissue groups (fat, muscle, rapidly perfused tissues, and liver) and assumes that all metabolism takes place in the liver where the rate of metabolism is described by the Michaelis-Menten equation. Detailed descriptions of this type of model are given elsewhere (Ramsey and Andersen, 1984; Andersen et al., 1987). An annotated copy of the source code for this model is available from the corresponding author (Reitz).⁶

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

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

Metabolic Constants for Rats: Metabolic parameters for male and female Sprague Dawley rats (body weights 200-400 grams) were obtained by computer optimization of gas uptake data sets (four experiments for each sex) according to procedures previously described by Gargas et al. (1986). Basically, given the physiology of the animals and the solubilities of VC in rat blood and tissues, the metabolic rate constants VMaxC and Km were varied until a satisfactory description of data gathered in several independent gas uptake experiments was obtained for both male and female rats (Figures 1a, 1b).

Metabolic Constants for Mice and Humans: Vinyl chloride belongs to a class of low molecular weight halogenated hydrocarbons which share the property of being metabolized by cytochrome P450 enzymes, and in particular, by the 2E1 subclass of these enzymes. Metabolism of this class of substrates by these enzymes appears to be the primary means of biotransformation in both animals and man (Raucy et al., 1993). Representative members of this class of compounds (methylene chloride, CH₂Cl₂ and chloroform, CHCl₃) have been studied extensively in rodents and humans (both *in vivo* and *in vitro*) and these studies provide a basis for estimating the *in vivo* metabolic rates for VC.

For example, methylene chloride is oxidized to carbon monoxide by cytochrome P450 in rodents and humans, and *in vivo* rates of metabolism have been reported by Andersen et al. (1987; 1991). Similarly, Corley et al. (1990) reported *in vivo* studies of the rates of metabolism of chloroform in rats and mice. These studies provide a basis for estimating the *in vivo* metabolic rate constants for VC in humans. The process by which these data are used to estimate *in vivo* metabolic rate constants for humans and mice for VC is outlined below:

1. *In vivo* maximum rates of metabolism (VMax's) in rats are obtained by experimentation (VC) or from the literature (CH₂Cl₂ CHCl₃). These values are listed in Table 2.
2. The weight of the liver in animals used in the *in vivo* studies is calculated for each chemical and each species from the percent liver and the body weight (Table 2).
3. The VMax for each species is divided by the weight of liver to give an *in vivo* rate per gram for each chemical. These VMax/g's are normalized to the rat for each chemical, and the "Ratio to Rat" is listed in the last row of Table 2 for each chemical.
4. For chloroform and methylene chloride, the *in vivo* "Ratio to Rat" is nearly constant (2.570, 2.707) suggesting that after normalization, the "Ratio to Rat" does not depend upon the chemical being studied (at least within this limited series of chemicals all metabolized by P450 2E1).
5. *In vivo* studies by Andersen et al (1991) with CH₂Cl₂ provide a basis for calculating the "Ratio to Rat" for humans of 0.208; Table 2. (Comparable *in vivo* studies for CHCl₃ in humans were not available.)
6. Finally, the experimentally determined *in vivo* VMax for VC in rats and the "Ratio to Rat" for mice and humans were used to calculate *in vivo* VMax's for VC by multiplying VMax/g (rat) by the "Ratio to Rat" and weight of liver in each species.

For example the *in vivo* VMax for VC in humans is calculated as: $(0.968/5.69) \times 0.208 \times 2198 = 77.7 \text{ mg/hr}$ (Table 2).

Model Validation

Validation in Rats

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

The model gave an excellent simulation of Watanabe et al.'s data. Over a range of concentrations from 1.4 ppm up to 4,600 ppm, the PB-PK model accurately predicted the levels of radioactive metabolites produced and successfully identified the region where saturation of VC metabolism occurs (200-500 ppm; Figure 2).

Validation in Mice

Metabolic rate constants for B₆C₃F₁ mice were estimated by extrapolation from in vivo results in B₆C₃F₁ mice obtained with model substrates as outlined in the Methods section. In order to test whether these estimated rate constants accurately reflected the in vivo metabolism of VC in mice, an independent set of gas uptake experiments conducted in male and female B₆C₃F₁ mice was used for validation.

For this validation, the basic PB-PK model for rats was adapted to mice by (a) incorporating the known physiological differences between rats and mice (see Andersen et al., 1987), (b) changing the blood/air partition coefficient for VC to that measured in the laboratory with samples of B₆C₃F₁ mouse blood (Gargas et al., 1989), and (c) setting the allometric constant describing the maximum rate of metabolic oxidation of VC to the value estimated from in vivo studies with other volatile, low molecular weight, halogenated hydrocarbons (Table 2). Other than these changes, the structure of the model was not altered.

Simulated and actual data from four experiments with male B₆C₃F₁ mice (initial concentrations of 345, 570, 1065, and 3190 ppm) and four experiments with female B₆C₃F₁ mice (initial concentrations of 280, 550, 975, and 2950 ppm) are shown in Figures

3a and 3b. As can be seen from inspection of these figures, the model gives a reasonable (but not perfect) simulation of the gas uptake data.

The highest concentrations of VC (~3000 ppm where metabolism is probably saturated) were well described by the PB-PK based on the metabolic rate constants estimated from model substrates. These data depend primarily upon the value chosen for VMax in the model and suggest that the extrapolation procedure employed for estimating the maximum in vivo rate of metabolism in B₆C₃F₁ mice was successful.

The low concentrations (~300 ppm, where metabolism is presumably first order) are also well described by the model. For rapidly metabolized substances, the uptake is largely flow-limited (i.e. depends upon how rapidly the material is delivered to the liver rather than the specific values of VMax and Km). Correspondence of model simulations with experimental data at low concentrations suggests that the physiological parameters for mice (flow rates and partition coefficients) used in the PB-PK models for B₆C₃F₁ mice are appropriate.

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

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

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

Validation in Humans

The human PB-PK model for VC (with in vivo metabolic rate constants estimated from experiments with CH₂Cl₂) was also validated by comparison of model simulations

with independently gathered human data. Physiological properties of humans were obtained from the International Commission on Radiation Protection's handbook on reference man (ICRP, 1975), the blood/air partition coefficient was set to the value measured in the laboratory (Gargas et al., 1989), and the *in vivo* metabolic rate constant VMaxC was estimated by the procedures outlined in Table 2. The PB-PK model for humans was not modified in any other way, and no attempt was made to "curve fit" experimental data by adjustment of model parameters when conducting simulations with the human PB-PK model.

The human PB-PK model was validated with data gathered by Baretta et al. (1969) in groups of human volunteers exposed to 59, 261, or 492 ppm for 7.5 hrs in a carefully controlled laboratory setting. Workers entered the chamber and were exposed to VC at the indicated concentrations for approximately 3.5 hrs. Then they left the chamber to have lunch in an area free of VC for approximately 0.5 hrs, following which they returned to the chamber for another 4 hrs (7.5 hrs total participation in the exposure). This exposure pattern was simulated by the PB-PK model during the validation exercises.

Samples of exhaled breath were collected from these volunteers (4-7 subjects in each exposure) at different times post-exposure for up to 20 hrs. The sampling procedure involved giving each person several glass tubes (20 mm diameter, approximately 23 cm in length) capped with screw cap septa. Each worker was asked to inhale through his nose and exhale by mouth into the glass tube four times. After the fourth breath, the workers quickly capped the glass tubes with impermeable septa and returned the tubes for analysis. A comparison of observed and simulated results for these exposures is presented in Figure 4.

The model for VC gave an good simulation of expired air data for all three concentrations over the period from 1 hr post exposure to 20 hr post exposure. It is noteworthy that these experiments included exposures at concentrations up to 500 ppm, a region where metabolic saturation occurs in rats (Figure 2). This validation exercise suggests that the PB-PK model should give a reliable representation of the disposition of VC in humans at concentrations up to 500 ppm.

Risk Estimation

Deriving Rat Potency Estimates

Maltoni conducted a series of inhalation bioassays of VC in male and female Sprague Dawley rats at concentrations ranging from 1 ppm to 30,000 ppm (Maltoni, 1974b). These animals were exposed to VC for 4 hr/day, 5 days/week, with exposures beginning in young adult animals and continuing until the animals reached one year of age. After the first year of exposure, animals were held until they died and then examined for the presence of tumors. Survival of the animals was compromised at the

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highest concentrations, so these results were not employed in derivation of a rat potency for VC. Results from exposures conducted at 0, 1, 5, 10, 25, 50, 100, 150, 200, 250, 500, 2500, and 6000 ppm were selected as the basis for fitting a dose response curve for induction of liver angiosarcoma by VC metabolites. Tumor incidence data used in constructing this curve are listed in Table 3.

The "dose surrogate" (measure of dose delivered to the target organ) chosen for risk analysis was the average daily amount of metabolite produced per day per liter of liver tissue. This type of dose surrogate is appropriate for risk analysis when the metabolite is highly reactive (as the chloroethylene oxide formed from VC would be) and either reacts with DNA or water in the target organ with a very short half-life (i.e. would not be expected to persist long enough to circulate to other organs in the body). Rationale for the selection of dose surrogates from PB-PK models have been discussed extensively elsewhere (Andersen et al., 1987) and the reader is referred to this publication for further details.

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

The risk estimation based on the PB-PK model (curved, heavy line in Fig 5) describes the tumor incidences observed by Maltoni over a broad range of doses, showing the ability of the PB-PK model to compensate for the effects of metabolic saturation in the activation of VC. In contrast, a risk estimation based on administered dose at the two highest concentrations (2500, 6000 ppm) significantly underpredicts the tumor incidences seen in rats by Maltoni at lower concentrations.

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

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

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

Estimation of Risk to Mice from Rat Data

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

VC was seen to increase the incidence of angiosarcoma of the liver (and angiosarcoma at other sites) in exposed mice in a dose-related fashion (Table 5). As with the rats, the tumor response at very high concentrations of VC reached a plateau and then declined. It is presumed that the decrease in tumor incidence is related to the fact that the animals in the highest dose groups showed very poor survival.

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

Two other studies of the effect of VC exposure on development of liver angiosarcoma have been reported. Lee et al. (1978) exposed CD-1 mice to VC for 6 hr/day, 5 days/week for 12 months, at which time the experiment was terminated (no

holding period after exposure). Lee reported combined incidences of hepatic angiosarcoma of 0%, 4.8%, 36.5%, and 44.9% after exposure to 0, 50, 250, and 1000 ppm of VC respectively. When subjected to GLOBAL83 calculations, the RSD associated with a lifetime increase in risk of 1×10^{-4} to mice (based on Lee et al.'s studies) was found to be 0.120 mg equivalents of metabolite per liter of liver per day, intermediate in potency between the RSD previously calculated for rats (0.177 mg equivalents/liter liver/day) and the RSD based on Maltoni's experiments in Swiss albino mice (0.0797 mg equivalents of metabolite per liter of liver per day). Thus these two mouse studies and the rat study gave quite consistent estimates of the RSD for VC metabolites.

However, when a bioassay of VC in B₆C₃F₁ and Swiss CD-1 female mice (and F344 rats and Syrian Golden Hamsters) conducted by the National Toxicology Program (NTP; Drew et al., 1983) was evaluated, quite different results were seen. In these studies, only one exposure concentration was studied (50 ppm, 6 hr/day, 5 days/week), but exposures were begun at different points in the animals life spans and were conducted for different durations (6 months, 12 months, etc.). One of the exposed groups of mice was subjected to an exposure paradigm similar to that employed by Maltoni et al. (1974) in that exposure began when the animals were 9 weeks old, they were exposed for 12 months, and then held until death.

In this group of female B₆C₃F₁ mice VC increased the incidence of angiosarcoma in the NTP study from 5.8% in controls (4/69 animals) to 76.7% (69/90 animals). In contrast, at this exposure concentration and paradigm, Maltoni et al. (1974) and Lee (1978) observed a 2-5% incidence of angiosarcomas in treated mice with no angiosarcomas seen in control animals. Thus the NTP has reported a considerably higher incidence of angiosarcomas in both control and treated animals than either Lee or Maltoni.

The RSD for VC in B₆C₃F₁ mice (calculated in the same manner as the RSD's for Maltoni's and Lee's studies) was 0.0032 mg equivalents of VC metabolites/day/liter of liver. This RSD is significantly lower (25-55 fold, implying more risk associated with a fixed concentration of VC) than the RSD's calculated from the Lee and Maltoni studies.

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

Estimation of Human Risk

Risk estimates for humans occupationally exposed to VC were prepared by the following procedure:

1. The validated PB-PK model for humans was used to construct a table of lifetime average daily doses (LADD) expressed in the same terms as used in the rat model: mg VC metabolites formed/day/liter of liver tissue for conditions thought likely to have been present in the workplace in past years (i.e. TWA's of 50 - 2,000 ppm and employment for 10 - 20 years).

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

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

The results of these estimations are presented in Table 4. The predictions range from about two hundred cases per 100,000 (for workers employed 10 years at a plant where the TWA was 50 ppm) to almost 4,000 cancers per 100,000 in workers employed for 20 years in a plant where TWA's were 2,000 ppm.

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

Simonato et al. (1991) reported a clear association between both duration of employment and ranked level of exposure. They estimated the absolute risk of angiosarcoma in exposed population with >25 years since first employment to be between 6.2 cases/100,000 for exposures less than 2,000 ppm.years to 280 cases/100,000 for individuals with more than 10,000 ppm.years. Relative risks in highly exposed populations were as high as 45.4:1. Absolute risks observed in the VC cohort with >25 years since first employment are listed in Table 4 for comparison with risks predicted by the linearized multistage model (LMS) using maximum likelihood estimates (MLE) from the PB-PK model.

In each case, the estimates from the procedure employing the PB-PK model are substantially higher than actually observed in humans. For example, based on the PB-PK prediction, workers exposed to 200 ppm TWA VC for 20 years (4,000 ppm years) would be expected to develop 1,465 cases of angiosarcoma/100,000. However, the

incidence of angiosarcomas observed in the group with >25 years since first employment and 2,000-5,999 estimated ppm.years was reported by Simonato et al. to be 42.2, which is about 35 fold lower than the PB-PK prediction. At higher levels of human exposure (e.g. 6,000-9,999 ppm.years, > 10,000 ppm.years) the PB-PK predictions are higher than the observed rates by a factor of ten or so (Table 4).

Potency factors derived from the studies of Drew et al. (1983) were also used to predict human risk (data not shown). When these potency factors were used, the discrepancy between predicted risk and observed incidence was much greater. Predictions based on the Drew studies were almost three orders of magnitude (1,000 fold) higher than the actual (observed) incidences of liver angiosarcoma in exposed workers, so these studies are clearly less consistent with human experience than the studies conducted by Maltoni and Lee.

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

DISCUSSION

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

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

Tumor Induction in Rats

The PB-PK model was used to analyze the patterns of tumor induction in rats exposed to a wide range of VC concentrations (1-30,000 ppm) for 4 hr/day, 5 days/week for 12 months by Maltoni et al. (1974). Excluding only those animals where toxicity of VC caused early mortality (10,000 and 30,000 ppm), the risk estimation procedure based on metabolized dose faithfully described the tumor incidence in Maltoni's rats (Figure 5).

Proper consideration of the dose dependency of VC metabolism is essential for the preparation of accurate risk assessments. If a risk assessment were based on the concentration of VC inhaled by the animals (instead of the amount of reactive metabolite formed by the animals) the results would lack consistency. For example, if a risk assessment were prepared from a cancer study which contained only high doses of VC (i.e. doses where metabolic activation was saturated), extrapolation to low doses would significantly underestimate the incidence of tumors in rats (Figure 5). On the other hand, if risk estimations were based doses of VC below the level of metabolic saturation, linear extrapolation to high doses would greatly overestimate the incidence of tumors in rats. As Gehring et al. (1979) pointed out, basing risk estimation on the amounts of VC metabolites produced instead of the exposure concentration produces a consistent estimate of carcinogenic potency across the entire dose range.

Tumor Induction in Mice

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

Mice are generally known to contain higher levels of the cytochrome P450 enzymes than either rats or humans and this is reflected by the higher rates of in vivo metabolism seen in mice versus rats for the halogenated hydrocarbon substrates listed in Table 2 (CH_2Cl_2 , CHCl_3 , VC). Based on this knowledge, mice would be expected to be more sensitive to the tumorigenic effects of exposure to a given concentration of VC than rats, and this has been widely verified (Maltoni et al., 1974; Lee et al., 1978; Drew et al., 1983; ECETOC, 1988).

The PB-PK model for rats was adjusted to predict VC metabolism in mice and validated by an independent experiments. Estimates of the carcinogenic potency of VC in different strains of mice were derived by regressing the metabolize dose in mice he model was used to estimate the carcinogenic potency of VC metabolites in mice (mg equiv/day/liter of liver) against the incidence of liver angiosarcomas in mice. GLOBAL83 was used to estimate the risk specific metabolized dose (RSD) associated

with a lifetime increase of 1×10^{-4} (extra risk) in cancer in mice. The RSD's calculated for the different species/strains of mice were:

Maltoni (rat potency)	0.177
Maltoni (Swiss mouse potency)	0.0797
Lee (CD-1 mice)	0.120
Drew (B ₆ C ₃ F ₁ mice).....	0.0032

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

The reason for the discrepancy in potency factors derived from the Drew et al. study is not clear. One possibility is that diagnostic criteria in this bioassay may have differed from those employed by other investigators, since angiosarcomas of the liver (a rare tumor) was not reported in any of the control mice from other groups but were reported in 2-5% of the control animals at NTP. This possibility could be evaluated by an expert committee of veterinary pathologists with access to slides from the archives of the different organizations.

It is also possible that the relatively high background incidence of liver tumors in the B₆C₃F₁ mouse has made it abnormally sensitive to the influence of liver carcinogens such as VC. This suggests that rodent strains with high background tumor incidences may not be good models to use when estimating human risk (if humans have much lower background incidences). In any case, as will be discussed later, the results from Maltoni's and Lee's groups appear to be much more consistent with the data from humans exposed to VC than the results of Drew et al. (1983).

Comparison of Potency Factors (PB-PK and Conventional)

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

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

The numbers calculated with the PB-PK model may be contrasted with the value reported in HEAST Table 3 (Carcinogenicity of VC, page 3-33). In each case the calculations represent the UCL for excess lifetime risk associated with continuous inhalation of 1 µg/m³ of VC:

HEAST Value (1994)	8.4×10^{-5} risk
PB-PK Based Value	5.7×10^{-7} risk

Thus the value calculated from the PB-PK based approach described here suggests that the potency factor currently listed in HEAST should be reduced approximately 147 fold.

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

Comparison with Human Epidemiology

It was noted above that considerable variation exists in the potency factors available for estimating the incidence of angiosarcomas in human populations exposed to VC. One of the most important questions, therefore, is: "Which of the alternative potency factors gives the most accurate description of the actual human experience?" To answer this question, we have compared the predicted risks to data reported by Simonato et al. (1990). This epidemiology study was chosen because we believe that it represents one of the most robust analyses available, both with regard to the number of individuals followed and the quality and length of follow up procedures employed.

A weakness in this database is the absence of a precise measure of the magnitude of VC exposures in the workplace. However, Simonato et al. (1991) have attempted to characterize the magnitude of occupational exposure by subdividing workers into groups based on their ppm.years of exposure (calculated as years on the job times TWA

ppm levels estimated to be present in the occupational setting during hours of work = ppm.years).

Simonato et al. had 24 cases of liver cancer in their cohort. The overall incidence of liver cancer was statistically different than expected, and the odds ratios as high as 45:1 were observed in some of the groups with the longest duration of exposure and highest exposure concentrations (Table 9 in Simonato et al., 1991). For the purpose of this comparison, we selected a subgroup of workers with more than 25 years since first exposure, subdivided by Simonato et al. into four exposure categories: (1) < 2,000 ppm.years, (2) 2,000-5,999 ppm.years, (3) 6,000-9,999 ppm.years, and (4) >10,000 ppm years. Although this exposure information is obviously imprecise, it allowed the calculation of a roughly equivalent exposure paradigm in Table 4 so that we could predict the approximate tumor incidence in the groups studied by Simonato to compare with the results he reported.

For example, in the subgroup estimated to have the lowest exposures by Simonato (0-2,000 ppm.years), the "reported" incidence of angiosarcoma was 6.2 per 100,000. In contrast, the risk assessment procedure described in this manuscript gave a maximum likelihood estimate (MLE) of between 188 and 736 cases per 100,000 for ppm.years between 500 and 2,000 (Table 4). Thus the PB-PK model predicted almost two orders of magnitude more cancer cases than actually occurred.

Similarly, individuals with 2,000-5,999 ppm years had a "reported" incidence of 42.2 cases per 100,000, while the PB-PK based procedure estimated the incidence in this group to be from 700 to 1,500 cases per 100,000. A similar disparity existed for the two most highly exposed groups from Simonato et al. (153-280 cases per 100,000 versus 1,500 to 4,000 cases per 100,000 predicted by the PB-PK based extrapolation. It is noteworthy that in the higher exposure group, the degree of overprediction by the PB-PK procedure seems to decrease (from almost two orders of magnitude overprediction to approximately one order of magnitude; Table 4). It also appears that the degree of overprediction (excess conservatism) is greatest at the lowest rates of VC exposure (below 6,000 ppm.years in Table 4).

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

Consequently, it appears that risk assessments based on estimates of the amounts of reactive metabolites of VC delivered to the liver of the target species (calculated with a PB-PK model) still significantly overestimate the potential of VC metabolites to induce liver cancer in humans. Since the PB-PK has been well validated in several species, we do not believe that this is because the PB-PK model has overpredicted the formation of

VC metabolites in human liver. Rather, it appears that the livers of humans less sensitive to the carcinogenic effect of reactive VC metabolites the livers of the commonly used inbred laboratory rodents.

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

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

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

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FIGURE LEGENDS

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

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

Figure 3: Predicted (solid line) and observed (open symbols) concentrations of vinyl chloride in a 9.1 liter recirculating exposure chamber containing 14 male mice (Figure 3a) or 14 female mice (Figure 3b) with values of V_{MaxC} and K_m calculated from in vitro studies ($V_{\text{MaxC}} = 9.04$, $K_m = 0.04$). Figure 3c shows the same data for 14 male mice after computer optimization of V_{MaxC} and K_m ($V_{\text{MaxC}} = 8.13$, $K_m = 0.28$).

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

Figure 5: Predicted (solid line) and observed (open symbols) incidences of liver angiosarcoma in rats following exposure to various concentrations of vinyl chloride for 4 hr/day, 5 days/week, for 12 months (animals were held until death for observation of tumor incidence). Data are taken from Maltoni et al., (1974b).

FOOTNOTES

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 - 2 To whom reprint requests should be addressed.
 - 3 U. S. Environmental Protection Agency, Res. Tri. Park, NC.
 - 4 Zeneca Central Toxicology Laboratory, Macclesfield, ENGLAND
 - 5 SimuSolv is a registered trademark of the Dow Chemical Co.
 - 6 To receive a copy of the source code, please send a self-addressed stamped envelope. If a copy on magnetic media is desired, please include a formatted 3.5" DOS diskette with your request.

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Table 1

Parameters used in the physiologically based pharmacokinetic model for vinyl chloride for Humans, Rats, and Mice. Alveolar ventilation and cardiac output are calculated from the allometric constants by multiplying the constant by the body weight (kg) of the animal raised to the 0.74 power. VMax is calculated from the allometric constant VMaxC by multiplying the constant by body weight raised to the 0.70 power.

	HUMAN	RAT	MOUSE
<u>WEIGHTS (% of Body Weight)</u>			
Liver	3.14%	2.53%	5.86%
Rapidly Perf.	3.71%	5.0%	5.0%
Slowly Perf.	62.1%	76.47%	76.14%
Fat	23.1%	7.0%	4.0%
<u>FLOWS (Allometric Constants)</u>			
Alveolar Ventilation	15	18	28
Cardiac Output	15	18	28
<u>% of Cardiac Output</u>			
Liver	24.0%	24.0%	24.0%
Rapidly Perfused	52.0%	52.0%	52.0%
Slowly Perfused	19.0%	19.0%	19.0%
Fat	5.0%	5.0%	5.0%
<u>PARTITION COEFFICIENTS</u>			
Blood/Air	1.16	1.68	2.41
Liver/Air	1.60	1.60	1.60
Rapidly Perfused/Air	1.60	1.60	1.60
Slowly Perfused/Air	2.10	2.10	2.10
Fat/Air	20.0	20.0	20.0
<u>METABOLIC CONSTANTS</u>			
VmaxC (Allometric)	3.97	2.75	8.13
Km (mg/liter)	0.04	0.04	0.28

Table 2

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

	Rat	Mouse	Human
Methylene Chloride:			
Body Wt (kg)	0.233	0.0275 ^a	83.0
Percent Liver	2.53%	5.86%	3.14%
Liver Wt (g)	5.895	1.612	2606
VMax (mg/hr)	1.500	1.054	138
Ratio to Rat	1.000	2.570	0.208
Chloroform:			
Body Wt (kg)	0.230	0.0285	-
Percent Liver	2.53%	5.86%	-
Liver Wt (g)	5.819	1.670	-
VMax (mg/hr)	2.431	1.889	-
Ratio to Rat	1.000	2.707	-
Vinyl Chloride:			
Body Wt (kg)	0.225	0.0285	70.0
Percent Liver	2.53%	5.86%	3.14%
Liver Wt (g)	5.69	1.67	2198
Ratio to Rat (Ave)	1.000	2.639	0.208
VMax (mg/hr)	0.968	1.889 ^b	77.7 ^b
VMaxC (allometric)	2.75	9.04	3.97

^a Andersen et al. (1987) listed 34.5 g as the body weight for mice in their Table 1 since this was the body weight for the mice in the NTP bioassay of methylene chloride and their PB-PK model was used for risk assessment. However, the average body weight of the mice used in the gas uptake studies was actually 27.5 grams and this body weight is used to calculate the VMax/VL ratio.

^b Calculated by multiplying the average mouse or human RATIO of VMax/VL 's to rat (methylene chloride and chloroform), the in vivo VMax/VL value for VC in rats, and the VL for humans.

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Table 3

Incidence of angiosarcomas of the liver observed in Sprague Dawley rats. Data are from Experiments BT1, BT2, BT9, & BT15 conducted by Caseare Maltoni (1974a,b). Data are given as # angiosarcomas/# animals examined for males, females, and combined males and females. Control animals from several experiments are combined in the 0 ppm group. Animals were exposed 4 hr/day, 5 days/week for 52 weeks and then held until they died (typically at least another year). Tumors were scored at the time of death.

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

¹ Eliminated from GLOBAL83 analysis because of mathematical limitations of the PC version of the computer fitting program (only 10 dose/response groups allowed).

Table 4

Lifetime Average Delivered Doses (LADD's) of VC metabolites in humans exposed to VC for 5 days/week, 50 weeks/year, for the indicated numbers of years. The LADD's are calculated from the PB-PK model for humans constructed as outlined in Methods, correcting for the fraction of a year exposed (50/52) and the fraction of a lifetime exposed (10, 20, or 30/70). Estimated lifetime risks (incidences/100,000) predicted for these exposures based on the rat potency factor are obtained from the GLOBAL83 computer program (specifying the Maximum Likelihood Estimate, NOT the 95% upper confidence limit). Observed cases per 100,000 at different levels of exposure (cumulative ppm.years) for the group having more than 25 years since first employment are obtained from Table 10 of Simonato et al., (1991).

PPM	Years Exposed	PPM Years	PB-PK LADD	PB-PK Prediction per 100,000	Observed Cases per 100,000
Ten Years Exposure					
50	10	500	3.33	188	---
100	10	1,000	6.63	374	(6.2) ^a
200	10	2,000	13.06	736	---
---	---	4,000	---	---	42.2
500	10	5,000	26.68	1,497	---
---	---	8,000	---	---	152.3
1000	10	10,000	31.28	1,753	---
---	---	>10,000	---	---	(280.0) ^b
2000	10	20,000	36.03	2,532	---
Twenty Years Exposure					
50	20	1,000	6.66	376	(6.2) ^a
100	20	2,000	13.26	747	---
200	20	4,000	26.11	1,465	42.2
---	---	8,000	---	---	152.3
500	20	10,000	53.35	2,971	---
---	---	15,000	---	---	(280.0) ^b
1000	20	20,000	62.57	3,476	---
2000	20	40,000	72.07	3,993	---

^a Group listed as having <2,000 cumulative ppm years by Simonato et al., entered at 1,000 ppm.years for comparison.

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

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Table 5

Incidence of angiosarcomas of the liver observed in Swiss albino mice. Data are from Experiment BT 4 conducted by Casare Maltoni (1974a,b) and summarized in ECETOC, 1988. Data are given as # angiosarcomas/# animals examined for males, females, and combined males and females. Animals were exposed 4 hr/day, 5 days/week for 30 weeks and then held until they reached 81 weeks of age. To calculate the dose surrogates for mice, the PB-PK model was configured according to Table 1 and a 4 hr exposure with 20 hours exposure free was simulated by the model. The simulated values of the dose surrogate were converted to lifetime average daily doses by multiplying by 5/7 (days/week) and 30/104 (fraction of lifetime exposed). Potency values were estimated with GLOBAL83 as described in the Methods section.

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

^a Eliminated from dose response regression because of the likelihood of poor survival at this dose.

Figure 1a

DLAF

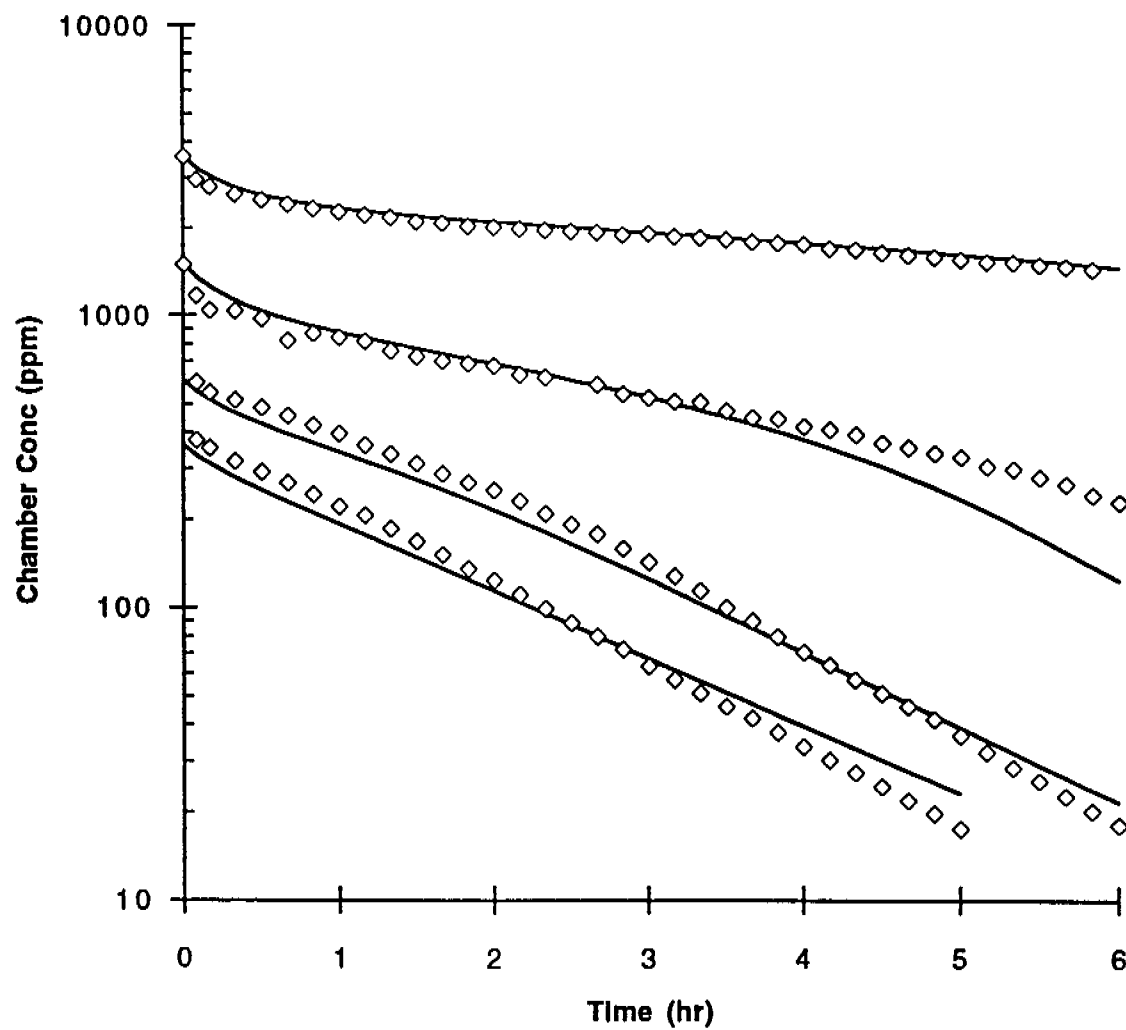


Figure 1b

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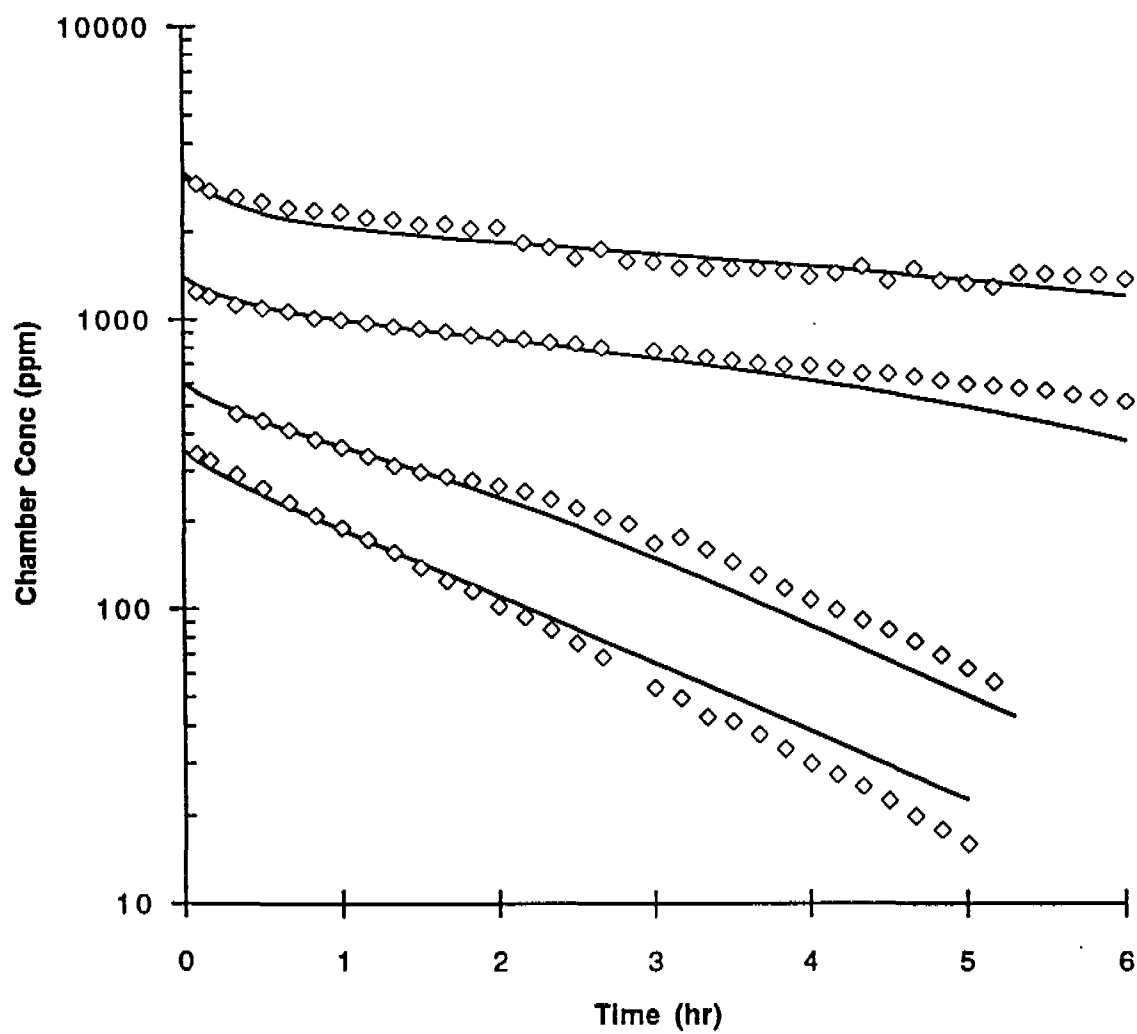
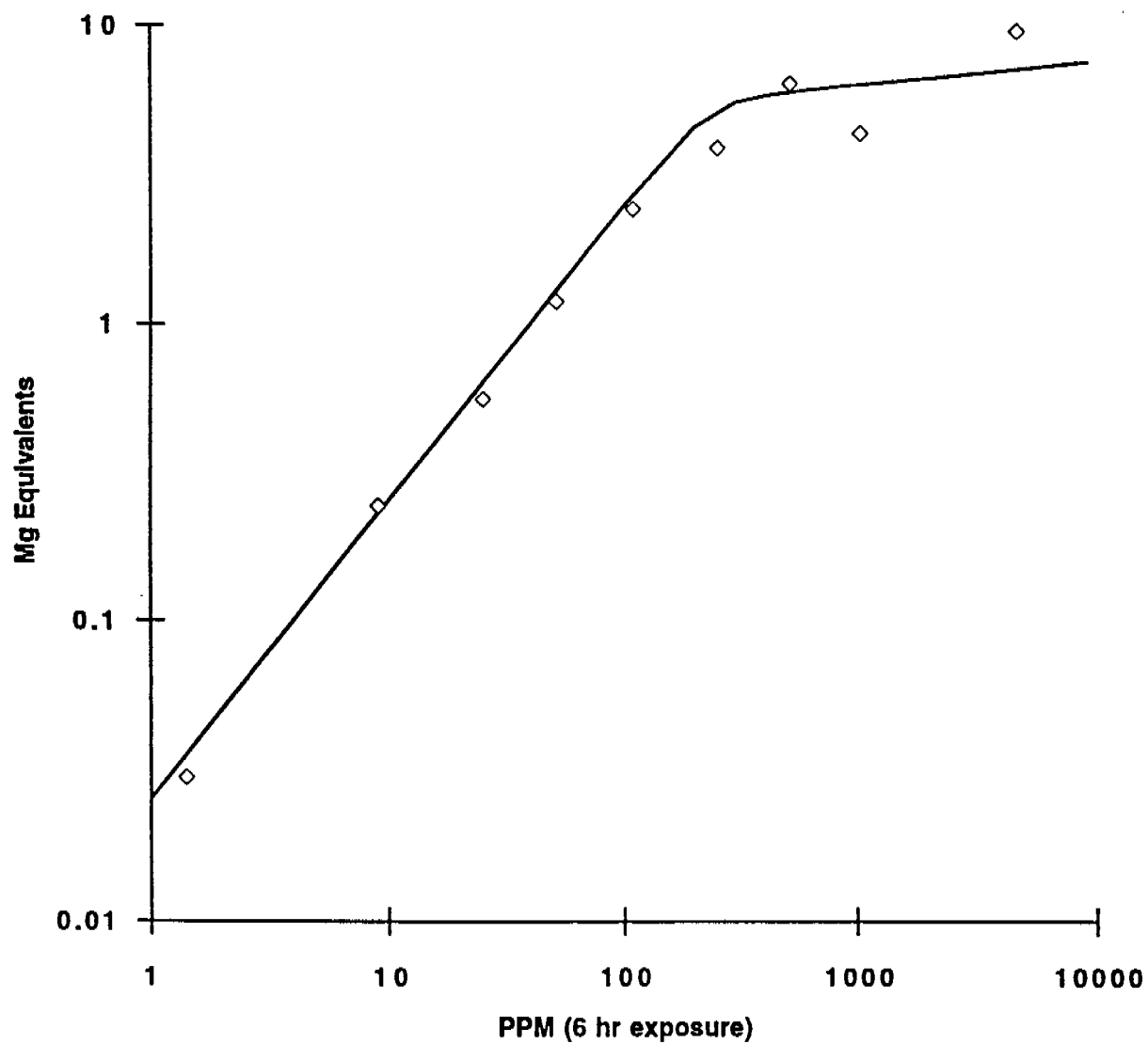


Figure 2

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Fig 3a

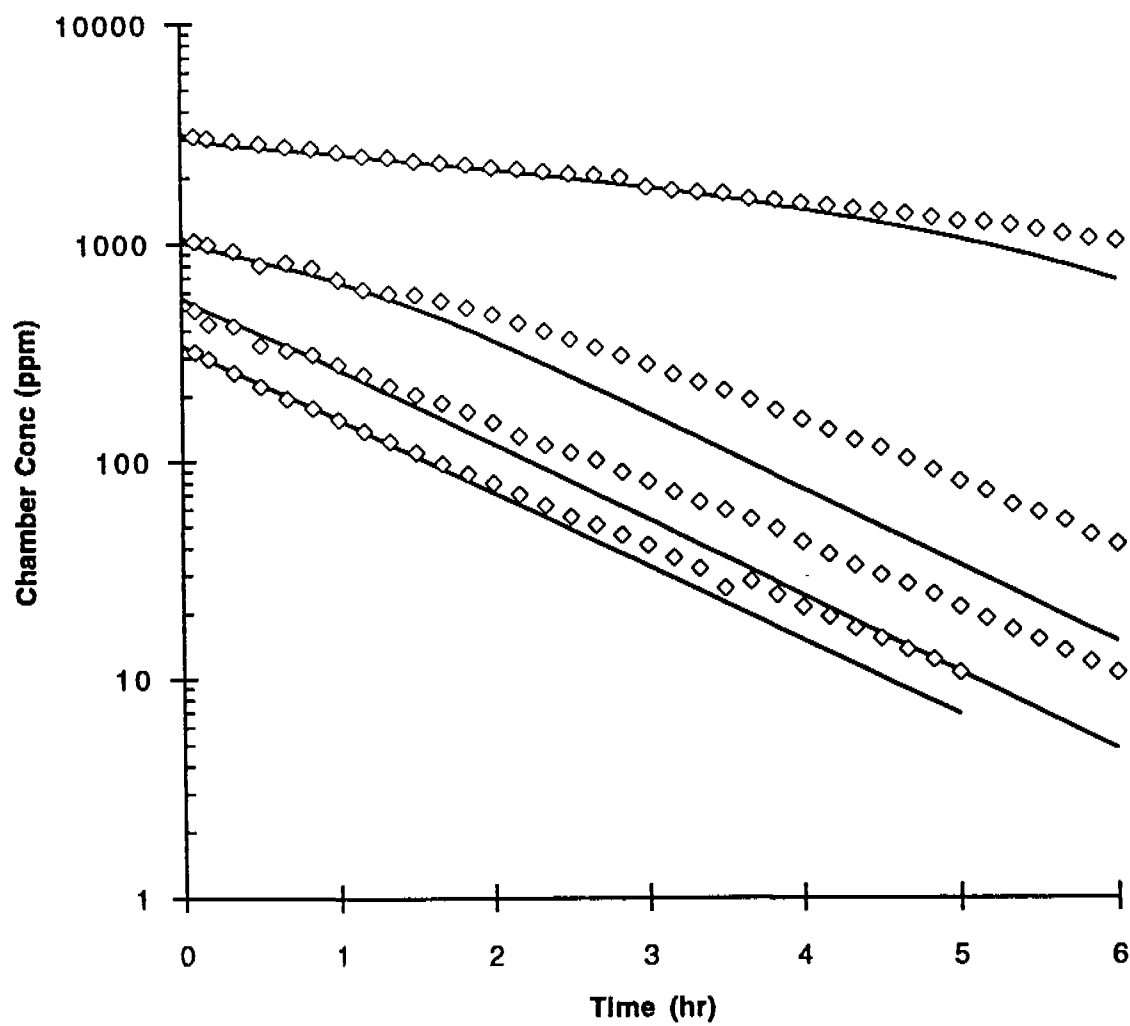


Fig 3b

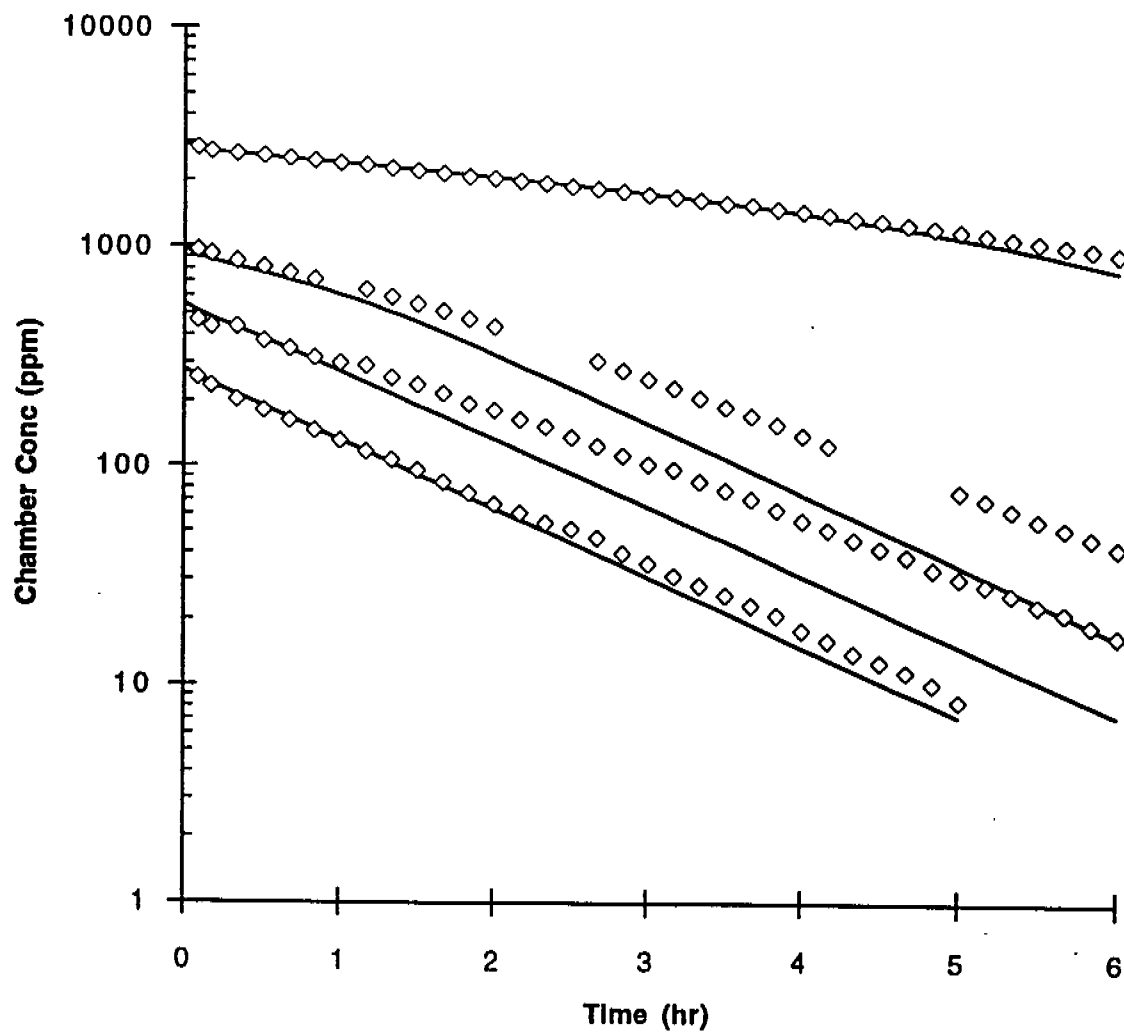
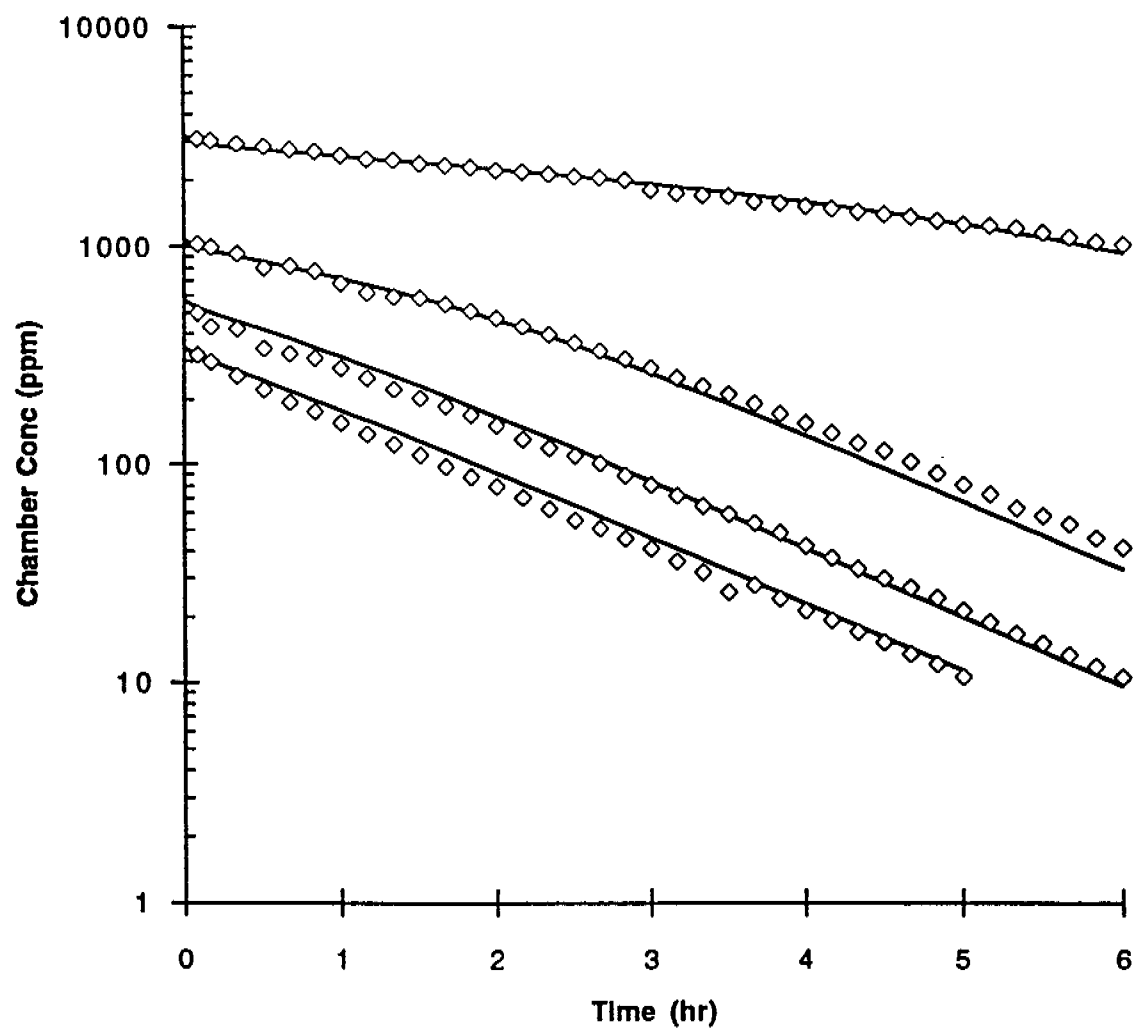


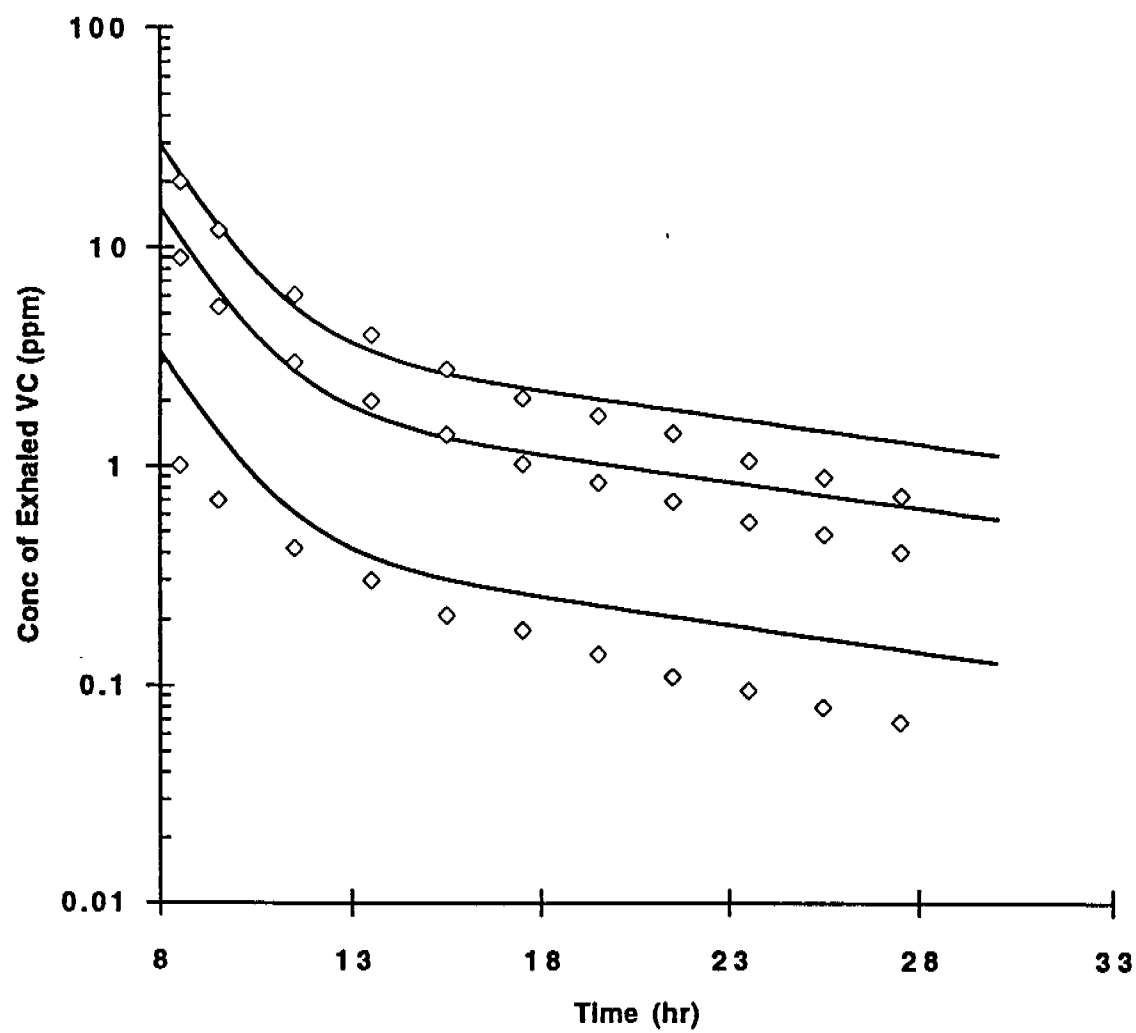
Fig 3c

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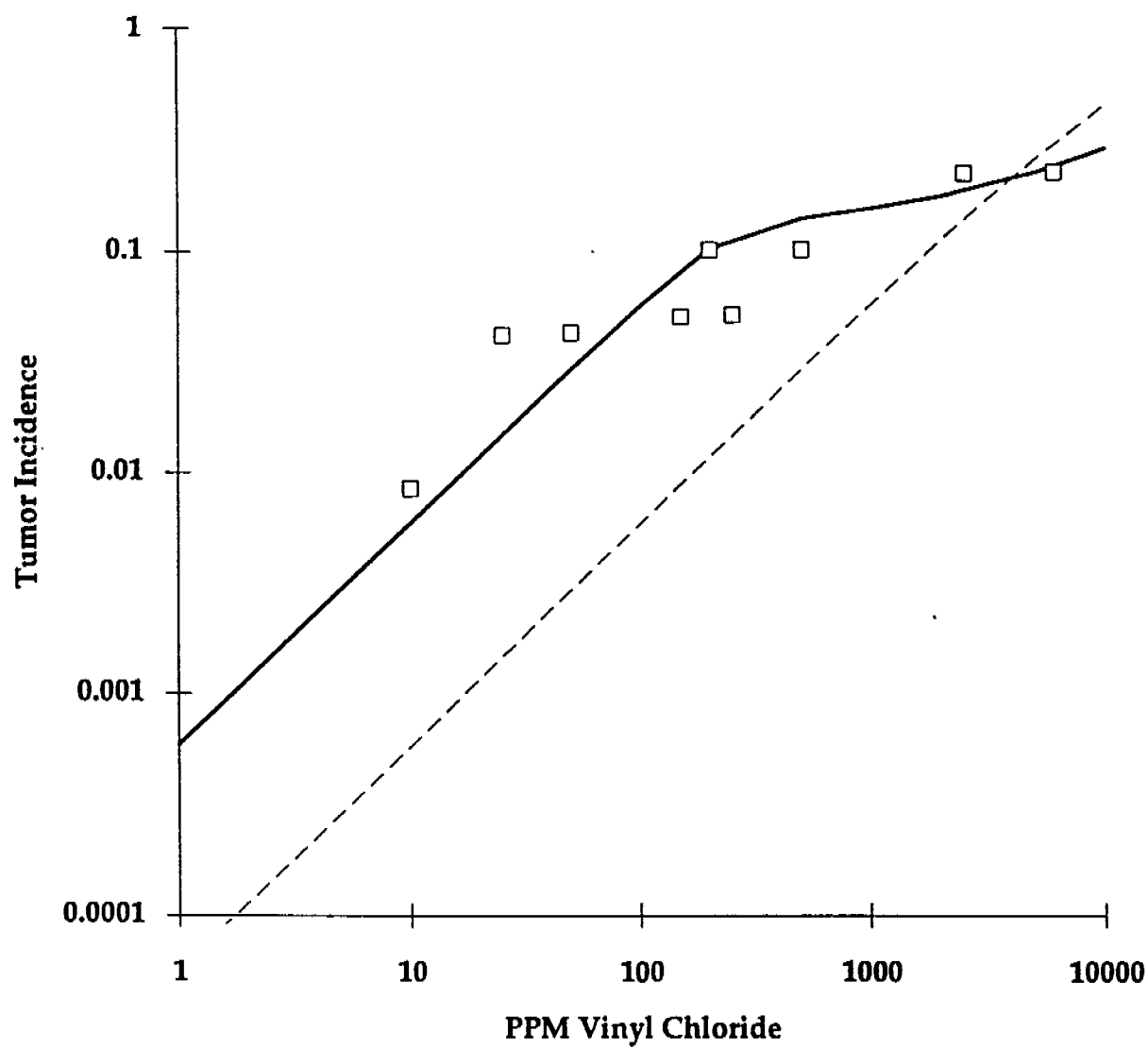
DATA

Figure 4



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Fig 5



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