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19. ABSTRACT (Continue on reverse if necessary and identify by block number) In pursuit of the goal of establishing a scientific basis for the interspecies extrapolation of pharmacokinetic data in health risk assessments, a series of studies have been conducted involving pharmacokinetic determinations in rats to several aliphatic halocarbons (with parallel studies initiated in the dog). Direct measurements of the uptake and elimination of halocarbon in rats have been completed during and following inhalation exposures and following oral administration of dichloroethylene (DCE) and perchloroethylene (PCE). One paper has been published on trichloroethane (TRI) pharmacokinetics in a peer-reviewed journal, and two additional manuscripts on trichloroethylene (TCE) and DCE have been composed. An assay for the measurement of halocarbons in the tissues of exposed animals has been successfully developed, and tissue concentration profiles in the liver, kidney, lung, fat, brain, muscle, and heart have been completed for oral and intraarterial administrations of PCE. The utility of the physiologically-based pharmacokinetic model for the accurate computer simulation of the pharmacokinetics of three halocarbons with wide variation in physicochemical properties (TRI, TCE, and PCE) has been demonstrated.				
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VALIDATION AND APPLICATION OF PHARMACOKINETIC MODELS FOR INTERSPECIES
EXTRAPOLATIONS IN TOXICITY RISK ASSESSMENTS OF VOLATILE ORGANICS

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I. OVERALL OBJECTIVE AND STATEMENT OF WORK

The overall objective of the proposed project is to investigate the scientific basis for interspecies extrapolation of pharmacokinetic and neurobehavioral toxicity data. Direct measurements of blood and tissue concentrations of halocarbons over time in two species will be used to formulate and validate physiologically-based pharmacokinetic models for inhalation and oral exposure. These models will be used for: (a) prediction of the time-course of blood and target organ levels in the absence of data; (b) interspecies extrapolations (i.e. scale-up from smaller to larger laboratory animals and ultimately to man). A combined physiological pharmacokinetic-toxicodynamic model for inhalation exposure to halocarbons will also be developed and evaluated for its ability to predict neurobehavioral effects under specified exposure conditions.

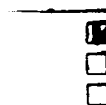
A series of experiments will be conducted to provide a pharmacokinetic data base of interspecies comparisons and for formulation of physiologically-based pharmacokinetic models. Adult male Sprague-Dawley rats and male beagle dogs will be administered equal doses/concentrations of selected halocarbons. Ingestion, inhalation and intravenous injection will be employed as routes of administration. concentrations of the parent compounds will be monitored in the blood (and in some cases in the exhaled breath) for appropriate periods during and after exposures. The cumulative uptake from exposure to each chemical will be determined. Relative rates and magnitude of elimination of the test chemicals by metabolism and respiration will be evaluated. For investigating the relative role of metabolism on the observed pharmacokinetics of volatile organic compounds, trichloroethylene (TCE), dichloroethylene (DCE), and trichloroethane (TRI) will be employed. Tetrachloroethylene (PER) and 1,1,2,2-tetrachloroethane (TET) will be employed for analyzing the role of pulmonary extraction/elimination. At least 2 doses and 2 vapor concentrations of each pair of test compounds will be utilized.

In order to determine the tissue disposition of halocarbons in two species, rats and dogs will receive equivalent exposures to halocarbons intravenously, orally and by inhalation. Concentrations of the parent compound in brain, liver, kidney, heart, lung, skeletal muscle and adipose tissue will be measured at selected intervals over time, in order to provide an assessment of the actual target organ dose for validation of physiologically-based model development and inter-species correlations with toxicity. A second series of tissue disposition experiments will be conducted to determine what adjustments in administered dose are necessary to achieve equal brain levels of test compounds in each species. An inhalation and oral exposure concentration will be administered to the rat that yields a brain level of halocarbon similar to that seen in the previous experiments in the dog.

Physiologically-based pharmacokinetic models will be developed and validated for oral and inhalation exposures to halocarbons, which will allow accurate prediction of the concentration of halocarbons in blood and tissues over time during and following exposure. Data from the direct measurements of blood and tissue concentrations will be compared



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to simulated values calculated from mass-balance differential equations comprising the model. Thereby, the accuracy of the model can be tested and adjustments made where necessary to improve the model simulations. Models for inhalation and oral exposures validated using pharmacokinetic data in rats will be employed to predict blood and tissue halocarbon concentrations in the dog. The accuracy of the model for this interspecies extrapolation will be assessed by comparing the predicted concentrations to values determined experimentally for the dog.

The neurobehavioral toxicity of inhaled solvents will be correlated with the target organ concentration in the two species. Rats and dogs will be exposed to selected halocarbons at defined inhaled concentrations and lengths of exposure. Two neurobehavioral tests, one for operant performance and one for acute motor performance, will be performed periodically during and/or after exposures. The magnitude of CNS effects of each solvent will be correlated with the target organ (i.e. brain) concentration, as determined in the tissue uptake studies, at each time-point. In this manner, the feasibility of toxicity extrapolations between species based on a common target organ dose will be evaluated.

Toxicodynamic models will then be developed and validated for inhaled halocarbons. To develop this model, rat brain halocarbon concentrations will be correlated with the magnitude of neurobehavioral effects in an appropriate equation and the validity of the equation tested by comparison with actual experimental values obtained from previous stages of the project. By incorporating predicted brain concentrations from the previously validated physiologically-based pharmacokinetic model, a combined physiological pharmacokinetic-toxicodynamic model can be developed. This combined model may allow the prediction of toxicity from the interspecies extrapolation of pharmacokinetic data, and in simulations in the absence of experimental data.

II. EVALUATION OF THE RELATIVE ROLE OF PROPENSITY FOR RESPIRATORY ELIMINATION ON THE PHARMACOKINETICS OF INHALED HALOCARBONS

In the first year of this research effort (as recorded in last year's annual report), a series of studies were conducted to evaluate the relative role of hepatic metabolism on the subsequent pharmacokinetics of inhaled halocarbons. Extensively metabolized trichloroethylene (TCE) and dichloroethylene (DCE) and poorly metabolized 1,1,1-trichloroethane (TRI) were used. While TCE and TRI are very similar structurally (differing only in a single double bond), differences in both volatility and metabolism were reflected in the resulting uptake and elimination of the test chemicals. It was important to eliminate the propensity for volatility as a factor in this focus on the impact of metabolism. DCE and TRI are of comparable volatility so they were expected to be eliminated similarly by the lung. The reported differences in pharmacokinetics were therefore more likely to be attributed to differences in metabolism. In this second year of the research effort supported by AFOSR, this factor of the propensity for respiratory elimination (due to the characteristic volatility of specific halocarbons) was investigated.

As a class of chemicals, halocarbons have low solubility in blood and high volatility (i.e. low blood:air partition coefficients), as well as rapid vascular-alveolar transfer. Thus, a substantial proportion of the blood's burden of halocarbons should be removed each pass through the lungs. It follows that halocarbons with low blood:air partition coefficients should be more efficiently eliminated (and have a less prolonged CNS depressant action) than halocarbons with relatively high partition coefficients. In order to test this premise, tetrachloroethylene (PCE) and 1,1,2,2-tetrachloroethane (TET) will be utilized. Both are poorly metabolized (Ikeda and Ohtsuji, 1972) and have similar oil:blood (i.e. fat/blood) partition coefficients, but PCE has a much lower blood:air partition coefficient (Sato and Nakajima, 1979). In this way, the factors of lipophilicity and propensity for hepatic metabolism are accounted for. Therefore, differences in the pharmacokinetics between PCE and TET in this experimental design are more likely to be attributed to differences in respiratory elimination.

For these inhalation exposures, the halocarbon was administered to unanesthetized male Sprague-Dawley rats previously prepared with an indwelling carotid artery cannula. These rats, weighing 325-375 g, inhaled the compound for 2 hr through a one-way breathing valve in an inhalation exposure system previously developed by this laboratory (see schematic for the inhalation exposure system in Fig. 1, Appendix C). Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently and analyzed for the test compound. Respiratory rates and volumes were continuously monitored during and following exposure, and were used in conjunction with the pharmacokinetic data to characterize profiles of uptake and elimination. This experimental protocol has provided a unique approach in this present study and in last year's experiments by combining direct measurements of the halocarbons PCE, TCE, TRI, and DCE in the exhaled breath and blood simultaneously with detailed measurements of respiration. The separation of the inhaled and exhaled breath streams by use of the one-way breathing valve afforded both sampling of the exhaled breath for halocarbon during and following exposure and measurement of the air flow in the breath stream. Emphasis on the pharmacokinetic measurements of these halocarbons in previous studies has focused primarily on measurements following the termination of exposure. Also, parameters of respiration were not monitored in these experiments. Accurate determination of the total amount of chemical absorbed or eliminated by inhalation requires monitoring of respiratory parameters. In the present study, measurement of halocarbon uptake was accomplished by calculation from either the blood level data or the exhaled breath data in conjunction with the monitored respiratory parameters.

III. STUDIES OF THE PHARMACOKINETICS OF PCE DURING AND FOLLOWING INHALATION EXPOSURE IN RATS

Toward this goal of investigating the relative role of the characteristic volatility of a halocarbon on its pharmacokinetics, the uptake, disposition, and elimination of perchloroethylene (tetrachloroethylene, or PCE) has been completed in the rat. The results of this study, together with the PBPK model simulations for inhaled PCE (described in Section VI), were presented at the most recent meeting of the Society of Toxicology in Atlanta, Georgia in March, 1989. A manuscript from this work is also in preparation for submission to a peer-reviewed journal this fall. The reference for this study (also listed in Section F) as presented in the abstract is as follows:

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., Manning, R.O., and Bruckner, J.V. "Direct measurements of perchloroethylene in the blood and exhaled breath of rats during and following inhalation exposure". 28th Annual Meeting of the Society of Toxicology, Atlanta, GA; Toxicologist 9: 78 (1989).

Specific objectives of this study were to: 1.) provide accurate direct measurements of the respiratory uptake and elimination of PCE during and following inhalation exposures by simultaneously measuring PCE in the blood and exhaled breath; 2.) determine the total dose of PCE absorbed systematically (cumulative uptake) during 2-hour inhalation exposures using inhaled and exhaled breath determinations and the monitored volumes of respiration; 3.) assess the effect of a 10-fold difference in exposure concentration (50 and 500 ppm) on PCE uptake and elimination from the blood, total cumulative uptake, and elimination in the breath; 4.) validate a physiologically-based pharmacokinetic (PBPK) model for PCE inhalation by comparing computer simulations of PCE uptake and elimination with experimentally observed values (see Section VI). Fifty or 500 ppm PCE was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 325-375 g (as described in detail in Section II). Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected during and following per inhalation and analyzed by gas chromatography.

In order to calculate the total received dose of PCE during inhalation exposures, the respiration of each animal was continuously monitored. The respiratory monitoring technique was conducted according to the methods previously published in solvent exposure studies by this laboratory (Dallas et al., 1983, 1986 and 1989). The airflow created by the animal's inspiration was recorded both during and following PCE inhalation exposure in terms of minute volume (volume of respiration per minute, or V_E), respiratory rate (f), and tidal volume (V_T). An average value for these parameters for each individual animal was obtained by averaging the measurements taken at 15-min intervals during the 2-hr exposure. The mean $\pm$ SD of these average values for the 500 ppm exposure group ($n=6$) were: $V_E = 189 \pm 21.5$; $f = 119.1 \pm 22.4$; $V_T = 1.62 \pm 0.34$. The mean $\pm$ SD for these average values for the 50 ppm exposure group ($n=6$) were: $V_E = 216 \pm 43.1$; $f = 134.5 \pm 14.9$; $V_T = 1.67 \pm 0.36$.

Significant respiratory elimination of unchanged PCE was evident during the inhalation exposure period, with near steady-state PCE levels achieved in the exhaled breath within 20-30 min. These near-steady state concentrations were approximately 2.1-2.4 $\mu\text{g/ml}$ in the exhaled breath of the 500 ppm exposed rats (Fig. 2, Appendix C, or Fig. C-2). In the 50 ppm inhalation exposure group, these exhaled breath levels at near-steady state were in the range of 0.20-0.22 $\mu\text{g/ml}$ (Fig. C-3). PCE was readily absorbed from the lung, in that substantial levels of PCE were present in the arterial blood at the initial sampling time (2 min). Unlike the exhaled breath data, the concentration of PCE in the blood progressively increased over the course of the 2-hr exposure in both exposure groups. The rate of increase was greater in the 500 ppm (Fig. C-4,) than in the 50 ppm group (Fig. C-5). Arterial PCE concentrations were not proportional to the inhaled concentration. After the initial rapid uptake phase over the first 30 minutes of exposure, blood levels in the 500 ppm rats were 12 to 17 times higher than 50 ppm rats. Measurement of the cumulative uptake of PCE by the rats was made by accounting for the quantity of unchanged PCE that was exhaled during the inhalation exposure period. The total cumulative uptake of PCE from the 2-hr exposure to 500 ppm (Fig. C-6) was $28.1 \pm 4.3 \text{ mg}$ ($x \pm \text{SD}$), or 79.9 mg/kg bw. The 2-hr exposure to 50 ppm PCE (Fig. C-7) resulted in a cumulative uptake of $3.9 \pm 0.9 \text{ mg}$ ($x \pm \text{SD}$), or 11.2 mg/kg bw.

IV. STUDIES OF THE PHARMACOKINETICS OF PCE FOLLOWING ORAL ADMINISTRATION IN RATS

Due to the potential for halocarbon exposures in humans to occur due to the ingestion of contaminated drinking water supplies, oral administrations of PCE in rats have also been conducted. Emissions from product manufacturing, usage activities, and spills are thought to be primary sources of halocarbon contamination of water supplies. Recently, the contamination of drinking water supplies by the leakage of solvents from storage tanks and chemical waste dumps has become of significant concern. There are large numbers of solvent and fuel storage tanks in the nation, many at U.S. Air force facilities. As the majority of these are located underground, leakage of solvents into groundwater supplies can proceed undetected for years. Despite the potential public health significance of halocarbon ingestion from contaminated drinking water supplies, there is presently insufficient information available concerning the systemic absorption and disposition of these and other halocarbons following their oral administration.

One of the primary objectives of this study was to examine the uptake, disposition, and elimination of ingested PCE over a wide range of concentrations. The transition from linear to non-linear kinetics is important in dose-response relationships and in toxic responses. Certain physiological and biochemical processes are linear over a wide range of substrate concentrations. Diffusion of lipophilic halocarbons across cellular membranes is a pertinent example of such a process. Other processes including solubility in the blood and microsomal enzyme metabolism are saturable. When the dose of halocarbon exceeds the capacity of these processes, there is a transition from linear to nonlinear pharmacokinetics. Under such conditions, there is no longer a

linear relationship between administered dose-exposure level and blood/target organ concentrations. Disproportionately high blood/target organ concentrations and accentuated toxicity are characteristic of nonlinear kinetics.

An investigation of the role of dose level on the pharmacokinetics of ingested PCE has been completed in rats. The results of this study were presented as part of a presentation at the Society of Toxicology meeting in March, 1989 in Atlanta, GA. The reference for this study (also listed in Section F of the Appendix) as presented in the abstract is as follows:

Ramanathan, R., Muralidhara, S., Dallas, C.E., Gallo, J.M. and Bruckner, J.V. "Influence of the pattern of ingestion on the pharmacokinetics of perchloroethylene (PER) in rats". 28th Annual Meeting of the Society of Toxicology, Atlanta, GA; Toxicologist 9: 78 (1989).

In order to procure repetitive blood samples following administration of single oral bolus doses of halocarbons to unanesthetized rats, an indwelling arterial cannula was surgically implanted prior to the halocarbon exposure. The cannula was tunneled subcutaneously to the back of the animal and existed just behind the head. The cannula was extruded through a steel spring that was attached to the back of the animal by a harness. After the surgery was complete, the animal was placed into a metabolism cage to recover for 24 hours before halocarbon dosing. PCE was given orally to unanesthetized male Sprague-Dawley rats in an aqueous Emulphor emulsion as a single bolus in doses of 10, 25, 50, and 100 mg/kg. Blood samples were collected from an indwelling carotid arterial cannula for up to 12 hours post administration. The blood samples were analyzed for PCE using a GC-ECD head space technique.

Absorption of PCE from the gut was very rapid, with peak blood levels achieved within 20 minutes of the oral administration for all four dose groups (Fig. C-8). Elimination of the halocarbon in the blood proceeded at a similar rate at each dose level, with the elimination curves roughly parallel up to 12 hours following the oral bolus. When considering all four dose groups, the maximum blood level achieved after the oral bolus (C_{max}) was not directly proportional to the dose level. Indeed, the 100 mg/kg dose group demonstrated an average C_{max} that was only slightly higher than the mean value for the 50 mg/kg dose group. The C_{max} levels for the 10 and 25 mg/kg groups, however, appeared to be somewhat more proportional to the dose level. A comparison of the area-under-the-blood-concentration-time-curve (AUC) between the four dose groups (Fig. C-9) reveals a linear relationship in AUC relative to dosage level between 10 and 100 mg/kg PCE. Fitting an equation for a regression analysis between these four points resulted in an R-square of 0.998, which would be a significantly good fit for a linear equation.

V. COMPLETION OF THE PHARMACOKINETIC STUDIES OF INHALED AND INGESTED 1,1-DICHLOROETHYLENE (DCE)

Pharmacokinetic studies of DCE that were initiated during the first year of this project have been completed in the course of the second project year. All of the results have been compiled in a manuscript that is to be submitted soon to a peer-reviewed toxicology journal. The manuscript is included as Section B of the Appendix (and listed in Section F), and the reference is as follows:

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V., "Comparative pharmacokinetics of inhaled and ingested 1,1-dichloroethylene in rats". (to be submitted to Toxicology and Applied Pharmacology, 1989).

In this study, the uptake, disposition, and elimination of DCE were determined during and following 100 and 300 ppm inhalation exposures for 2 hours. Oral exposures to DCE were investigated utilizing 10 and 30 mg/kg doses administered as either a) a single oral bolus; b) a gastric infusion for 2 hours; or c) three equally divided bolus doses over a 2 hour period. Significant respiratory elimination of unchanged DCE was evident during the inhalation exposure period, with steady-state DCE levels achieved in the exhaled breath within 20 min at both dose levels. DCE respiratory elimination was proportional to the inhaled concentration during exposure. As a result of the 2-hr exposure to 100 ppm DCE the cumulative uptake was 3.3 ± 0.3 mg ($\bar{x} \pm \text{SE}$), or 10 mg/kg bw. The total cumulative uptake of DCE from the 2-hr exposure to 300 ppm was 10.2 ± 0.6 mg ($\bar{x} \pm \text{SE}$), or 30 mg/kg bw. Percent uptake of DCE during inhalation exposure was similar in magnitude at both exposure concentrations. The magnitude of pulmonary elimination was proportional to the inhalation exposure concentration.

Arterial DCE concentrations, however, were not proportional to the inhalation concentration. After the initial rapid uptake phase over the first 20 minutes of exposure, blood levels for the 300 ppm-exposed rats were 4 to 5 times higher than DCE blood concentrations of rats that received 100 ppm exposures. The maximum blood levels achieved during single oral bolus or gastric infusion administration were also not proportional to the administered dose. As with the inhalation exposures, these blood values for the high dose group were at least 4 to 5 times higher than for the low dose group for both oral administrative routes. The C_{max} achieved for the multiple bolus administration of 30 mg/kg, however, was nearly 9 times more than that achieved following 10 mg/kg.

The AUC values for the single bolus and gastric infusion groups were similar at both dose levels (nearly identical for the high dose). These oral administration values were only 60-80% of the corresponding inhalation AUCs. The bioavailability (F) of DCE was determined by the ratio of the AUC value of each experimental group to the corresponding dose administered by intravenous administration. The high dose groups consistently had a higher F than the groups administered the low dose of DCE by any of the exposure routes. At both the high and low doses, F was higher for animals inhaling DCE than for orally administered rats.

VI. APPLICATION OF A COMMON PBPK MODEL FOR APPLICATIONS WITH DIFFERENT HALOCARBONS TCE, TRI, PCE

In the development and validation of physiologically-based pharmacokinetic (PBPK) models in this project for predicting the pharmacokinetics of various halocarbons, an important goal has been to define a single model that has the capability of producing accurate simulations for more than one chemical. Of course, required changes in physicochemical constants are dictated as different chemicals are employed, but the utility of a validated model will be enhanced considerably if additional adjustments in model parameters can be minimized. In the previous year of this project, therefore, a PBPK model was developed that has been tested for its utility in providing reasonably accurate simulations for three different halocarbons: TRI, TCE, and PER.

The initial PBPK model was developed for predicting the pharmacokinetics of TRI during and following inhalation exposures. Data from the direct measurements of blood and exhaled breath levels of halocarbon have been compared to simulated values calculated from mass-balance differential equations comprising the PBPK model. In this way, the accuracy of the model has been tested by comparison to observed blood and exhaled breath concentrations. The PBPK model for inhaled TRI and the laboratory data used for the model validation has been published in a peer-reviewed journal. A reprint of the publication is included as Section A of the Appendix. The reference for this publication (also listed in Section F of the Appendix) is as follows:

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "The uptake and elimination of 1,1,1-trichloroethane (TRI) during and following inhalation exposures in rats. Toxicology and Applied Pharmacology 98: 385-397 (1989).

It was assumed that a blood flow-limited model was adequate to characterize the tissue distribution of TRI. Compartmental volumes and organ blood flows were obtained from the literature (Gerlowski and Jain, 1983; Ramsey and Andersen, 1984) and scaled to 340 g, the mean body weight of rats used in the present study. Partition coefficients and the metabolic rate constant for TRI were taken from Gargas et al. (1986, 1989), except for the richly perfused tissue:blood and lung:blood partition coefficients, which were assumed to be the same as the liver:blood partition coefficient. The lung:air partition coefficient was then derived by multiplying the blood:air coefficient from Gargas et al. (1986) by the lung:blood coefficient. The alveolar:lung mass transfer coefficient was estimated from the value used for methylene chloride (Angelo and Pritchard, 1984). Differential mass balance equations that described the transport of TRI in the rat were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Authier, Concord, MA). The solution to the equations provided predicted TRI concentrations over time. The model-predicted cumulative uptake values were the sum of the simulated amounts of TRI in each tissue compartment in the model.

Concentrations of TRI in the expired air were well simulated by the model during and following the 50 and 500 ppm exposures. Model predictions that TRI levels in the exhaled breath would quickly reach near steady state after the exposures began were consistent with the observed data, with the observed levels slightly lower than simulated levels over the course of the 50 and 500 ppm exposures. The model accurately predicted both rapid and slow elimination phases of expiration of TRI postexposure. When the model was used to describe the time course of TRI in the arterial blood, a relatively good fit was obtained during the 500 ppm exposure (Fig. 4, Appendix A). Arterial blood concentrations were overpredicted by approximately 20% during the 50 ppm exposure (Fig. 3, Appendix A). The model predicted a slightly more rapid decline in blood levels postexposure in both groups than was observed during the period of 130-200 min, but levels at subsequent time points were accurately predicted.

This PBPK model that was validated for TRI inhalation was then evaluated for its utility in predicting TCE levels in rats during and following inhalation exposures. TCE and TRI are interesting test chemicals to compare and contrast since they are so structurally similar, yet differ significantly in metabolic capacity. TRI is only slightly metabolized by the rat, so the impact of liver metabolism on the model simulations was not of great importance. However, since TCE is so highly metabolized, it is essential to incorporate accurate estimates of the Michaelis-Menten parameters into the PBPK model so that the model predictions would correctly estimate metabolic rates. The PBPK model for TCE inhalation was evaluated by comparison to direct measurements of TCE in the blood and exhaled breath of exposed rats. The model and the direct measurements used for validation have been included in a manuscript ready for submission to a peer-reviewed journal. A manuscript including a majority of the present text was included in last year's annual report. The reference for this manuscript (also included in Section F of the Appendix) is as follows:

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M. and Bruckner, J.V.: "Physiological pharmacokinetic model for trichloroethylene inhalation exposure in rats". (now being submitted to Toxicology and Applied Pharmacology, 1989).

In general, the PBPK model predictions for TCE are in good agreement with the actual data. The prediction of exhaled breath levels during TCE inhalation are in close agreement with the direct measurements of expired TCE at both dose levels. Post-exposure exhaled breath predictions were accurate for the 50 ppm group and slightly under predicted for the first hour following exposure to 500 ppm TCE (predictions thereafter are accurate). During TCE inhalation exposure, blood levels of rats inhaling 500 ppm were very accurately predicted while blood level simulations for the 50 ppm group were slightly overpredicted (about 0.1 $\mu\text{g/ml}$). Post-exposure blood levels were overpredicted during the first hour following exposure to 500 ppm TCE. After the first hour post-exposure, all predicted values are in excellent agreement with the observed TCE concentrations.

The present model incorporated the dynamics between the venous, alveolar and arterial compartments that has been used for a methylene chloride PBPK model (Angelo and Pritchard, 1984, 1987). The representation is appealing in that venous and arterial blood pools are distinct, and a physiologically realistic membrane transport term (h) controls chemical uptake and elimination at the alveolar-lung interface. The blood flow-limited tissue compartments and the Michaelis-Menten liver elimination are similar to other models on metabolized volatile organic compounds (Andersen et al., 1987). The experimentally measured model parameters, alveolar ventilation, and the inhaled gas concentration, were the only values that were altered for the predictions obtained at the 50 and 500 ppm exposures to TCE. The mean of the measured alveolar ventilation rates for TRI and TCE were used for the oral simulations. First-order absorption rate constants (K_a) and bioavailabilities (f) were empirically estimated, and the value of V_d for TCE was adjusted from the reported value to permit better agreement between observed and predicted TCE blood concentrations.

The PBPK model applications for TCE and TRI were presented together at the latest meeting of the Society of Toxicology that was held in Atlanta, GA in March, 1989. In addition to the model development and data validation for inhalation exposures, PBPK model simulations for oral administrations of TCE and TRI were also presented. The reference for this abstract (also listed in Section F of the Appendix) is as follows:

Gallo, J.M., Dallas, C.E., and Bruckner, J.V.: "Physiological pharmacokinetic models for 1,1,1-trichloroethane (TRI) and 1,1,1-trichloroethylene (TCE) in rats following inhalation and oral exposures". 28th Annual Meeting of the Society of Toxicology, Atlanta, GA; Toxicologist 9: 230 (1989).

The PBPK model appears to be a suitable predictor for orally administered TCE or TRI. However, the current absorption rate constant parameters differ for the low and high oral doses, and is not consistent with a linear pharmacokinetic model. Utilizing the same K_a and f values for both doses produces predicted blood TCE concentrations considerably different from the observed concentrations. Resolution of this problem could be achieved by further experimentation (including iv and oral dosing and tissue concentration determinations), and possibly by optimization of the absorption parameters based on the global model.

A third test chemical, PCE, has been employed demonstrating the versatility of the PBPK model for the prediction of halocarbon pharmacokinetics. Observed values from measurements of PCE in the blood and exhaled breath of rats during and following inhalation exposure (see Section III of this report and Section C of the Appendix) were used to compare to simulations by the model to establish its accuracy for inhaled PCE. The only changes necessary in the model were the physicochemical constants specific to PCE, and the values measured in the laboratory for the alveolar ventilation and inhaled concentrations specific to the validation experiments. Partition coefficients for PCE for input into the model were obtained from Ward et al. (1988), as well as estimates for the Michaelis-Menten parameters (Table 1, Appendix C).

Model-simulated values for elimination of inhaled PCE in the breath were in close agreement with direct measurements of expired PCE during inhalation exposure at both dose levels. Post-exposure exhaled breath predictions were slightly lower than observed values. PCE blood levels were overpredicted during inhalation exposure. The computer-simulated levels approached near steady-state more rapidly than did observed values. Post-exposure blood concentrations were either in close agreement or only slightly underpredicted. Predictions of the cumulative uptake of inhaled PCE during the course of 500 ppm inhalation exposure were very close to the uptake values calculated from the observed exhaled breath data and monitoring of respiratory volumes. Uptake during inhalation of 50 ppm PCE was underpredicted relative to calculated values.

VII. DEVELOPMENT OF AN ASSAY FOR HALOCARBON CONCENTRATIONS IN TISSUES

Although knowledge of the deposition of chemicals in target tissues is of major importance in risk assessment, PBPK models have to date been used primarily to forecast uptake and elimination of VOCs from the bloodstream. Blood concentration over time, as a measure of bioavailability, is routinely accepted as an index of the level of chemical in the body, and therefore a representative index of toxic effects. This assumption can be misleading, in that blood concentrations may not be reflective of the concentration of chemical or active metabolite at the local site of effect in a target tissue. Thus, a more logical measure of target organ exposure is the area under the tissue versus time curve for the reactive chemical (Andersen, 1987). Relatively little has been published on the use and validation of PBPK models for prediction of time integrals of tissue exposure to VOCs, because of a paucity of tissue concentration versus time data sets. This lack of a data base is due to the considerable effort required in such studies and to technical problems with quantitation of the volatile chemicals in solid tissues. For example, the only tissue data Reitz et al. (1988b) had to use for validation of their PBPK model were levels of radioactivity measured by Schumann et al. (1982) in the liver and fat of mice and rats at the termination of 6-hour inhalation exposures to ¹⁴C-TRI.

It has therefore been a goal of this project to develop an accurate assay for the direct measurement of the parent halocarbon in the tissues of exposed animals. These measurements will be of significant utility for the validation of PBPK models for the prediction of halocarbon pharmacokinetics. Each compartment of the model can therefore be represented (and validated) by a tissue that is sampled in a laboratory experiment. Compartments that are single organ-specific like the liver, kidney, and brain would be directly represented. Predictions for a physiologically-generalized compartment, such as the one that has been utilized for "poorly perfused" tissues, would be validated by measurements from a representative tissue group, the muscle tissues.

A technique for the analysis of halocarbons in tissues has now been successfully developed and employed in tissue measurement in several experiments. Extensive analyses have been conducted to determine the

efficiency of the measurements for both PER and TET in the following tissues: brain, liver, kidney, lung, fat, heart, and muscle. One-gram tissue samples are placed into 8 ml of chilled isooctane for PER or ethyl acetate for TET. The samples are maintained in an ice bath at all times, even during homogenization. A polytron is used to homogenize the tissue samples. In order to insure that the percent recovery of halocarbon is maximal and reproducible, a specific period of homogenization is required for each tissue. Brain, liver, and fat are the most easily homogenized, requiring only 4 or 5 seconds. Kidney, lung and heart homogenization require 8 to 10 seconds. Muscle is the most difficult, requiring 15 to 20 seconds. Samples are extracted with 8 ml of isooctane for PER or ethyl acetate for TET and vortexed for 30 seconds. Samples are then centrifuged at 3000 rpm for 5 min at 4°C in a Sorvall RC 2-B centrifuge. Twenty μ l of the organic phase is withdrawn with a microsyringe and transferred to headspace vials (Perkin-Elmer, Norwalk, CT). One interesting and important methodological finding was that 20 μ l was the optimal amount of the organic phase homogenate to add to the headspace vial. Adding aliquots greater than 30 μ l resulted in a decrease in the efficiency of recovery of the halocarbon, as determined by comparison to standards with known concentration.

Standards are made and assayed by diluting a calculated amount of pure test chemical in the appropriate solvent. The column used is an 8' X 1/8" stainless-steel column packed with FFAP Chromasorb W-AW (80-10 mesh). Operating temperatures are: injection port, 200°C; electron capture detector, 360°C; column 110°C; headspace control unit, 90°C.

This analytical technique has been used in pilot studies of the tissue disposition of PCE and TET in rats following oral and ia administration. Efficiency of recovery from tissues spiked with the halocarbons ranged from approximately 70% for muscle to nearly 100% for liver and brain. Reproducibility between different spiked tissue samples has been found to be very consistent.

VIII. MEASUREMENTS OF PCE IN THE TISSUES OF RATS FOLLOWING ORAL AND INTRAARTERIAL ADMINISTRATION

Groups of male S-D rats (mean body wt = 350 g) were administered PCE via a surgically-implanted carotid artery cannula. As blood flow in the right carotid artery ceases when the cannula is inserted and the vessel ligated, a solution injected through the cannula flows back to the heart and enters the systemic arterial circulation. Thus, this technique allows direct intraarterial (ia) injection. Serial blood and tissue samples have been taken following dosing, in order to characterize uptake and elimination profiles for PCE. Blood and tissues sampling will be carried out beyond the normal 5 to 6 half-lives to ensure that the terminal elimination phase in all tissues is adequately characterized. A minimum of 4 rats were sacrificed at each of the following time points: 1/4, 1/2, 1, 2, 4, 6, 12, 18, 24, and 48 hours after the administration of a single bolus dose. One gram samples of brain, liver, kidney, lung, fat, heart, and muscle were then procured and analyzed as described in Section VII. Profiles of the tissue uptake, disposition, and elimination of PCE following ia and oral administration are compiled in Appendix D.

Absorption of PCE into the tissues following intraarterial (ia) administration was very rapid, as indicated by the significant levels detected in all tissue samples at just 15 minutes following exposure (Appendix D, Fig. 1, or Fig. D-1). The highest levels were achieved in the fat (35.6 $\mu\text{g PCE/g}$), with levels of 20-26 $\mu\text{g PCE/g}$ in the liver, kidney, and brain. By 30 minutes post-administration (Fig. D-2), PCE concentrations in all sampled tissues had declined by 25-50% except for those in the fat, which remained relatively equivalent to that measured after 15 minutes. PCE levels in the fat achieved a peak concentration of 67 $\mu\text{g/g}$ after 1 hour, while PCE concentrations in the other tissues continued to steadily decline (Fig. D-3). Fat levels did begin to decline by 2 hours following exposure (Fig D-4), while all other tissue groups decreased by more than 60% relative to an hour before. The rate of diminution in non-fat tissue concentrations began to level off by 4 hours (Fig. D-5) and 6 hours (Fig. D-6) post administration. By 6 hours following ia administration, fat levels of PCE had diminished to about 64% of the peak level achieved 5 hours before. Between 6 and 12 hours following PCE administration, levels in non-fat tissues diminished by more than 50%, while fat concentrations decreased by only 9% (Fig. D-7). After another six-hour interval, PCE concentrations in the liver, kidney, heart, and brain remained relatively constant (Fig. D-8). These tissue groups probably have reached an approximate equilibration within the same time frame because they represent the well-perfused tissue groups we have sampled in this study. The muscle tissues, representing the poorly-perfused tissues in this investigation, continued to decline significantly after 18 hours. Tissue measurements taken after 24 (Fig. D-9) and 48 hours (Fig. D-10) appeared to indicate that PCE levels in non-fat tissues were decreasing at a rate of 50% in a 24 hour period, while fat levels demonstrated an 85% decline.

Examination of the tissue concentration-time profiles for ia administration separately for each tissue group sampled indicated definitive similarities and differences between the tissues in PCE disposition and elimination. Profiles for the liver (Fig. D-11), kidney (Fig. D-12), and brain (Fig. D-13) were nearly identical. There was a distinct similarity in both the magnitude of PCE concentration and the rate of elimination as reflected by the tissue concentration-time profile for these three well-perfused organs. The lung (Fig. D-14) and the heart (Fig. D-15) also displayed a marked similarity in these parameters. These two tissue groups had PCE concentrations consistently less than in the tissue concentration-time profiles for the three well-perfused tissues. However, concentrations at the final sampling point (48 hours) converged for these five tissue groups. The muscle tissue profile (Fig. D-16) was similar to that of the heart and lung, but was slightly higher between the 6 and 24 hour points. The fat tissue concentration-time profile (Fig. D-17) was completely different from the other tissue groups. The highly lipophilic nature of PCE obviously resulted in much higher levels in the fat, as well as a much slower rate of decline during the extended elimination phase.

Perhaps the most pressing risk assessment concern presently for exposure to halocarbons by human populations is the potential for oral exposure due to the increasingly reported incidence of contaminated drinking water supplies. Oral exposures were conducted in rats in the

present context by administering a 10 mg/kg dose in a single oral bolus. Male Sprague-Dawley rats with a mean body weight of 350 grams were administered the halocarbon with a gavage intubation needle. Sampling times and procedures were identical to those employed for the parallel ia studies.

Absorption of the PCE from the gut was very rapid, as indicated by the significant levels of the halocarbons measured in all tissues after just 15 minutes (Fig. D-18). PCE levels at this initial sampling point were 3-10 times higher after ia administration than after an equivalent dose given orally. Also, liver and brain levels after oral dosing were actually slightly higher than levels in the fat, a situation which never occurred following ia administration. PCE levels decreased only slightly in most of the non-fat tissues by the 30 minute sampling point (Fig. D-19). Levels of PCE in the lung and fat increased slightly. There was a dramatic increase of PCE in the fat after 1 hour (Fig. D-20), however, while the not-fat tissue levels demonstrated only slight increases or decreases. Indeed, the concentration of PCE in the heart, muscle, lung, and brain had still not begun to decline by the measurements conducted 2 hours after oral dosing (Fig. D-21). All the non-fat tissue concentrations were thus not in a consistent state of decline until 4 hours after oral dosing (Fig. D-22). Peak levels of PCE in the fat were 44.4 $\mu\text{g/g}$ at the 6 hour point, while the decline in not-fat tissue levels in that 2 hour interval was minimal (Fig. D-23). Consistent decreases in fat concentration of PCE did not occur until the following six hour intervals, at the 12 hour (Fig. D-24), 18 hour (Fig. D-24) and 24 hour (Fig. D-26) sampling points. by 48 hours after oral dosing with 10 mg/kg of PCE (Fig. D-27), though, the halocarbon could only be detected in the liver (barely) and in the fat.

Absorption, distribution, and elimination of PCE in each tissue has also been recorded in tissue concentration-time profiles in Appendix D. Comparison of the liver tissue profile for oral (Fig D-28) and ia (Fig. D-11) administrations revealed almost identical levels and rate of elimination between 1 and 18 hours after exposure. There were higher ia levels within the first hour and at the 24 and 48 hour points. Levels of PCE in the kidney were only similar over the time interval between the 4 and 12 hour points for oral (Fig. D-29) and ia (Fig. D-12) exposure. Levels after ia administration were higher at the earlier and later time points. This same pattern was demonstrated in the tissue levels after oral administration in the brain (Fig. D-30), heart (Fig. D-31), and lung (Fig. D-32), and muscle (Fig. D-33) relative to the results following ia administration. This pattern was also exhibited in a comparison of fat concentration-time profile by the two routes of administration, except that fat PCE levels continued to be the same proportion lower in the oral exposures (Fig. D-34) relative to the ia data at the later time points.

IX STUDIES OF THE BIOAVAILABILITY OF PCE IN DOGS

One of the primary goals of this project has been to provide a pharmacokinetic data base for interspecies comparisons and for formulation of physiologically-based pharmacokinetic models. Adult, male Sprague-Dawley rats and male beagle dogs are being utilized in the project. The rats and dogs are being administered equal doses/concentrations of selected halocarbons by ingestion, inhalation and intraarterial injection. Studies toward this end for the rat have been reported in previous sections of this Annual Report. These comprehensive experiments provide the information necessary for the most efficacious design of experimental protocols for the dog. This has been useful in avoiding inefficient or redundant work in the dog experiments, an important factor in view of the high cost of conducting experiments in dogs. The original physiologic model for the rat will be scaled up to predict halocarbon pharmacokinetics in the dog. The direct measurements of blood and tissue levels of the halocarbons in the dog will then be compared to corresponding values predicted by the scaled-up physiologic model. This procedure will provide a means of assessing the utility of the physiologic model in extrapolating halocarbon pharmacokinetics between two species. Such a validation would establish greater merit in employing the established model in making extrapolations from animal test species to the human.

Male beagle dogs of approximately 10 kg were first surgically prepared with an indwelling cannula in the jugular vein. The cannulated animals were then allowed to recover from the surgical anesthesia overnight. These dogs were then administered 10 mg/kg of PCE as a single oral bolus dose, using polyethylene glycol as a dosage vehicle. The dog received the oral dose through a teflon tube which extended to the stomach, thus insuring that the animal received the complete dose that was intended to be administered. Results from these initial studies are presented in Appendix E.

Studies conducted thus far in the dog have indicated that PCE is absorbed very rapidly from the gut following oral administration. Blood samples taken from the venous cannula demonstrated that substantial PCE is present in venous blood at the first sampling point (i.e. 4 min.). Blood concentration-time data from individual dogs indicates that the orally-absorbed PCE reaches a peak concentration from 10 minutes (Fig. E-1) to 15 minutes (Fig. E-2) following a single oral bolus. Rapid elimination from the blood was evident over the following 24 hours. Tissue concentrations have also been determined in the dog 24 hours after oral exposure to PCE (Fig. E-3 and E-4). Fat concentrations measured 24 hours after a single oral bolus to PCE were very similar in the rat and dog. PCE levels in the liver, kidney, heart, lung, muscle, and brain were all significantly higher in the beagle dog than in the Sprague-Dawley rat. It is clear that a well-defined study of the tissue disposition of these halocarbons in different species will have a significant impact on the manner in which the basic principle of interspecies extrapolation is employed in health risk assessments.

X. COLLABORATIVE ARRANGEMENTS

As Principal Investigator, Dr. Cham E. Dallas has been responsible for the overall supervision of the project during the previous year. Dr. Dallas has personally conducted all of the inhalation exposures, including experiments with TCE, TRI, DCE, and PCE. He has also developed the novel mathematical approaches to the analysis of the respiratory monitoring data for the halocarbon inhalation studies (see Appendix A). Dr. James V. Bruckner, as co-Principal Investigator, has been responsible for the design and conduct of the pharmacokinetic studies of halocarbon ingestion. Dr. James Gallo has had the primary responsibility for the development and validation of the physiologically-based pharmacokinetic model from the experimental studies. All halocarbon pharmacokinetic studies with the dog in this project are under the authority of Dr. Randall Tackett. In the experiments conducted thus far, he has personally performed the surgery on the dogs for the implantation of venous and arterial cannulas. Dr. Xiao Mei Chen is a postdoctoral associate who is supported by this project. She has developed the assay for the measurement of halocarbons in the tissues of exposed animals, and has conducted these tissue measurements thus far for PCE and TET in both rats and dogs. Dr. R. Ramanathan has participated in the analysis of blood samples for halocarbon uptake and disposition from the test animals. This effort was in conjunction with an EPA project on the effect of exposure route on the toxicity of volatile organics, which is intended for use in setting drinking water standards. Mr. S. Muralidhara has conducted the rat surgery required, analytical determinations of blood samples, oral exposures to halocarbons, and data analysis. Dr. Tom Reigle, who has extensive operant behavior testing experience, has given valuable assistance in the selection and purchase of the appropriate testing equipment that can be used for both rats and dogs. Miss Elizabeth Lehman is an undergraduate chemistry student who assisted in the conduct of the laboratory studies, glassware washing and record keeping. Very useful technical information and counsel on the development of the PBPK models and the analysis of pharmacokinetic data in halocarbon inhalation exposures has been received in consultation with Drs. Melvin Andersen, Harvey Clewel, and Michael Gargas at the Biochemical Toxicology Branch, Toxic Hazards Division, Harry G. Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base. Data from the PCE inhalation experiments has been shared with Dr. Alan Vinegar of the Biological Simulation Program at NSI Technology Services, also located at Wright-Patterson Air Force Base in Dayton, Ohio.

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APPENDIX A

REPRINT OF PAPER PUBLISHED IN THE JOURNAL
TOXICOLOGY AND APPLIED PHARMACOLOGY

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V., "The uptake and elimination of 1,1,1-trichloroethane (TRI) during and following inhalation exposures in rats". Toxicology and Applied Pharmacology 98: 385-397(1989).

The Uptake and Elimination of 1,1,1-Trichloroethane during and following Inhalation Exposures in Rats^{1,2}

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The Uptake and Elimination of 1,1,1-Trichloroethane during and following Inhalation Exposures in Rats. DALLAS, C. E., RAMANATHAN, R., MURALIDHARA, S., GALLO, J. M., AND BRUCKNER, J. V. (1989). *Toxicol. Appl. Pharmacol.* 98, 385-397. The pharmacokinetics of 1,1,1-trichloroethane (TRI) was studied in male Sprague-Dawley rats in order to characterize and quantify TRI uptake and elimination by direct measurements of the inhaled and exhaled compound. Fifty or 500 ppm TRI was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 325-375 g. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently both during and following TRI inhalation and analyzed for TRI by gas chromatography. Respiratory rates and volumes were continuously monitored during and following exposure and were used in conjunction with the pharmacokinetic data to characterize profiles of uptake and elimination. TRI was very rapidly absorbed from the lung, in that substantial levels were present in arterial blood at the first sampling time (i.e., 2 min). Blood and exhaled breath concentrations of TRI increased rapidly after the initiation of exposure, approaching but not reaching steady state during the 2-hr exposures. The blood and exhaled breath concentrations were directly proportional to the exposure concentration during the exposures. Percentage uptake of TRI decreased 30-35% during the first hour of inhalation, diminishing to approximately 45-50% by the end of the exposure. Total cumulative uptake in the 50 and 500 ppm groups over the 2-hr inhalation exposures was determined to be 6 and 48 mg/kg body wt, respectively. By the end of the exposure period, 2.1 and 20.8 mg, respectively, of inhaled TRI was eliminated from rats inhaling 50 and 500 ppm TRI. A physiological pharmacokinetic model for TRI inhalation was utilized to predict blood and exhaled breath concentrations for comparison with observed experimental values. Overall, values predicted by the physiological pharmacokinetic model for TRI levels in the blood and exhaled breath were in close agreement with measured values both during and following TRI inhalation. © 1989 Academic Press, Inc.

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1,1,1-Trichloroethane (TRI), also known as methyl chloroform, has been used in large quantities for decades in industry as a solvent and metal degreasing agent. Other applications include its use in adhesives, spot removers, aerosols, and water repellents. The toxicity of TRI is considered to be of a relatively low order of magnitude, with depression of the central nervous system (CNS) (Torkelson

and Rowe, 1981; Kleinfeld and Feiner, 1966; Stewart, 1968) and cardiac arrhythmias (Dornette and Jones, 1960; Reinhardt *et al.*, 1973; Herd *et al.*, 1974) the major effects seen after high doses in animals and humans. Hepatic and renal toxicity have been demonstrated only after very high acute doses in animals (Plaa and Larson, 1965; Klaassen and Plaa, 1966, 1967; Gehring, 1968). Historically, human exposures to TRI have been of greatest significance in industry and other occupational settings, where exposures are primarily by inhalation. Workers are routinely exposed to TRI vapors in open or closed (i.e., recirculating) work environments. Employees may be inadvertently exposed to high concentrations when there has been a spill or equipment malfunction.

Studies of the pharmacokinetics of inhaled solvents such as TRI are playing an increasingly important role in toxicology. Knowledge of the uptake, disposition, and elimination of these chemicals is quite useful in health risk assessments. There is presently little kinetic data available involving direct measurements of TRI in laboratory animals during inhalation exposures. The fate of ^{14}C -TRI has been investigated following the termination of single 6-hr 150 or 1500 ppm inhalation exposures in rats and mice (Schumann *et al.*, 1982a). By 72 hr postexposure, 87–98% of the total recovered radioactivity was eliminated as unchanged TRI in the expired air. Respiratory elimination and metabolism of TRI remained approximately the same after TRI inhalation exposures were repeated 5 days/week for 18 months (Schumann *et al.*, 1982b). The fraction of the total inhaled dose which is eliminated during ongoing inhalation exposures, however, has not been delineated in laboratory animals. Likewise, the rate and magnitude of uptake have not been quantified over time during the course of TRI inhalation exposures in animals. It was necessary, for example, for Schumann *et al.* (1982a) to base estimates of pharmacokinetic parameters for rats on an as-

sumed constant uptake of 60% of inhaled TRI over 6 hr of exposure.

Therefore, an objective of the current investigation was to provide accurate measurements of the respiratory uptake and elimination of TRI during inhalation exposures. Inhaled and exhaled breath concentrations were monitored at frequent intervals in rats both during and following TRI inhalation, as were the minute volume and respiratory rate. Blood levels of TRI were monitored concurrently, so systemic uptake and elimination could be correlated with the respiratory measurements. The exhaled breath and blood TRI concentrations were then utilized to assess the accuracy of values predicted by a physiologically based pharmacokinetic model for TRI inhalation.

METHODS

Animals. Adult, male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. They were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided *ad libitum*. The rats were used after at least a 14-day acclimation period, at which time they were approximately 12 weeks old and their body weight ranged from 325 to 375 g. Solvent exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test material. 1,1,1-Trichloroethane, 98.3% minimum purity, was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). The purity of the chemical during the conduct of the study was verified by gas chromatography to be slightly less than 99%.

Animal preparation. An indwelling carotid arterial cannula was surgically implanted into each animal. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml):acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). The cannulated animals were maintained in a harness and pulley system that allowed relative freedom of movement in metabolism cages during a 24-hr recovery period.

Inhalation exposures. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneve, Switzer-

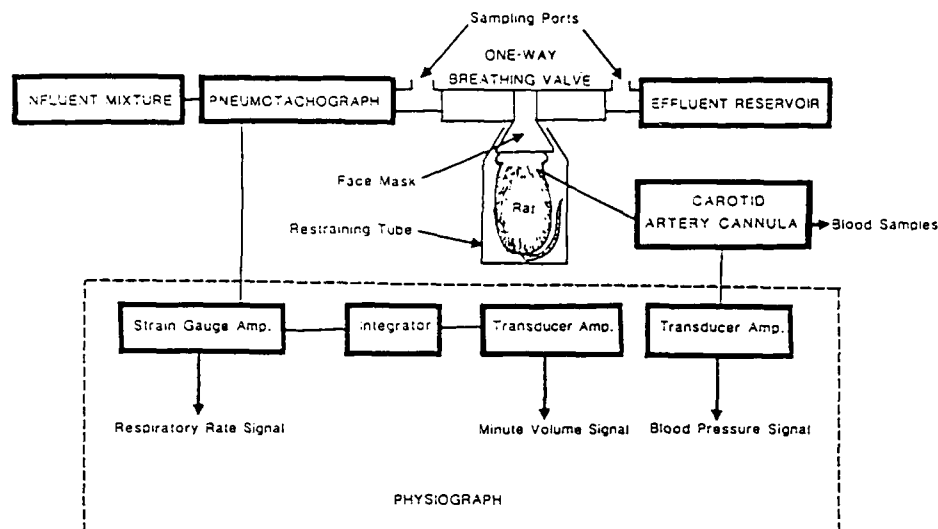


FIG. 1. Schematic diagram of the inhalation exposure system. An unanesthetized rat in the restraining tube inhaled TRI through the one-way breathing valve attached to the face mask. TRI was inhaled from the influent mixture gas sampling bag and exhaled into the effluent reservoir bag. Inhaled and exhaled breath samples were taken from their respective sampling ports and arterial blood samples from an indwelling cannula. The rate and volume of respiration were monitored on a physiograph. For sake of clarity, the breathing valve, gas sampling bags, and other components are not drawn to scale.

land). A face mask designed to fit the rat was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. A miniaturized one-way breathing valve (Hans Rudolph, Inc., St. Louis, MO) was attached to the face mask so that the valve entry port was directly adjacent to the nares of the test animal. The dead space of the valve was 0.5 ml. The valve was designed so that the negative pressure generated by the animal's inspiration pulled the inhalation diaphragm open and the exhalation diaphragm closed against its seat. Upon expiration, the positive pressure generated within the device pushed the exhalation diaphragm open and the inhalation diaphragm closed against its seat. This established separate and distinct airways for the inhaled and exhaled breath streams with no significant mixing of the inhaled and exhaled air. The use of such a device for pharmacokinetic studies of inhaled halocarbons in small animals has been described in detail (Dallas *et al.*, 1986). Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. A known concentration of TRI was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of the test chemical into the bag filled with air. Uniform dispersion of the vapor was ensured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, a

three-way connector, the breathing valve, and an empty 70-liter gas collection bag (Fig. 1). The latter bag served as a reservoir to collect exhaled gas. Thereby, a closed system was maintained to prevent release of the agent into the laboratory. TRI inhalation exposures were initiated only after stable breathing patterns were established for the cannulated animals in the system. Just before the initiation of exposure, the solvent vapor was first drawn out of the gas sampling bag by an air pump attached to the three-way connector. In this manner, the animal was assured of being subjected at the very start of the exposure to a TRI concentration equivalent to the target concentration in the bag, without significant dilution from dead space air in the system. The test animals then were subjected to 2-hr TRI inhalation exposures. During this period and for up to 4 hr afterward, inhaled and exhaled breath samples were taken from the sampling ports at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for TRI content by gas chromatography.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored. The respiratory monitoring was conducted according to the methods previously used in solvent exposure studies by this laboratory (Dallas *et al.*, 1983, 1986). The airflow created by the animal's inspiration was detected by a pneumotachograph located in the inhaled airstream be-

tween the influent bag and the breathing valve. The signal from the pneumotachograph and accompanying transducer was employed in recording the number of respirations per minute (f) in one channel of a physiograph. This signal was then integrated over a 1-min interval to yield the volume of respiration per minute, or minute volume (V_E). A value for the average tidal volume (V_T) during that 1-min interval was determined by dividing V_E by f for that minute. An average value for these parameters for individual animals was obtained by averaging the measurements taken at 10-min intervals during the 2-hr exposure. The mean $\pm$ SE of these values for the 500 ppm exposure group ($n = 6$) were $V_E = 236.3 \pm 22.9$ ml/min; $f = 135.3 \pm 6.6$ breaths/min; and $V_T = 1.74 \pm 0.18$ ml. The mean $\pm$ SE of these values for the 50 ppm exposure group ($n = 6$) were $V_E = 252 \pm 14.7$ ml/min; $f = 129.5 \pm 13.5$ breaths/min; and $V_T = 1.96 \pm 0.1$ ml.

Since the V_E and the TRI exhaled breath concentration at each sampling point were measured, subtraction of the quantity of TRI exhaled from the amount inhaled yielded an approximation of the quantity of TRI taken up each sampling period (cumulative uptake, or Q_{upt}).

$$Q_{upt} = (C_{inh} V_E t) - (C_{exh} V_E t), \quad (1)$$

where C_{inh} is the inhaled concentration; V_E and C_{exh} are the minute volume and exhaled breath measurements, respectively, made at each time point; and t is the interval of time between sampling points (every 10 min for Q_{upt}). The successive Q_{upt} values are summed to determine cumulative uptake over the 2-hr exposure.

Determination of the cumulative elimination of TRI during inhalation exposure was made as a function of the Q_{upt} and measurements of the inhaled dose. In their calculation of exhaled breath concentration, Ramsey and Andersen (1984) assumed that alveolar respiration accounts for 70% of total respiration, with 30% of total respiration delegated to the inhaled air that does not participate in alveolar ventilation. By adding instrumental dead space of the breathing valve in the exposure system in the present study to this assumed physiological dead space, a value of 50% of total respiration was assigned to alveolar ventilation. Therefore, cumulative elimination (Q_{elim}) of TRI was estimated by

$$Q_{elim} = (C_{inh} V_{alv} t) - Q_{upt}, \quad (2)$$

where the alveolar ventilation is $V_{alv} = 0.5 V_E$ and t is the time interval between sequential sampling of the exhaled breath. As for Q_{upt} , with sequential determination of Q_{elim} it is possible to measure the cumulative elimination of TRI during inhalation exposures. The successive elimination of TRI following exposure was calculated as $Q_{elim} = C_{exh} V_E t$.

The percentage uptake (% Upt) of the total inhaled dose at each successive time point during the inhalation exposure period was calculated as

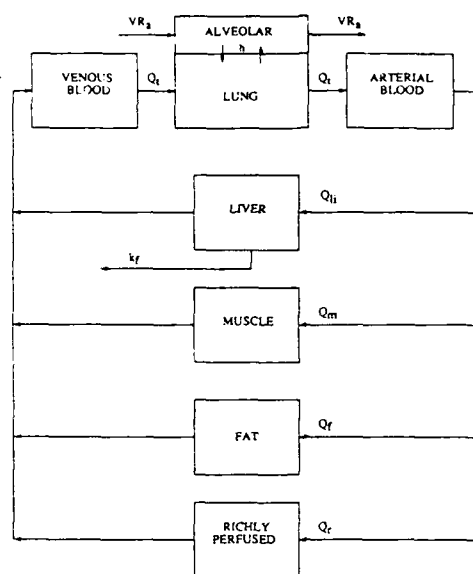


FIG. 2. Diagram of the physiologically based pharmacokinetic model used to simulate the uptake and elimination of inhaled TRI. The symbols and parameters used to describe the model are included in Table I and in the equations given under Methods.

$$\% \text{ Upt} = \frac{(C_{inh} - C_{alv}) \times 100}{C_{inh}}, \quad (3)$$

where the TRI alveolar concentration is $C_{alv} = C_{art}/N$, in which C_{art} is the measured TRI arterial blood level and N is the blood:air partition coefficient for TRI.

A physiologically based pharmacokinetic (PBPK) model was used to describe the disposition of TRI in the rat (Fig. 2). It was assumed that a blood flow-limited model was adequate to characterize the tissue distribution of TRI. Compartmental volumes and organ blood flows were obtained from the literature (Gerlowski and Jain, 1983; Ramsey and Andersen, 1984) and scaled to 340 g, the mean body weight of rats used in the present study. Partition coefficients and the metabolic rate constant for TRI were taken from Gargas *et al.* (1986, 1989), except for the richly perfused tissue: blood and lung: blood partition coefficients, which were assumed to be the same as the liver: blood partition coefficient. The lung: air partition coefficient was then derived by multiplying the blood: air coefficient from Gargas *et al.* (1986) by the lung: blood coefficient. The alveolar: lung mass transfer coefficient was estimated from the value used for methylene chloride (Angelo and Pritchard, 1984). Differential mass balance equations, incorporating the parameters listed in Table I, that described the transport

TABLE I
PARAMETERS FOR THE PHYSIOLOGICAL
PHARMACOKINETIC MODEL OF TRI IN THE RAT (340 g)

Parameter	Value
Alveolar ventilation rate (ml/min), VR_a	126 (50 ppm exposure) 118 (500 ppm exposure)
Inhaled gas concentration ($\mu\text{g}/\text{ml}$), C_{inh}	0.279 (50 ppm exposure) 2.70 (500 ppm exposure)
Blood flows (ml/min)	
Cardiac output, Q_b	106.4
Fat, Q_f	9.4
Liver, Q_l	39.8
Muscle, Q_m	12.8
Richly perfused, Q_r	44.4
Tissue volumes (ml)	
Blood, V_b	25.4
Fat, V_f	30.5
Liver, V_l	13.6
Muscle, V_m	248.0
Richly perfused, V_r	17.0
Alveolar, V_a	2.0
Lung, V_i	3.97
Partition coefficients	
Lung:air, R_a	8.6
Fat:blood, R_f	47.7
Liver:blood, R_l	1.49
Lung:blood, R_i	1.49
Muscle:blood, R_m	0.55
Richly perfused:blood, R_r	1.49
Miscellaneous constants	
Lung:alveolar mass transfer coefficient, k	500 ml/min
Metabolic rate constant, K_r	0.115 min^{-1}

of TRI in the rat were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted TRI concentrations over time. The model-predicted cumulative uptake values were the sum of the simulated amounts of TRI in each tissue compartment in the model.

Analysis of TRI in air and blood. The concentration of TRI in the inhaled and exhaled air samples collected during and following the inhalation exposures were measured with a Tracor MT560 gas chromatograph (GC) (Tracor Instruments, Austin, TX). Analyses for the 500 ppm exposures were conducted using a flame ionization detector (FID), while the analyses for the 50 ppm exposures were conducted using an electron capture detector (ECD). In either case, air samples were procured with a

gas-tight, 1-ml syringe and injected directly onto an 8 ft $\times$ $\frac{1}{8}$ in. stainless-steel column packed with 0.1% AT 1000 on GraphPak. Standards were prepared in each of four 9-liter standard bottles with Teflon stoppers containing needles used for taking the air samples with the syringe. Operating temperatures were 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; and 110°C, isothermal column operation. When using the ECD, gas flow rates of 40 ml/min were employed for nitrogen (carrier gas), with an additional makeup gas flow rate of 30 ml/min to the detector.

TRI levels in the blood were measured by GC headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock by a 1-ml syringe. Depending on the anticipated blood concentration, between 25 and 200 μl of the blood was taken from the stopcock with an Eppendorf pipet and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE-lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 autosampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer, Norwalk, CT), where it was heated to 80°C by a high-precision thermostat device. A predetermined volume of the vapor was then injected automatically into the column for analysis. Standard solutions were made and assayed by diluting calculated amounts of pure TRI in toluene, transferring to vials, and analyzing as previously described. The concentration of TRI in the blood samples was then determined from a standard curve generated from blood that was spiked with these standard solutions. The column used was an 8 ft $\times$ $\frac{1}{8}$ in. stainless-steel column packed with FFAP Chromasorb W-AW (80-100 mesh). Operating temperatures were 200°C, injection port; 350°C, ECD detector; and 85°C, column oven. The carrier gas was 5% argon-methane, at a flow rate of 40 ml/min with a makeup gas flow rate of 20 ml/min to the detector.

RESULTS

The target concentrations for the TRI inhalation exposures were 50 and 500 ppm. The starting concentration of TRI in the bag from which the test animal inhaled the test compound was measured just prior to the initiation of each exposure. TRI bag concentrations were 515.8 ± 20.6 and 53.6 ± 2.2 ppm ($\bar{x} \pm \text{SE}$) for the 500 and 50 ppm groups, respectively. The actual concentrations inhaled by the animals were determined by measurements of air samples taken from the airway

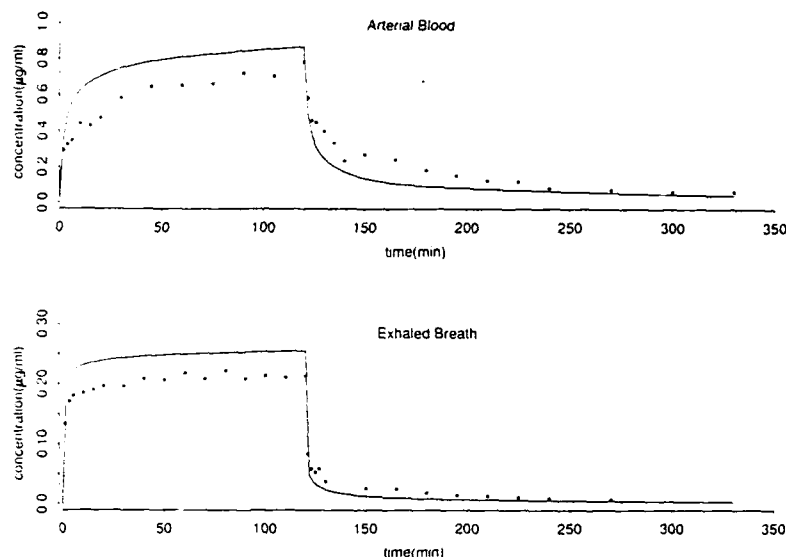


FIG. 3. Observed (●) and model-predicted (—) TRI concentrations in the blood (top graph) and exhaled breath (bottom graph) of rats during and following a 2-hr 50 ppm inhalation exposure. Each point represents the mean value for six rats.

immediately adjacent to the breathing valve. Inhaled TRI concentrations for the six rats in each group were 491.6 ± 11 ppm for the 500 ppm exposures and 51.2 ± 1.2 ppm ($\bar{x} \pm \text{SE}$) for the 50 ppm exposures.

TRI concentrations in the blood and exhaled breath of rats during and following inhalation of TRI are shown for 50 ppm exposures in Fig. 3 and for 500 ppm exposures in Fig. 4. Concentrations of TRI in the exhaled breath generally paralleled concentrations in the arterial blood, though some differences were noted. TRI was rapidly absorbed from the lungs and readily available for distribution to tissues of the body, in that arterial blood concentrations of TRI were quite high at the first sampling time (i.e., 2 min). After an initial rapid rise, the blood levels increased steadily but did not reach steady state by the end of the 2-hr exposures. Exhaled breath levels increased even more rapidly than blood levels after the initiation of exposures, attaining near steady state within 10 to 15 min. The exhaled breath versus time curves were as-

ymptotic, in that they gradually increased throughout the remainder of the 2-hr inhalation period. An increase in the inhaled concentration from 50 to 500 ppm produced an equivalent (i.e., 10-fold) increase in the observed blood and exhaled breath concentrations of TRI. Upon cessation of TRI inhalation, the chemical was rapidly eliminated. As can be seen in Figs. 3 and 4, TRI concentrations in the exhaled breath initially diminished more rapidly than did blood concentrations. Disappearance of TRI from the blood paralleled that in the expired air during the latter part of the postexposure period.

PBPK model-generated blood and exhaled breath concentrations of TRI are shown as solid lines in Figs. 2 and 3. Concentrations of TRI in the expired air were well represented by the model during and following the 50 and 500 ppm exposures. Model predictions that TRI levels in the exhaled breath would quickly reach near steady state after the exposures began were consistent with the observed data, with the observed levels slightly lower

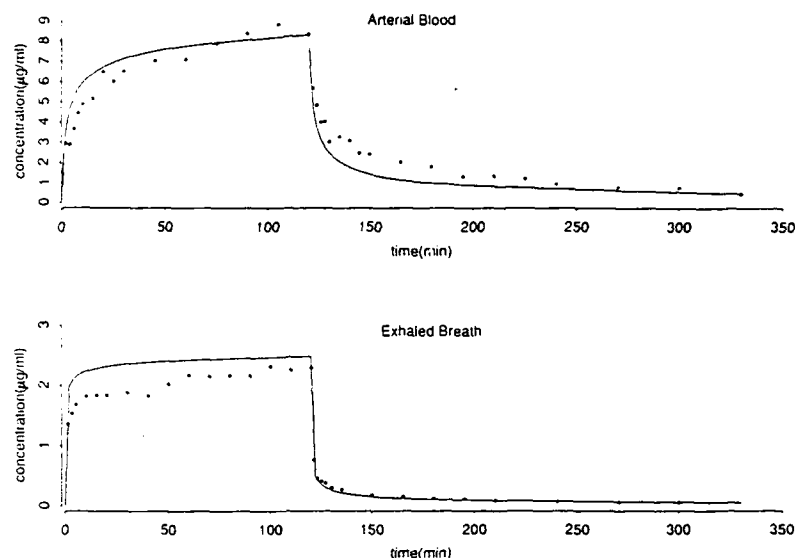


FIG. 4. Observed (●) and model-predicted (—) TRI concentrations in the blood (top graph) and exhaled breath (bottom graph) of rats during and following a 2-hr 500 ppm inhalation exposure. Each point represents the mean value for six rats.

than simulated levels over the course of the 50 and 500 ppm exposures. The model accurately predicted both rapid and slow elimination phases of expiration of TRI postexposure. When the model was used to describe the time course of TRI in the arterial blood, a relatively good fit was obtained during the 500 ppm exposure (Fig. 4). Arterial blood concentrations were overpredicted by approximately 20% during the 50 ppm exposure (Fig. 3). The model predicted a slightly more rapid decline in blood levels postexposure in both groups than was observed during the period of 130–200 min, but levels at subsequent time points were accurately predicted.

Percentage systemic uptake of TRI appeared to be both concentration- and time-dependent (Fig. 5). Although percentage uptake was quite high during the initial minutes of inhalation of 50 and 500 ppm TRI, a decrease of 30–35% occurred during the first hour. Percentage uptake diminished more slowly during the remainder of the exposure

period. As can be seen in Fig. 5, the mean values after 10 min are slightly but consistently lower in the 500 ppm group.

Plots of cumulative uptake of TRI during the inhalation sessions, as calculated by Eq.

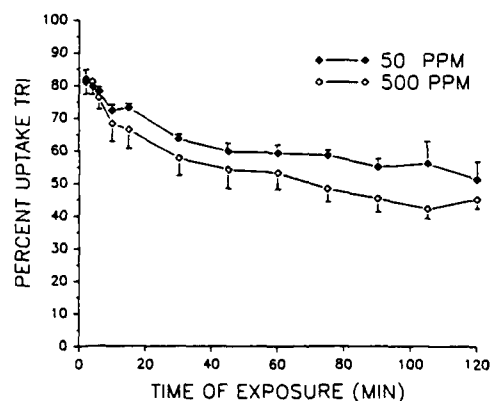


FIG. 5. Percentage uptake of TRI during inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. Each point represents the mean $\pm$ SE for six rats. The percentage uptake of the inhaled dose over time was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter.

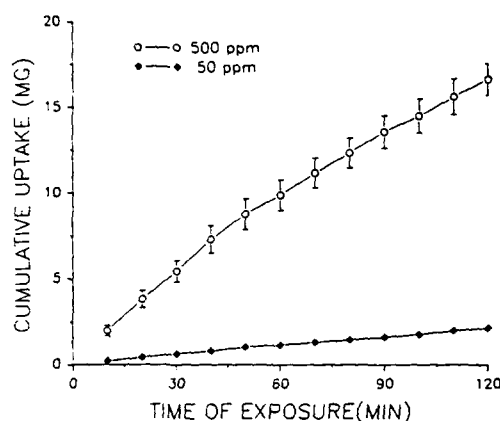


FIG. 6. Cumulative uptake of TRI during inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. The quantity of inhaled TRI retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled TRI concentrations. Each point represents the mean $\pm$ SE for six rats. The diminutive SE bars are omitted from the 50 ppm values for sake of clarity.

(1), are shown in Fig. 6. Cumulative uptake, as determined from direct measurements of the minute volume and TRI concentrations in the inhaled and exhaled breath, was not linear in either the 50 or 500 ppm animals. The departure from linearity was more apparent at the higher exposure level. Total cumulative uptake during the 2-hr exposures, as ascertained from the direct measurement data, was 2.2 ± 0.2 and 16.7 ± 0.9 mg ($\bar{x} \pm$ SE) in the 50 and 500 ppm groups, respectively. Predicted values for uptake, derived by summing the predicted levels of TRI in the model compartments, were significantly less than these measured uptake values (i.e., after 2 hr exposure to 500 ppm TRI, predicted uptake was 50% of measured uptake).

Cumulative elimination of TRI in the exhaled breath during and following inhalation exposure is shown in Fig. 7. During TRI exposure, TRI in the pulmonary blood and TRI not absorbed from the alveolar space each contribute to the TRI eliminated in the exhaled breath. Cumulative pulmonary elimi-

nation, as determined by Eq. (2), was proportional to the inhaled concentration. By the end of the 2-hr exposure to 50 and 500 ppm TRI, 2.1 ± 0.2 and 20.8 ± 3.0 mg ($\bar{x} \pm$ SE), respectively, were eliminated from the rats in the exhaled breath. Model-predicted elimination at the end of 2 hr in the 50 and 500 ppm groups was approximately 40 and 50% greater, respectively, than these measured values. Following the termination of exposure, TRI was eliminated in the exhaled breath in progressively smaller quantities, as reflected by postexposure plateaus in the elimination curves. During the 2-hr postexposure period, an additional 0.3 and 3.3 mg of TRI were eliminated from the animals in the 50 and 500 ppm exposure groups, respectively.

DISCUSSION

Pharmacokinetic studies are playing an increasingly important role in toxicology and

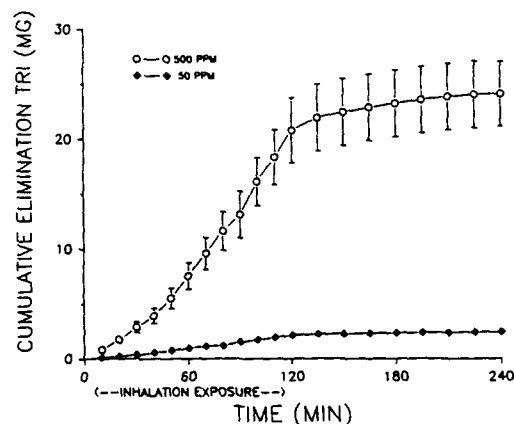


FIG. 7. Cumulative elimination of TRI during and following inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. The quantity of inhaled TRI eliminated in the breath over time was calculated using direct measurements of the minute volume and TRI concentrations in the inhaled and exhaled breath. Each point is the mean $\pm$ SE for six rats. Cumulative elimination was determined for successive 10-min intervals during the 2-hr exposure, and for successive 15-min intervals postexposure.

in health risk assessments (Clark and Smith, 1984; Clewell and Andersen, 1985; NRC, 1987). Unfortunately, there is relatively little information available on the uptake and disposition of TRI and many other VOCs during ongoing inhalation exposures. Most pharmacokinetic studies of TRI have focused on the elimination of the chemical and its metabolites following the cessation of exposures (Stewart *et al.*, 1969; Ikeda and Ohtsuji, 1972; Eben and Kimmerle, 1974; Seki *et al.*, 1975; Holmberg *et al.*, 1977; Caperos *et al.*, 1982; Schumann *et al.*, 1982a,b). Although Schumann *et al.* (1982a) utilized rats with an indwelling jugular cannula, just three blood samples were taken and analyzed for TRI content during a 6-hr inhalation session. Similarly, blood levels appear to have been taken for TRI analysis only three times from human volunteers during 6 hr of TRI inhalation (Nolan *et al.*, 1984). These few time points are not sufficient to accurately define blood concentration versus time profiles, or to recognize changes which may occur in kinetics during the course of exposures.

The monitoring of blood and breath levels of VOCs in animals has been primarily restricted to times after termination of exposures, due to problems involving restricted access to subjects in inhalation chambers and metabolism cages and technical difficulties in working with small animals. In the present study we have utilized a technique which allowed direct, simultaneous measurements of respiratory parameters, inhaled and exhaled breath concentrations of TRI, and TRI blood levels in unanesthetized rats during exposures. The separation of the inhaled and exhaled breath streams, by use of a miniaturized one-way breathing valve, facilitated accurate serial determinations of airflow and TRI concentrations in the inhaled and exhaled breath. A similar non-rebreathing valve has been used previously for assessing respiratory volumes and gas exchange (Mauderly *et al.*, 1979), though it has apparently not been used in pharmacokinetic studies. This

approach allowed us to directly monitor respiratory uptake and elimination of TRI, a VOC for which pulmonary clearance is the major route of elimination. Indeed by the end of the 2-hr 50 and 500 ppm inhalation exposures, we determined that 52.5 and 56.3%, respectively, of the inhaled dose had been exhaled.

Percentage uptake of inhaled TRI was highly time-dependent. The percentage uptake of inhaled TRI has apparently not been previously determined in laboratory animals. Schumann *et al.* (1982a) assumed that 60% of inspired TRI was absorbed by rats throughout a 6-hr exposure. The rate of transfer of TRI from alveoli to blood should initially be very rapid, but become progressively slower as the chemical accumulates in the blood and tissues. This pattern was reflected by the time course of systemic uptake of TRI in the current study, where percentage uptake decreased from more than 80% at the beginning to less than 50% at the end of the 2-hr exposure. Initial uptake of inhaled TRI is governed by tissue loading and metabolism. Once the tissues have reached steady state, continued uptake will be dependent upon the rate of metabolism of the chemicals. Since TRI is very poorly metabolized by the rat and by humans (Ikeda and Ohtsuji, 1972; Schumann *et al.*, 1982a; Nolan *et al.*, 1984), percentage uptake would be expected to be very low once steady state was reached. Steady state was not reached in our study, as percentage uptake progressively decreased over the 2 hr of exposure. Monster *et al.* (1979) found that percentage uptake of inhaled TRI by humans decreased rapidly from approximately 95% at the onset to 30% at the end of 4-hr exposures. Nolan *et al.* (1984) reported that human volunteers exposed for 6 hr to 35 or 350 ppm TRI retained about 25% of the chemical to which they were exposed. The greater percentage uptake in rats than in humans is consistent with a higher TRI blood:air partition coefficient and greater cardiac output/pulmonary blood flow in rats

than in humans (Reitz *et al.*, 1988). Since systemic uptake of TRI is time-dependent, average values of percentage uptake for short intervals may be misleading and have little relevance for health risk assessments.

TRI exhibits linear kinetics over a wide dosage range. Exhaled breath levels and blood levels of TRI were directly proportional to the inhaled concentration (i.e., 50 and 500 ppm) of TRI throughout the 2-hr exposures in the current study. Similar findings were reported in humans exposed for 6 hr to 35 and 350 ppm TRI (Nolan *et al.*, 1984). Schumann *et al.* (1982a) found that the amount of TRI exhaled by rats and mice increased eight- to ninefold when the inhaled concentration of TRI was increased from 150 to 1500 ppm. These investigators also observed that blood levels, tissue levels, and body burden of ^{14}C -TRI were each proportional to exposure level in both species. Although TRI was poorly metabolized, Schumann *et al.* (1982a) demonstrated that its biotransformation by mice and rats was a dose-dependent, saturable process. Metabolic saturation in rats was believed to occur between 500 and 1500 ppm, if not near 500 ppm. Metabolic saturation, however, had little apparent effect on the overall pharmacokinetics of TRI, since biotransformation was a minor route of elimination. The kinetics of TRI is governed largely by its partition coefficients (e.g., blood:air, tissue:blood) and the physiology (e.g., respiratory rate and volume, cardiac output, tissue volumes, and blood flow rates) of the animal.

The major route of elimination of TRI in laboratory animals and in man is exhalation of the parent compound (Schumann *et al.*, 1982a; Nolan *et al.*, 1984). Nolan and his colleagues measured TRI in the exhaled breath of male human volunteers during and after 6-hr inhalation sessions. The exhaled breath levels after 1.5 hr of exposure to 35 and 350 ppm TRI were 0.14 and 1.28 $\mu\text{g}/\text{ml}$, respectively. Assuming a linear scaleup to a 50 and 500 ppm exposure, the exhaled breath levels

in humans would be 0.2 and 1.83 $\mu\text{g}/\text{ml}$. These values are quite comparable to exhaled breath levels measured in the present study after 1.5 hr of exposure of rats to 50 and 500 ppm TRI (i.e., 0.21 and 2.16 $\mu\text{g}/\text{ml}$, respectively). The similarity in magnitude in exhaled breath levels of TRI between rats and man is an unexpected finding. It would be anticipated that alveolar and presumably exhaled breath concentrations of TRI would be lower in rats than in man, due to the rat's higher blood:air partition coefficient and greater percentage uptake of inhaled TRI.

The aforementioned physiological parameters and biochemical constants were used to input into a PBPK model for inhalation of TRI. Our model accurately predicted the time courses of TRI in the blood and exhaled breath of rats both during and following exposure to 50 and 500 ppm TRI. Cumulative uptake over the 2-hr exposure, however, was underpredicted by our model. The source of the discrepancy between the predicted and the measured uptake value is unclear. Revisions of the model may be warranted by findings in ongoing studies of TRI concentrations in tissues of exposed animals. These data should be useful in verifying tissue:blood partition coefficients, tissue compartments, and tissue volumes. Reitz *et al.* (1988), for example, found that most of the changes in the pharmacokinetics of TRI in older rats could be accounted for by increasing the size of the fat compartment in their PBPK model. Reitz *et al.* (1988) used their model to accurately predict blood and exhaled breath concentrations measured in humans subjected to TRI inhalation exposures. The investigators also utilized the PBPK model to predict TRI blood levels and amounts metabolized post-exposure in mice, rats, and humans, as well as to describe the kinetics of TRI in rats after iv injection, bolus gavage, and drinking water administration. Thus, it appears that PBPK models can be quite useful in predicting the time course of TRI concentrations in the

body of different species under different exposure conditions.

Major species differences have been observed in the pharmacokinetics of inhaled TRI. After 1.5 hr of exposure to 35 or 350 ppm TRI, humans had mean blood levels of 0.14 and 1.62 $\mu\text{g/ml}$, respectively (Nolan *et al.*, 1984). After being normalized for differences in inhaled concentrations, mean blood levels in rats in the present investigation were approximately 3.6-fold higher than the levels measured in humans. Schumann *et al.* (1982a) reported blood levels of TRI in rats similar to those observed in the present study (when normalized for inhaled concentration). The lower blood levels in humans are consistent with a lower TRI blood:air partition coefficient for man (2.53 versus 5.76 for rats) and the greater adipose tissue volume in man (23.1% versus 11% in rats). In a comparison with the normalized data of Schumann *et al.* (1982a), Nolan *et al.* (1984) noted that blood levels in mice and rats inhaling TRI were 17.3 and 3.5 times higher, respectively, than those measured in humans. When determining the actual inhaled dose at equivalent inhaled concentrations, one must consider the wide variation in volume of respiration and body weight between species. Assuming a 4.2 liters/min alveolar ventilation and 70 kg body wt for man (Ganong, 1979), the rats in the present study received an inhaled dose approximately six times greater than that of the humans in the study by Nolan *et al.* (1984). Nolan and his colleagues determined following a 6-hr exposure to 150 ppm TRI that mice, rats, and humans metabolized 0.16, 0.06, and 0.014 $\mu\text{mol/kg/ppm}$, respectively. Thus, mice and rats should be more susceptible than humans to TRI toxicity at equivalent inhaled concentrations, due to significantly greater systemic absorption and metabolism of the chemical.

Meaningful health risk assessments require a careful selection of the measure of dose. In the present study systemic uptake of TRI is measured directly during the initial phase of

inhalation exposure, when significant loading of tissues is occurring. Once tissue loading is completed (i.e., steady state is reached), very little uptake of TRI should occur because of the poor metabolism of the chemical. Thus, the common practice of assessing dose by multiplying ventilation rate by inhaled concentration would be very misleading during prolonged exposures. A more logical measure of target organ dose or tissue exposure would be the area under the tissue concentration versus time curve. The concept and rationale for selection of appropriate target organ dose measures (i.e., tissue dosimetry) are discussed in detail by Andersen (1987). It is important that target organs and mechanisms of toxicity be elucidated, so that the agent(s) responsible for toxic effects are identified and can subsequently be quantified and correlated with the magnitude of toxicity in the target tissue(s). It is not clear for TRI whether the parent compound or its metabolites should serve as the dose measure, or surrogate. Near-lethal exposures are required for effects on most target organs. Carcinogenicity bioassays have been negative, or inconclusive. Reitz *et al.* (1988) decided to use the average concentration of TRI in the liver over a lifetime (ACL) as a dose surrogate. These investigators used a PBPK model to calculate ACLs for comparison of internal doses received by mice and rats in long-term toxicity studies versus humans drinking TRI-contaminated water. Unfortunately, there is a paucity of data on actual concentrations of TRI in the liver and other organs. Direct measurement studies are needed to generate tissue concentration versus time data sets for rigorous validation of PBPK model predictions of dose surrogates.

APPENDIX

*Mass Balance Differential Equations for Physiological Pharmacokinetic Model**

Arterial Blood

$$V_b \frac{dC_b}{dt} = Q_l \left(\frac{C_l}{R_l} - C_b \right).$$

Venous Blood

$$V_b \frac{dC_v}{dt} = Q_{li} \frac{C_{li}}{R_{li}} + Q_m \frac{C_m}{R_m} + Q_f \frac{C_f}{R_f} + Q_r \frac{C_r}{R_r} - Q_t C_v.$$

Alveolar Space

$$V_a \frac{dC_a}{dt} = VR_a C_{inh} u_1(t)^{**} - VR_a C_a + h \left(\frac{C_l}{R_a} - C_a \right).$$

Lung

$$V_l \frac{dC_l}{dt} = Q_l \left(C_v - \frac{C_l}{R_l} \right) + h \left(C_a - \frac{C_l}{R_a} \right).$$

Liver

$$V_{li} \frac{dC_{li}}{dt} = Q_{li} \left(C_b - \frac{C_{li}}{R_{li}} \right) - k_r \frac{X_{li}}{R_{li}}.$$

Muscle

$$V_m \frac{dC_m}{dt} = Q_m \left(C_b - \frac{C_m}{R_m} \right).$$

Fat

$$V_f \frac{dC_f}{dt} = Q_f \left(C_b - \frac{C_f}{R_f} \right).$$

Richly Perfused

$$V_r \frac{dC_r}{dt} = Q_r \left(C_b - \frac{C_r}{R_r} \right).$$

Note. $*C_i$ = concentration of TRI in compartment i . See Table 1 for the definition of

the other symbols. $**\mu(t) = 1$ for $t \leq 120$ min and 0 for $t > 120$ min.

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APPENDIX B

MANUSCRIPT TO BE SUBMITTED TO THE JOURNAL
TOXICOLOGY AND APPLIED PHARMACOLOGY

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V., "Comparative pharmacokinetics of inhaled and ingested 1,1-dichloroethylene in rats". (to be submitted to Toxicology and Applied Pharmacology, 1989).

Comparative Pharmacokinetics of Inhaled and Ingested
1,1-Dichloroethylene in Rats

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Volatile organic chemicals (VOCs) are of concern to public health due to potential human exposures in both an occupational setting during their manufacture and from residential exposure due to subsequent contamination of drinking water supplies. The primary route of exposure in occupational environments is by inhalation, and until recently most prior interest in the health hazards of VOCs has been by inhalation exposure. Current toxicological knowledge of volatile organics is thus largely based upon situations and experiments involving inhalation exposures. In light of the increasing detection of these halocarbons in drinking water supplies in the United States (Symons et al., 1975, NOMS, 1977), there is now considerable interest in the toxicity of these contaminants following oral exposure.

It is unclear, however, whether the results of inhalation studies can be used to accurately predict the consequences of ingestion of the chemicals. In one report (NAS, 1980), the use of inhalation data was avoided in making risk assessments of drinking water contaminants, with the reasoning that the disposition and ensuing bioeffects of inhaled chemicals may differ markedly from that which occurs when the agents are ingested. It was concluded that while inhalation studies may be of value from a qualitative standpoint, such studies may be of limited utility quantitatively in predicting consequences of ingestion of many chemicals. In contrast, the model by Stokinger and Woodward (1958) has been applied to use inhalation data to derive adjusted acceptable daily intake (ADI) for several short-chain aliphatic halocarbon VOCs (Federal Register, 1984). Unfortunately, there is a limited pharmacokinetic and

toxicological data base from which to judge the validity of such route to route extrapolations.

One VOC of particular concern for potential oral and inhalation exposures in humans is the short-chain halocarbon 1,1-dichloroethylene (vinylidene chloride, DCE). DCE is of interest due to a variety of modes of toxicity such as hepatotoxicity (Jenkins, et al., 1972, Reynolds et al., 1975; Andersen et al., 1979), nephrotoxicity (Jenkins and Andersen, 1978; NTP, 1982), and carcinogenicity in one study (Maltoni et al., 1977). DCE has been measured at low levels (< 0.2 $\mu\text{g/liter}$) in well water and municipal drinking water supplies (U.S. EPA, 1975; Shakelford and Keith, 1976). However, groundwater contamination has been detected at considerably higher levels (Page, 1981; U.S. EPA, 1982). Comparable doses of DCE have been administered to rats by inhalation (McKenna et al., 1978a) and by gastric intubation (McKenna et al., 1978b). The question of influence of routes of administration on tissue disposition was not addressed directly. Emphasis was placed on the roles of dose and fasting on metabolism, elimination and toxicity. However, tissue levels were measured at only a single time-point post exposure (i.e. 72 hours). 1,1-DCE has been shown to markedly alter calcium homeostasis in hepatocytes of rats within 20 to 30 minutes of dosing (Moore, 1982; Luthra et al., 1984). Overt cytotoxicity has been observed as early as 2 hours, with maximal injury manifest within 4 to 8 hours of 1,1-DCE exposure by inhalation (Reynolds et al., 1980) or by ingestion (Jaeger et al., 1973). Therefore, it is essential that the kinetics of DCE be compared between the two routes of administration during the critical early hours when cytotoxicity occurs.

The approach utilized in the current study was to administer

equivalent doses of DCE orally and by inhalation in unanesthetized rats within the same time frame. Detailed physiologic measurements conducted during DCE inhalation exposures were used to determine the total uptake of DCE over time, accounting for the significant respiratory elimination of the inhaled compound. An equivalent dose was then administered to rats by single and multiple oral bolus dosing regimens and the systemic uptake, disposition, and elimination of DCE by the two administrative routes compared. A physiologically-based pharmacokinetic model for inhalation and oral DCE exposures was validated by comparison to the observed data and evaluated for use in interroute extrapolation of pharmacokinetic data.

MATERIALS AND METHODS

Animals. Adult, male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. They were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided ad libitum. The rats were used after at least a 14-day acclimation period, at which time their body weight ranged from ~375 g. Solvent exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test material. 1,1-Dichloroethylene (DCE), of 99% purity, was obtained from Aldrich Chemical Co. (Milwaukee, WI) and the purity verified by gas chromatography prior to use in animal exposures.

Animal preparation. All rats were surgically prepared with an indwelling carotid arterial cannula, which exited the animal at the back

of the neck. The rats were anesthetized for the surgical procedure by an injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml): acepromazine maleate (10 mg/ml): xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v:v:v). The cannulated animals were maintained in metabolism cages with total freedom of movement during a 24-hr recovery period. For animals requiring intravenous administration, animals were also prepared with an indwelling jugular cannula simultaneously with the arterial cannula. For gastric infusion experiments, a ventral incision was made and a flared-tip cannula inserted into the fundus of the stomach simultaneously with carotid arterial surgery and exited through the same port as the arterial cannula.

Inhalation exposures. A known concentration of the test chemical was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of the solvent into the bag filled with air. Uniform dispersion of the vapor was insured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, a miniaturized one-way breathing valve (Hans Rudolph, Inc. St. Louis, MO), and an empty 70-l gas collection bag. The latter bag served as a reservoir to collect exhaled gas. The breathing valve was attached to a face mask designed to fit the rat so that the valve entry port was directly adjacent to the nares of the test animal, thus establishing separate and distinct airways for the inhaled and exhaled breath streams. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. A cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneve, Switzerland) and the face mask held

firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. DCE inhalation exposures were initiated only after stable breathing patterns were established for the cannulated animals in the system. The test animals then inhaled DCE vapors for a 2-hr period. During this exposure period and for up to 3 hr afterward, inhaled and exhaled breath samples were taken from the sampling ports at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for DCE content by gas chromatography.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored according to the methods previously published in solvent exposure studies by this laboratory (Dallas et al., 1983 and 1986). The airflow created by the animal's inspiration was recorded both during and following DCE inhalation exposure in terms of minute volume (volume of respiration per minute, or V_E), respiratory rate (f), and tidal volume (V_T). An average value for these parameters for each individual animal was obtained by averaging the measurements taken at 10-min intervals during the 2-hr exposure. The mean $\pm$ SE of these average values for the 100 ppm exposure group ($n=6$) were: $V_E = 229.5 \pm 8.9$ ml/min; $f = 136.2 \pm 2.9$ breaths/min; and $V_T = 1.72 \pm 0.12$ ml. The mean $\pm$ SE of these average values for the 300 ppm exposure group ($n=6$) were: $V_E = 215.7 \pm 18.8$ ml/min; $f = 134.5 \pm 11.5$ breaths/min; and $V_T = 1.60 \pm 0.09$ ml.

Calculations of DCE uptake and elimination from inhalation exposure were conducted according to equations presented in previous pharmacokinetic determinations of TRI inhalation in rats by this laboratory (Dallas et al., 1988). Since the V_E and the TCE exhaled

breath concentration at each sampling point were measured, subtraction of the quantity of TCE exhaled from the animal from the amount inhaled yielded an approximation of the quantity of uptake of TCE for each sampling period (cumulative uptake). In the determination of the cumulative elimination of TCE during inhalation exposure, the cumulative uptake for each time interval was subtracted from the total inhaled dose for that interval. The percent uptake of the total inhaled dose up to each successive time point during the inhalation exposure period was determined by dividing the cumulative uptake by the inhaled dose for that time period.

Oral and intravenous dosage regimens. The value for the total cumulative uptake achieved for a two hour inhalation exposure was then used for the administration of an equivalent oral dose using three different regimens. A single oral bolus of 30 mg/kg (equivalent to 300 ppm DCE for 2 hr) and 10 mg/kg (equivalent to 100 ppm for 2 hr) was thus administered to unanesthetized rats with an indwelling carotid arterial cannula. The DCE was administered in an aqueous emulsion, Emulphor, intragastrically with a blunt-tipped intubation needle. For the multiple oral bolus administrative regimen, the 10 and 30 mg/kg doses were each subdivided into 4 equal doses and administered sequentially at 30-min intervals in Emulphor. For intragastric infusions, the animals previously prepared with an intragastric cannula received the 10 or 30 mg/kg dose in Emulphor over a 2-hr infusion period. The infusion was conducted using a microprocessor-controlled P22 syringe infusion pump (Harvard Apparatus, No. Natick, MA). The intravenous administration of DCE was conducted using the animals previously prepared with an indwelling jugular vein cannula. The intravenous doses were delivered

as a single bolus in PEG 400 through the jugular cannula. Blood samples were taken during and following the oral and iv administrations of DCE from the indwelling carotid arterial cannula.

Analysis of DCE in air and blood. The concentration of TCE in the inhaled and exhaled air during the inhalation exposures were measured with a Tracor MT560 gas chromatograph (Tracor Instruments, Austin, TX) using an electron capture detector (ECD). Air samples were procured with a gas-tight, 1-ml syringe and injected directly onto an 8-ft X 1/8-in stainless-steel column packed with 0.1% AT 1000 on GraphPak. Standards were prepared in each of four 9-liter standard bottles which have Teflon[®] stoppers containing needles used for taking the air samples with the syringe. Operating temperatures were: 150°C, injection port; 360°C, ECD detector; 70°C, isothermal column operation. Gas flow rates were employed of 40 ml/min for nitrogen (carrier gas), with an additional make-up gas flow rate to the detector of 30 ml/min.

DCE levels in the blood from inhalation and iv administration and the various oral dosing regimens were measured by gas chromatographic headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock by a 1-ml syringe. Depending on the anticipated blood concentration, between 25 and 200 µl of the blood was taken from the stopcock with an Eppendorf pipette and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 autosampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer, Norwalk, CT), where it was heated to a preset temperature by a high precision thermostat device. A precise volume of the vapor was then injected automatically

into the column for analysis. The column used was an 8-ft X 1/8 in stainless-steel column packed with FFAP Chromasorb W-AW (80-100 mesh). Operating temperatures were: 200°C, injection port; 360°C, ECD detector; and column oven, 70°C. The carrier gas was 5% argon-methane, at a flow rate of 40 ml/min with a make-up gas flow rate to the detector of 20 ml/min.

RESULTS

While 100 and 300 ppm were the target DCE inhalation concentrations, the actual concentration inhaled by the animals was determined by measurements of air samples taken from the airway immediately adjacent to the breathing valve. Inhaled DCE concentrations for the six rats in each group were 310.0 ± 3.5 ppm for the 300 ppm exposures and 101.6 ± 0.8 ppm for the 100 ppm exposures.

During and following DCE inhalation, concentrations of the parent compound were measured in the exhaled breath (Fig. 1) of 100 ppm and 300 ppm exposed rats. Significant respiratory elimination of unchanged DCE was evident during the inhalation exposure period, with steady-state DCE levels achieved in the exhaled breath within 20 min at both dose levels. DCE respiratory elimination was proportional to the inhaled concentration during exposure, as indicated by the exhaled breath values during 30-120 min of the exposure period (near steady-state) of 71.6 ± 4.1 ppm and 204.6 ± 9.3 ppm ($\bar{x} \pm \text{SE}$) for the 100 and 300 ppm exposure groups, respectively. Upon cessation of DCE inhalation, the concentration of DCE declined very rapidly in the expired air of both exposure groups.

Measurement of the cumulative uptake of DCE by the rats (Fig. 2)

was made by accounting for the quantity of unchanged DCE that was exhaled during the inhalation exposure period. As a result of the 2-hr exposure to 100 ppm DCE the cumulative uptake was 3.3 ± 0.3 mg ($x \pm SE$), or 10 mg/kg bw. The total cumulative uptake of DCE from the 2-hr exposure to 300 ppm was 10.2 ± 0.6 mg ($x \pm SE$), or 30 mg/kg bw. Percent uptake of DCE during inhalation exposure was similar in magnitude at both exposure concentrations (Fig. 3). The % uptake decreased rapidly in the first 30 min to a near-steady state equilibrium thereafter. Mean values for % uptake during the second hr of exposure (near-steady state) were between 61 and 66% for both dose groups.

The cumulative elimination of DCE in the exhaled breath both during and following inhalation exposure is shown in Fig. 4. During DCE exposure, the cumulative elimination is dependent on DCE in the blood and on DCE eliminated from the alveolar and physiologic dead space that was not absorbed into the blood. The magnitude of pulmonary elimination was proportional to the inhalation exposure concentration. By the end of the 2-hr exposure to 100 and 300 ppm DCE, 2.2 ± 0.2 and 6.1 ± 1.3 mg ($x \pm SE$), respectively, were eliminated from the rats in the exhaled breath. Following the termination of exposure, DCE that is eliminated in the breath is solely from unchanged DCE from the systemic circulation. During the 3-hr post-exposure period, an additional 0.14 and 0.37 mg of DCE were eliminated from the animals in the 100 and 300 ppm exposure groups, respectively.

Evaluation of the ratio of DCE concentration in the blood to DCE concentration in the exhaled breath over the duration of the inhalation exposure is shown in Fig. 5. After the first few minutes of exposure, this ratio was consistently higher for the 300-ppm-exposed rats relative

to the 100 ppm group, though the difference is slight until the point at the termination of exposure (120 min).

The arterial blood concentrations of DCE during and following inhalation exposure are compared to blood levels following equivalent oral doses using the three oral dosing regimens for a 300 ppm inhalation and 30 mg/kg oral dose (Figs. 6 a-c) and for a 100 ppm inhalation and 10 mg/kg oral dose (Fig. 7 a-c). After the initiation of inhalation exposure, substantial concentrations of DCE were found in the blood of all animals at the first sampling time (2 min). Uptake of DCE following single or multiple oral bolus administration was very rapid, as peak blood levels were achieved within 2 to 4 minutes of administration. Arterial DCE concentrations were not proportional to the inhalation concentration. After the initial rapid uptake phase over the first 20 minutes of exposure, blood levels for the 300 ppm-exposed rats were 4 to 5 times higher than DCE blood concentrations of rats that received 100 ppm exposures. The $\bar{x} \pm \text{SE}$ for the blood concentrations from 30 to 120 min, during near-steady state, were 0.56 ± 0.03 and 2.19 ± 0.14 $\mu\text{g/ml}$ for the 100 and 300 ppm exposure groups, respectively. At the high dose, peak blood levels were approximately 4 to 5 times higher following single oral bolus dosing than maximum blood levels (C_{max}) achieved during the corresponding inhalation exposures (Fig. 6a). The maximum blood levels achieved during single oral bolus or gastric infusion administration were also not proportional to the administered dose. As with the inhalation exposures, these blood values for the high dose group were at least 4 to 5 times higher than for the low dose group for both oral administrative routes. The C_{max} achieved for the multiple bolus administration of 30 mg/kg (Fig. 6c), however, was nearly 9 times more than that achieved following 10 mg/kg (Fig. 7c).

The AUC (area-under-the-blood-concentration-time curve) for each of the groups administered DCE orally was lower than the AUC seen for the rats inhaling an equivalent DCE dose by inhalation (Table 1). The AUC values for the single bolus and gastric infusion groups were similar at both dose levels (nearly identical for the high dose). These oral administration values were only 60-80% of the corresponding inhalation AUCs. The bioavailability (F) of DCE was determined by the ratio of the AUC value of each experimental group to the corresponding dose administered by intravenous administration. The high dose groups consistently had a higher F than the groups administered the low dose of DCE by any of the exposure routes. At both the high and low doses, F was higher for animals inhaling DCE than for orally administered rats.

The terminal elimination half-life ($t_{1/2}$) was similar for both inhalation exposure concentrations. Following a single oral bolus administration of DCE, the $t_{1/2}$ was also similar to the corresponding inhalation exposure concentration. The administration of DCE by gastric infusion, however, resulted in two to three-fold increases in $t_{1/2}$ relative to inhalation or single oral bolus DCE exposures. Apparent clearance and volume of distribution did not vary significantly with inhalation exposure concentration. Both of these pharmacokinetic parameters, however, were considerably lower for animals inhaling DCE than for the orally-administered rats.

DISCUSSION

Although there have been pharmacokinetic studies in which VOCs have been administered orally and by inhalation, the experimental design of

most of the studies has been such that the results are of limited utility in making route to route extrapolations. Pyykko et al. (1977), for example, monitored the uptake and elimination of ^3H -toluene in various tissues of rats for 24 hours after the animals were dosed by inhalation or gastric intubation. As different doses of ^3H -toluene of different specific activity were given by each route of administration, no conclusions could be drawn about the relative uptake of the chemical by the two exposure routes. Typically, studies of the tissue disposition and binding of VOCs following inhalation and oral exposure involve oral administration of the compound as a single oral bolus, while inhalation exposures occur over a 6-hour period. As evidenced by this approach with perchloroethylene (Pegg et al., 1979) and 1,2-dichloroethane (Reitz et al., 1982), tissue concentrations and levels of covalently-bound ^{14}C are often measured only at a single time-point post-exposure (i.e. 72 hours). The relevance of these practices to interroute extrapolations of kinetic VOC data has thus been limited by questions of variations in the administered dose, the time elapsed between dosing and tissue analysis, and by the dissimilarity between the inhalation and oral dosing regimens.

Several approaches have been undertaken in the past in an attempt to utilize inhalation exposure data to estimate toxicity and acceptable levels of exposure to VOCs by ingestion. Some moderate correlations were established in comparisons between inhalation LC_{50} data for single exposures to individual chemicals and mixtures (Pozzani et al., 1959). Stokinger and Woodward (1958) utilized the threshold limit value (TLV) for human inhalation of a chemical in workplace air in an equation for deriving a value that would represent an acceptable standard for

ingestion exposure. Other variables incorporated were the retention, or absorption factor (% uptake), respiratory volume, and a safety (uncertainty) factor. An adaptation of this model for applications using animal inhalation data has been employed for estimating safe drinking water levels of perchloroethylene (Olson and Gehring, 1976). The accurate determination of the absorption factor (% uptake) has been recognized as essential to the extrapolation of dosages from inhalation data to the ingestion route (Khanna, 1983). It was pointed out that in previous interroute extrapolations, the absorption factor was either estimated based on physicochemical similarities with other agents, indirectly calculated from available data, or even unsupported by any rationale. In the present investigation, direct monitoring of minute volume and the magnitude of inhaled and exhaled DCE was utilized to determine the retention factor during inhalation exposures. This approach thus offers the advantage of direct measurements of respiratory volumes, the inhaled dose, and the retention factors that were previously estimated on a more tenuous basis in most interroute extrapolations.

The total cumulative uptake of DCE during inhalation exposures was determined from these summated measurements over time of the inhaled dose and the magnitude of pulmonary elimination. Administration of this amount as an equivalent oral dose to rats by several oral administrative routes revealed several differences in pharmacokinetics between these equivalent oral and inhalation exposures. Peak blood levels achieved after single oral bolus administration of DCE were 3 times higher than for the corresponding inhalation dose. Under the

assumption that a minimum expression of certain kinds of acute toxicity for a chemical may require that a threshold level is achieved at a target organ, this finding would appear to attribute relatively greater risk to the oral bolus administration of DCE relative to the equivalent inhalation exposure. It is conceivable that a single high dose might produce injury by exceeding the metabolic detoxification pathway for the animal. Following its metabolic activation, DCE is inactivated by complexation with glutathione (Jones and Hathaway, 1978). However, there is evidence that a single high dose of certain halocarbons may be less toxic than the equivalent dose given over a prolonged interval. Indeed, the findings of Chieco et al. (1981) suggest that this may be the case for DCE. With a boiling point of only 31.7°C (Merck, 1976), DCE is one of the most volatile halocarbons. Together with its poor solubility in blood, this characteristic high volatility leads to a high rate of exhalation as a route of elimination for DCE. It is thus conceivable that a large portion of a bolus dose of DCE would be rapidly eliminated by exhalation before GSH stores are depleted to result in cytotoxicity. For inhalation exposures to DCE, however, it has been concluded that the metabolic capacity of rats to metabolize and eliminate DCE can be exceeded during continuous inhalation of 150 ppm or more of DCE (Dallas et al., 1983, Filser and Bolt, 1979). Therefore, despite higher peak blood levels from the single oral bolus administration relative to an equivalent inhalation dose, saturation of the metabolic capacity for DCE elimination and the subsequent increased risk for resulting toxicity may be more likely to occur from the inhalation exposure.

A promising new approach to the extrapolation of pharmacokinetic data between different routes of exposure is the use of physiologically-based pharmacokinetic (PBPK) models. The uptake of some VOCs into the tissues of exposed animals can be predicted with significant accuracy using these models. It has been pointed out that these models can thus be utilized in interroute extrapolations and risk assessments provided they have sufficient biological detail to describe differences in absorption between the exposure routes (NAS, 1986). Ramsey and Andersen (1984) described the kinetics of styrene by both inhalation and oral routes of exposure using a PBPK model. The uptake and disposition of 1,1,1-trichloroethane in the blood of rats has been accurately predicted by a PBPK model for inhalation and oral gavage administrations (Reitz et al., 1988). A PBPK model for DCE has been published in which accurate predictions of the uptake, metabolism, and elimination of DCE by various routes of exposure (including inhalation and oral administration) were made (D'Souza and Andersen, 1988). In this and the previous citations, however, the fundamental objective was not necessarily the interroute extrapolation of data but the development and validation of the models for predicting the pharmacokinetics of the VOCs. A PBPK approach has been utilized for determining the equivalent no-effect levels for inhalation and drinking water exposures to trichloroethylene, or TCE (NAS, 1986). The concept involved predicting the target tissue levels that would be obtained from inhalation and oral exposures to TCE, and matching the predictions with observed tissue level and toxicity data associated with the lowest-observed-effect dose levels from the TCE inhalation literature (Kimmerle and Eben, 1973). In this way, an oral exposure level that would be expected to yield a

non-toxic tissue dose could be calculated based on inhalation exposure data. PBPK models thus have a significant potential for interroute extrapolation of pharmacokinetic and toxicity data of VOCs.

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Figure Legends

1. Exhaled breath concentrations of DCE during and following inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each point represents the mean $\pm$ SE for 6 rats.
2. Cumulative uptake of DCE during inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. The quantity of inhaled DCE retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled DCE concentrations. Each point represents the mean $\pm$ SE for 6 rats.
3. Percent uptake of DCE during inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each point represents the mean $\pm$ SE for 6 rats. The percent uptake of the inhaled dose over time was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter.
4. Cumulative elimination of DCE during and following inhalation exposures. Rats inhaled 100 or 200 ppm DCE for 2 hr. The quantity of inhaled DCE eliminated in the breath over time was calculated using direct measurements of the minute volume and DCE concentrations in the inhaled and exhaled breath. Each point is the mean $\pm$ SE for 6 rats. Cumulative elimination was determined for successive 10-min intervals during the 2-hr exposure, and for successive 15-min intervals post-exposure.

5. Ratio of the DCE arterial blood concentration to the DCE exhaled breath concentration at each sampling point during inhalation exposure to DCE. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each observed value represents the mean ratio for 6 rats.
6. Comparison of DCE blood levels during and following 2 hr inhalation exposures to 300 ppm DCE with: a.) blood levels following a single oral bolus of 30 mg/kg; b.) blood levels following four repeated oral bolus administrations of 7.5 mg/kg each at 30 min intervals; c.) blood levels during and following intragastric infusion of 30 mg/kg over a 2 hr period. Each value represents the mean $\pm$ SE for 6 rats.
7. Comparison of DCE blood levels during and following 2 hr inhalation exposures to 100 ppm DCE with: a.) blood levels following a single oral bolus of 10 mg/kg; b.) blood levels following four repeated oral bolus administrations of 2.5 mg/kg each at 30 min intervals; c.) blood levels during and following intragastric infusion of 10 mg/kg over a 2 hr period. Each value represents the mean $\pm$ SE for 6 rats.

Footnotes

- 1 Research sponsored by U.S. EPA cooperative Agreement CR 812267 and the Air Force Office of Scientific Research, Air Force Systems Command, USAF, under grant number AFOSR 87-0248. The US Government is authorized to reproduce and distribute reprints for Governmental purposes notwithstanding any copyright notation thereon. This manuscript is submitted for publication with the understanding that the US Government is authorized to reproduce and distribute reprints for Governmental purposes.
- 2 Presented at the 27th Annual Meeting of the Society of Toxicology, Dallas, TX, February, 1988.
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Index Terms

1,1-dichloroethylene

Vinylidene Chloride

Physiologically-based Pharmacokinetic Model

Respiratory Elimination

Pharmacokinetics

Inhalation Exposure

Oral Exposure

Interoute Extrapolation

TABLE 1

COMPARISON OF PHARMACOKINETIC PARAMETERS FOR INHALATION, ORAL,
AND INTRAVENOUS ADMINISTRATIONS OF 1,1-DICHLOROETHYLENE IN RATS^a

Dose/Administrative Route	AUC μg min/ml	F	t _{1/2} (min)	V _{dss} l/kg	Cl _r (ml/kg min)
300 ppm Inhalation ^b	304.1±	0.58±	50.1±	0.091	4.0
30 mg/kg Gastric Infusion ^b	238.6	0.46	179.7	27.3	125.8
30 mg/kg Single Oral Bolus	239.5	0.46	62.1	6.3	125.3
30 mg/kg Multiple Oral Bolus ^c					
30 mg/kg Intravenous Bolus	519.4	---	164.5	10.8	136.8
100 ppm Inhalation	72.9	0.36	55.6	0.11	5.52
10 mg/kg Gastric Infusion	41.5	0.21	122.9	43.2	241.2
10 mg/kg Single Oral Bolus	50.0	0.25	47.2	10.4	20.0
10 mg/kg Multiple Oral Bolus ^d					
10 mg/kg Intravenous Bolus	202.6	---	105.7	6.82	49.4

^a All values are x ± SD.

^b Inhalation and gastric infusion administrations were over 2 hr.

^c 30 mg/kg multiple oral bolus administered as three 10 mg/kg bolus doses at 40 min intervals.

^d 10 mg/kg multiple oral bolus administered as three 3.3 mg/kg bolus doses at 40 min intervals.

Fig. B-1

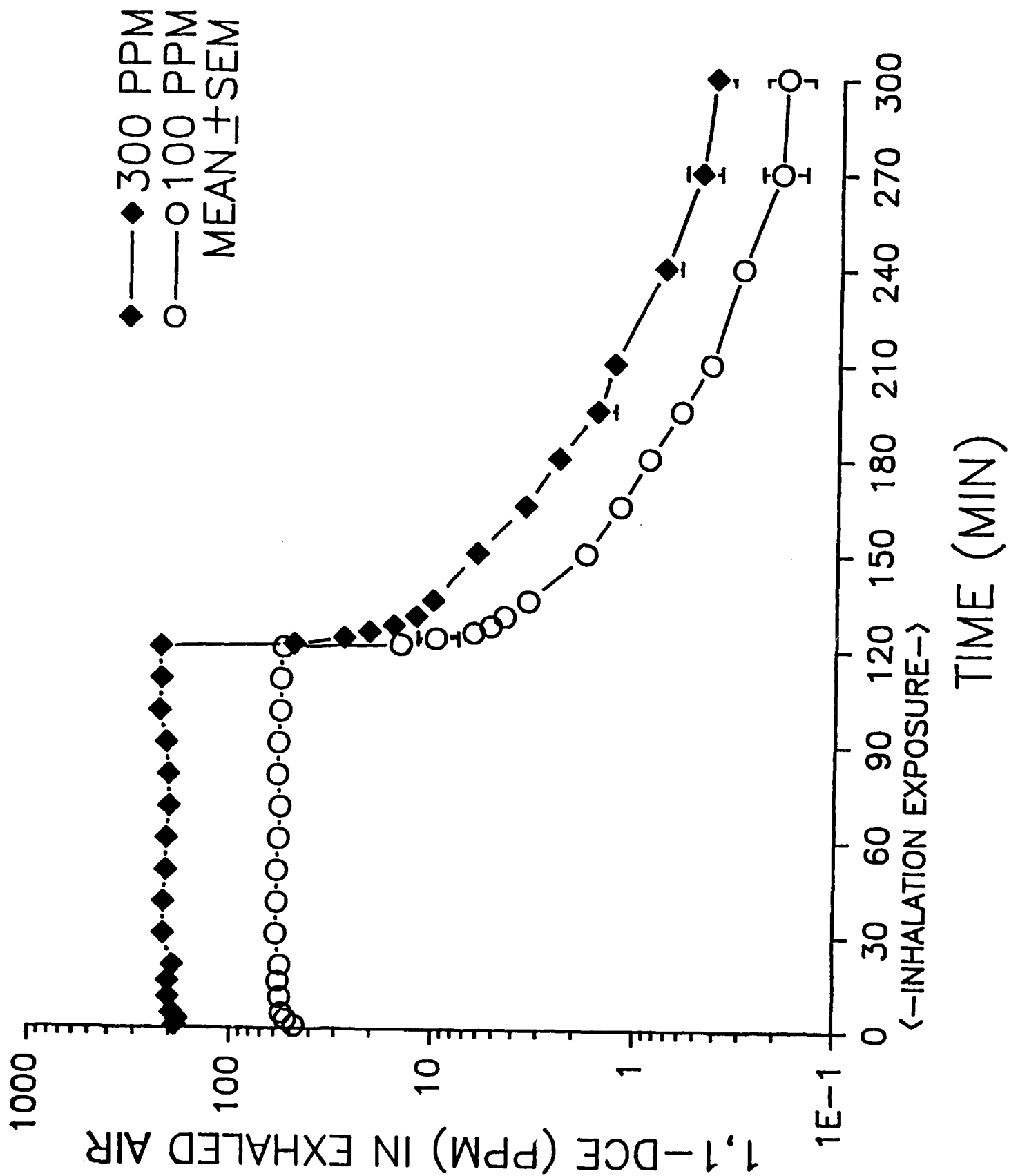


Fig. B-2

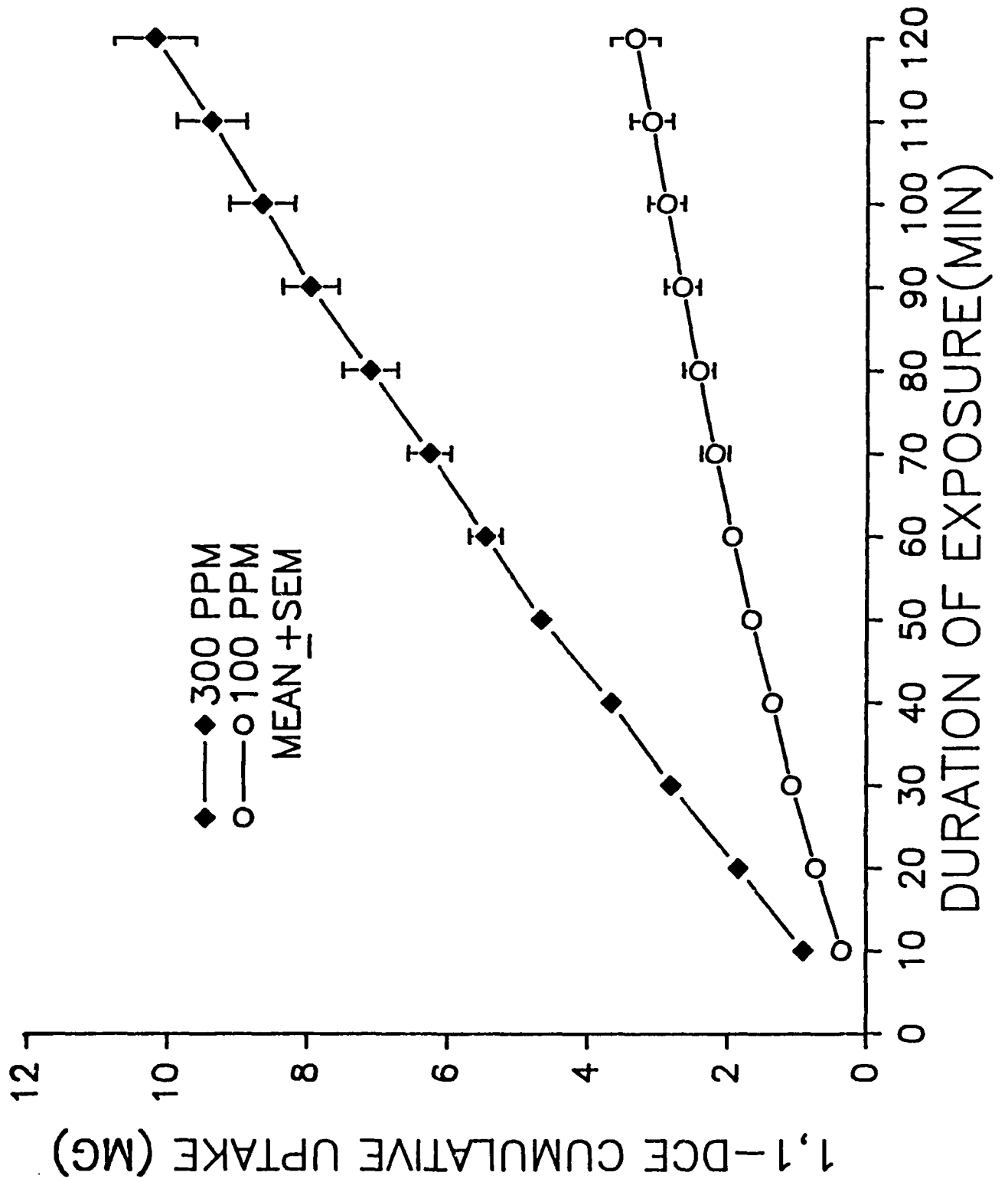


Fig. B-3

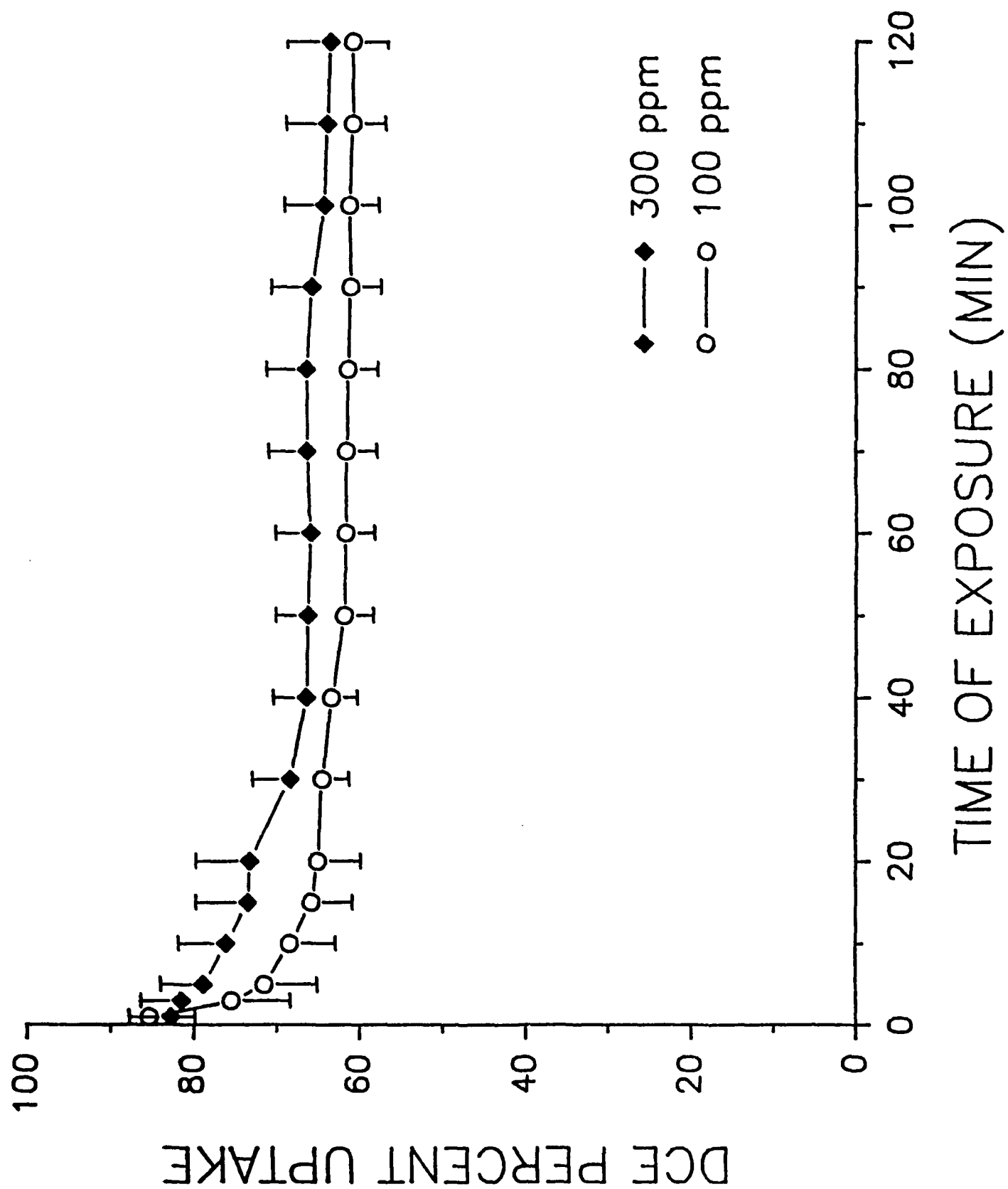


Fig. B-4

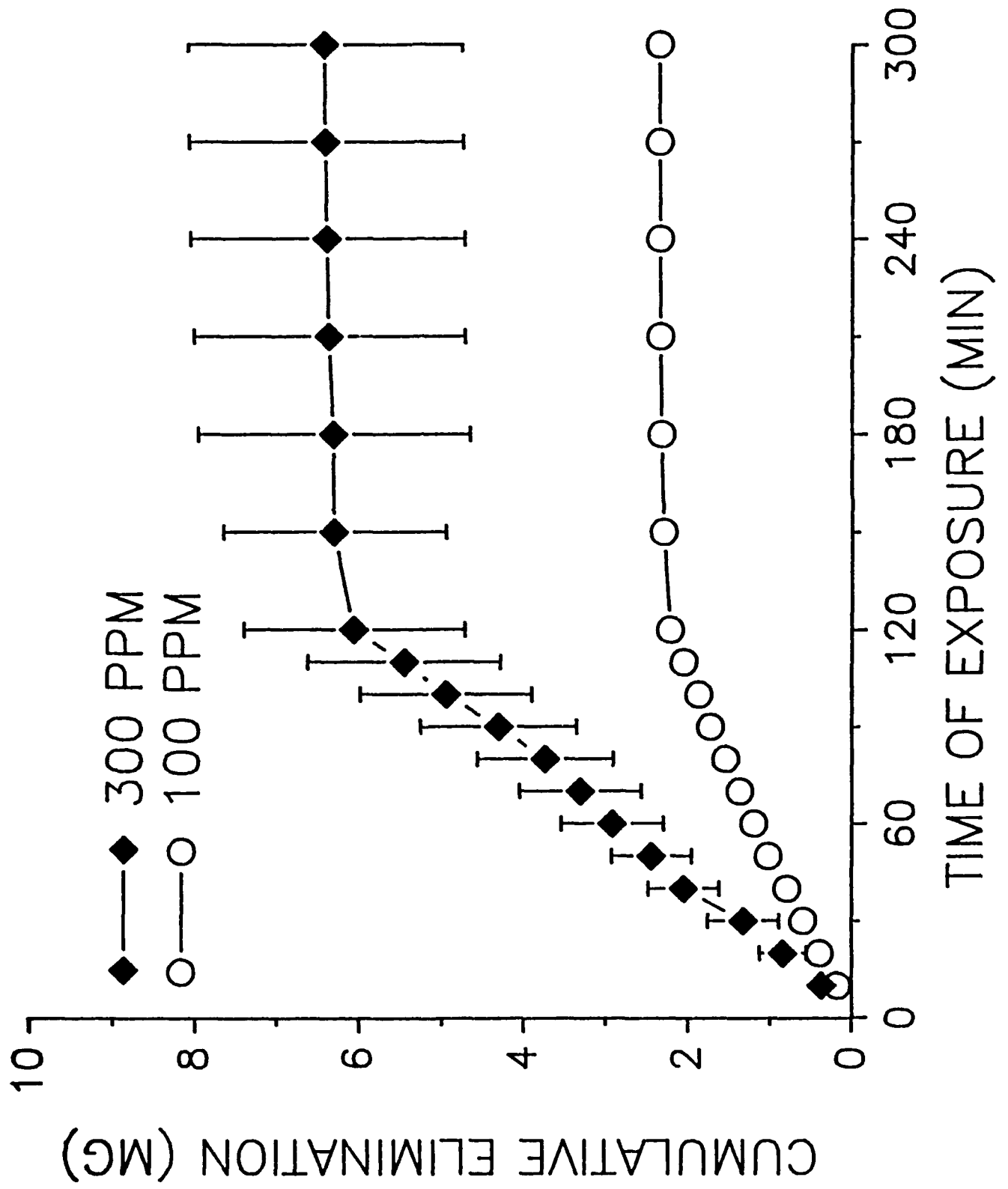
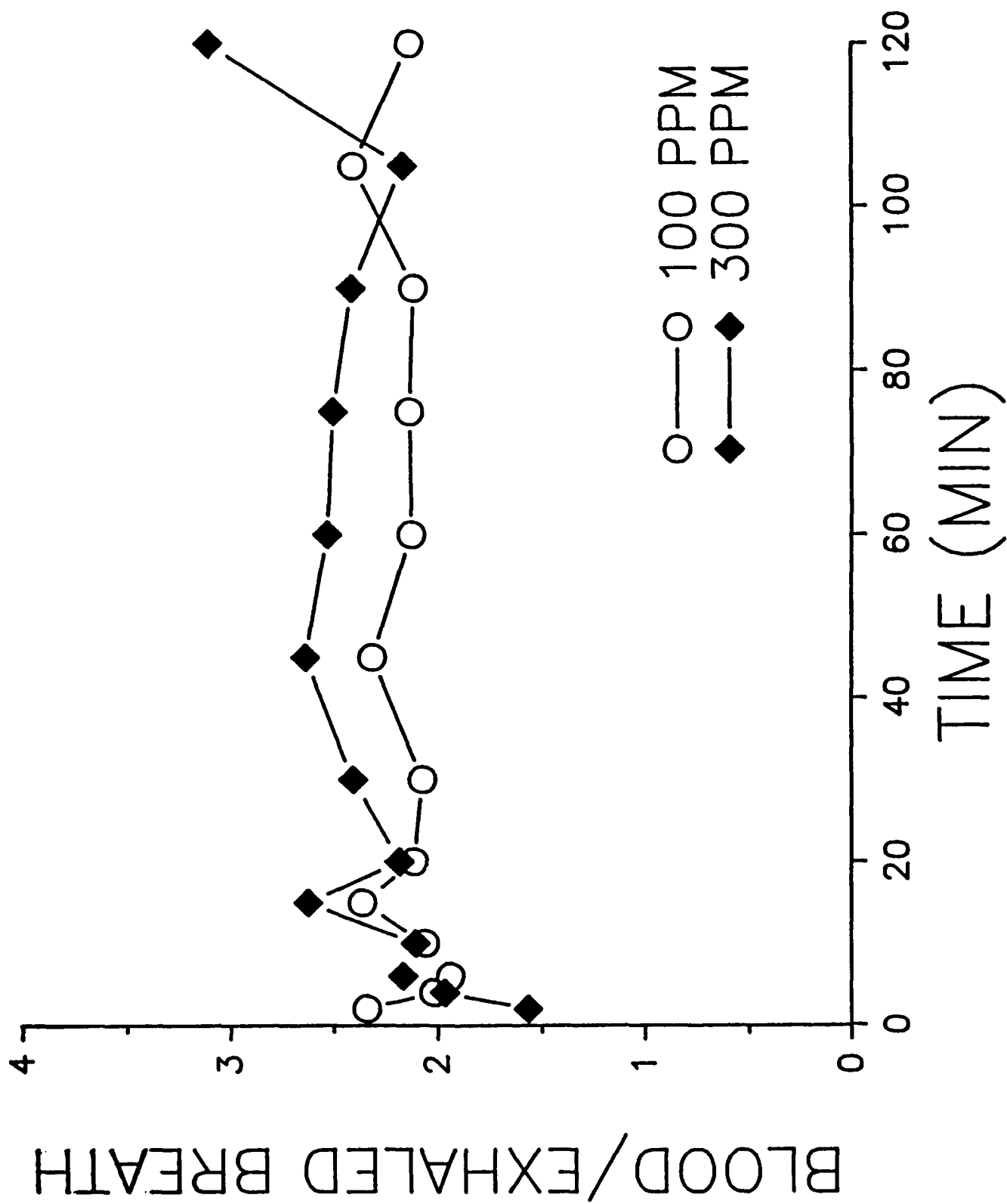


Fig. B-5



1,1-DICHLOROETHYLENE: INHALATION VS. ORAL BOLUS
(300 PPM VS. 30 MG/KG)

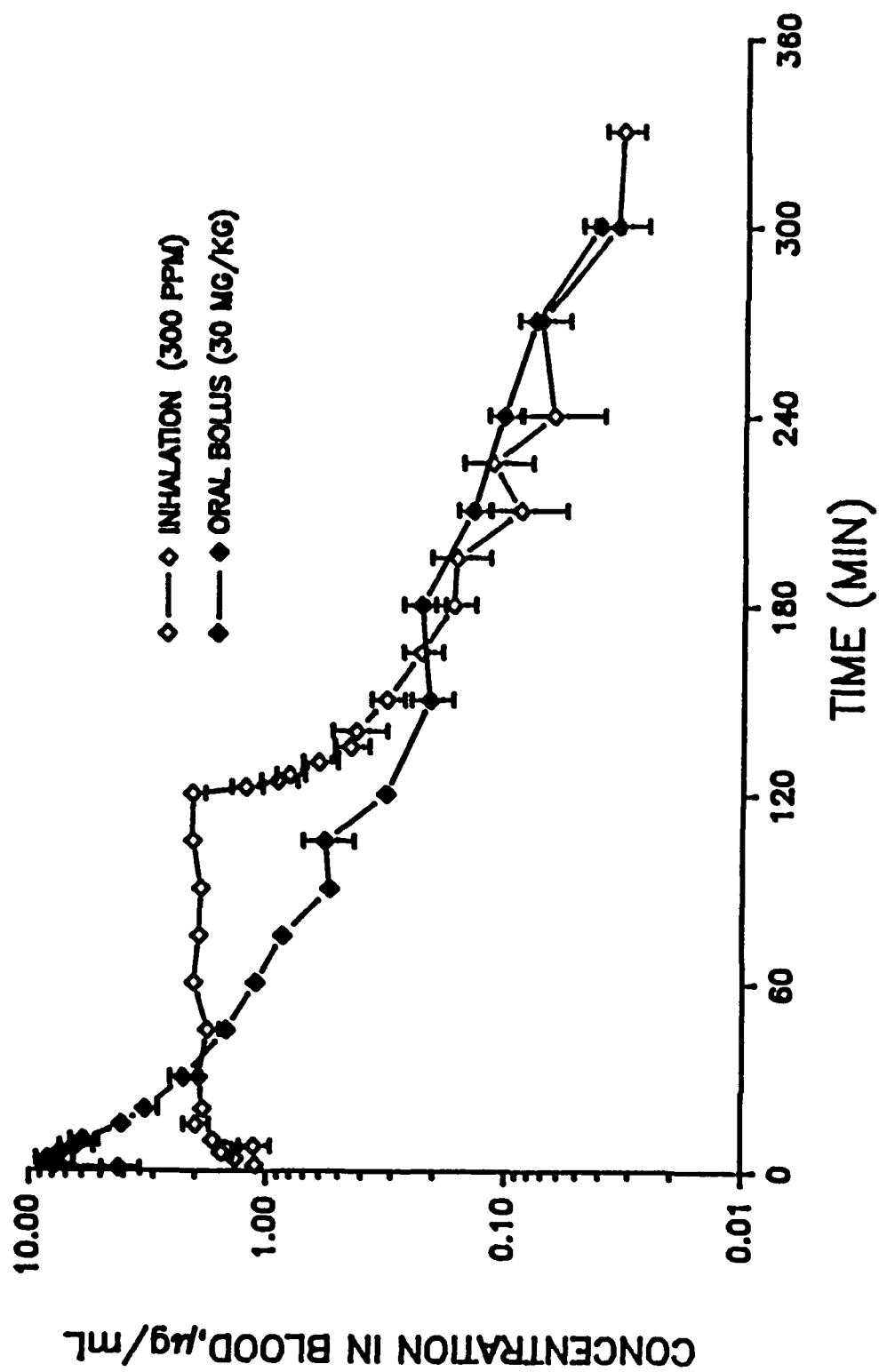


Fig. B-6a

1,1-DICHLOROETHYLENE: INHALATION VS. GASTRIC INFUSION
(300 PPM VS. 30 MG/KG)

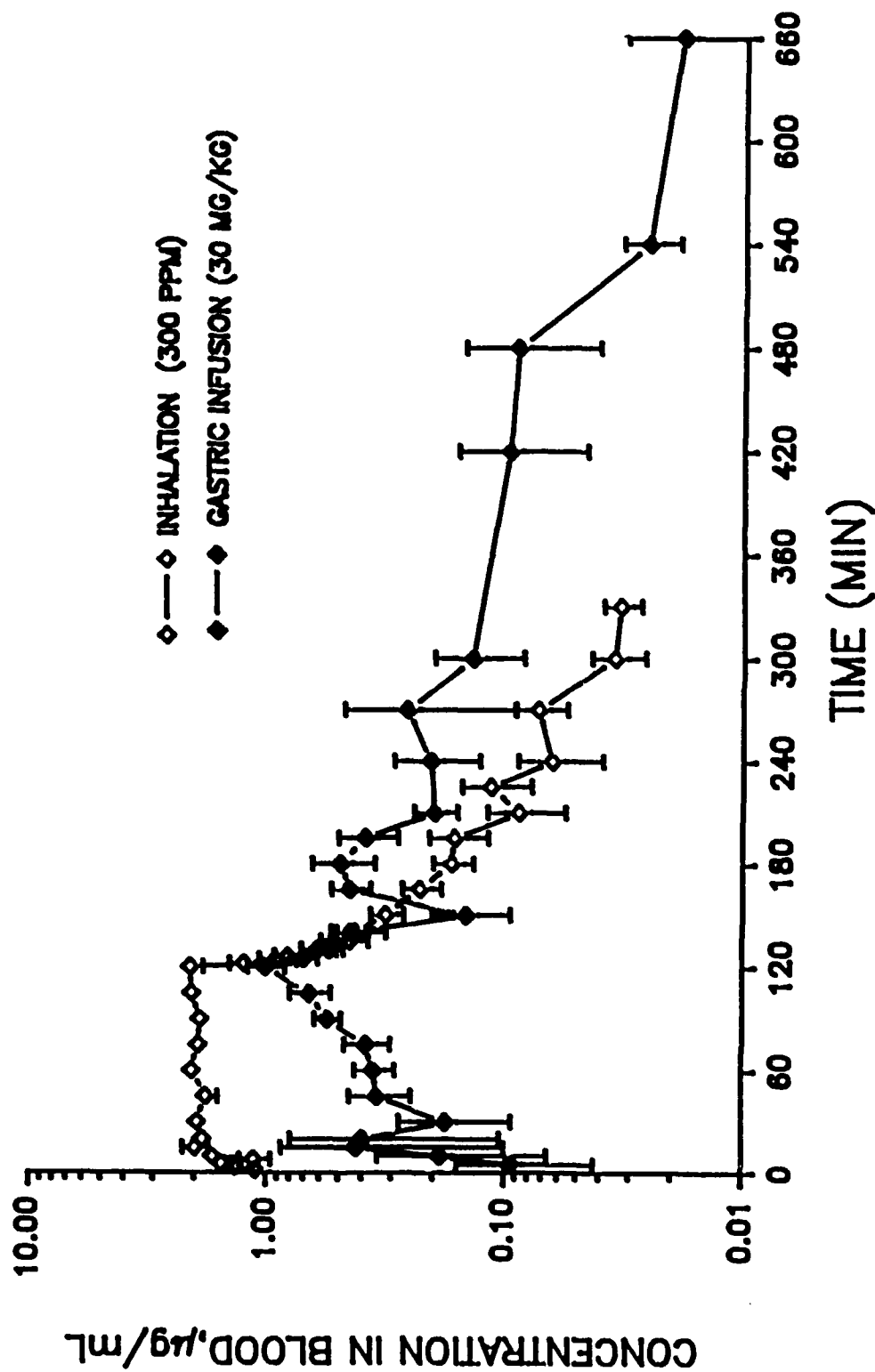


Fig. B-6b

1,1-DICHLOROETHYLENE: INHALATION VS. MULTIPLE BOLUS
(300 PPM VS. 30 MG/KG)

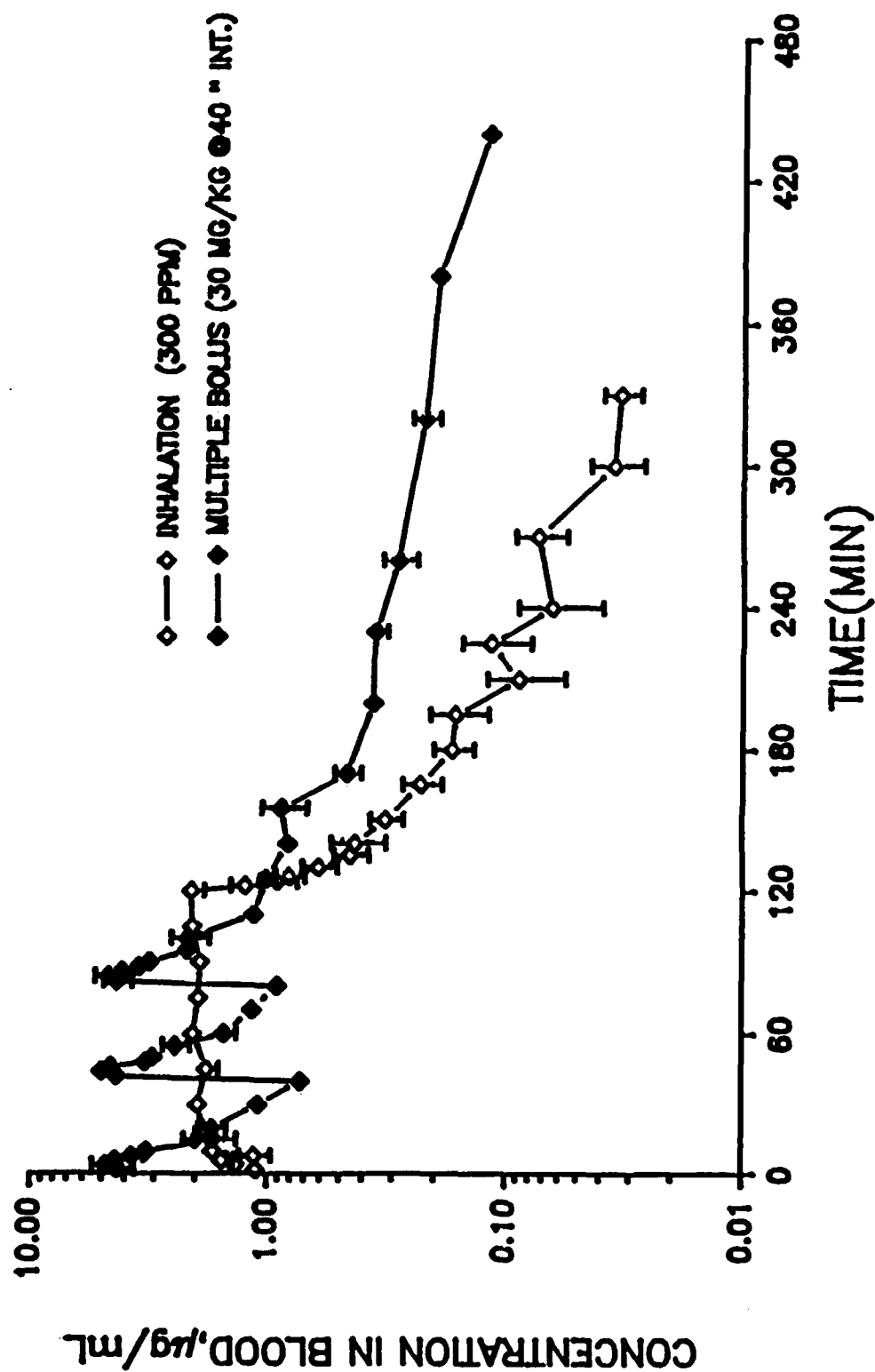
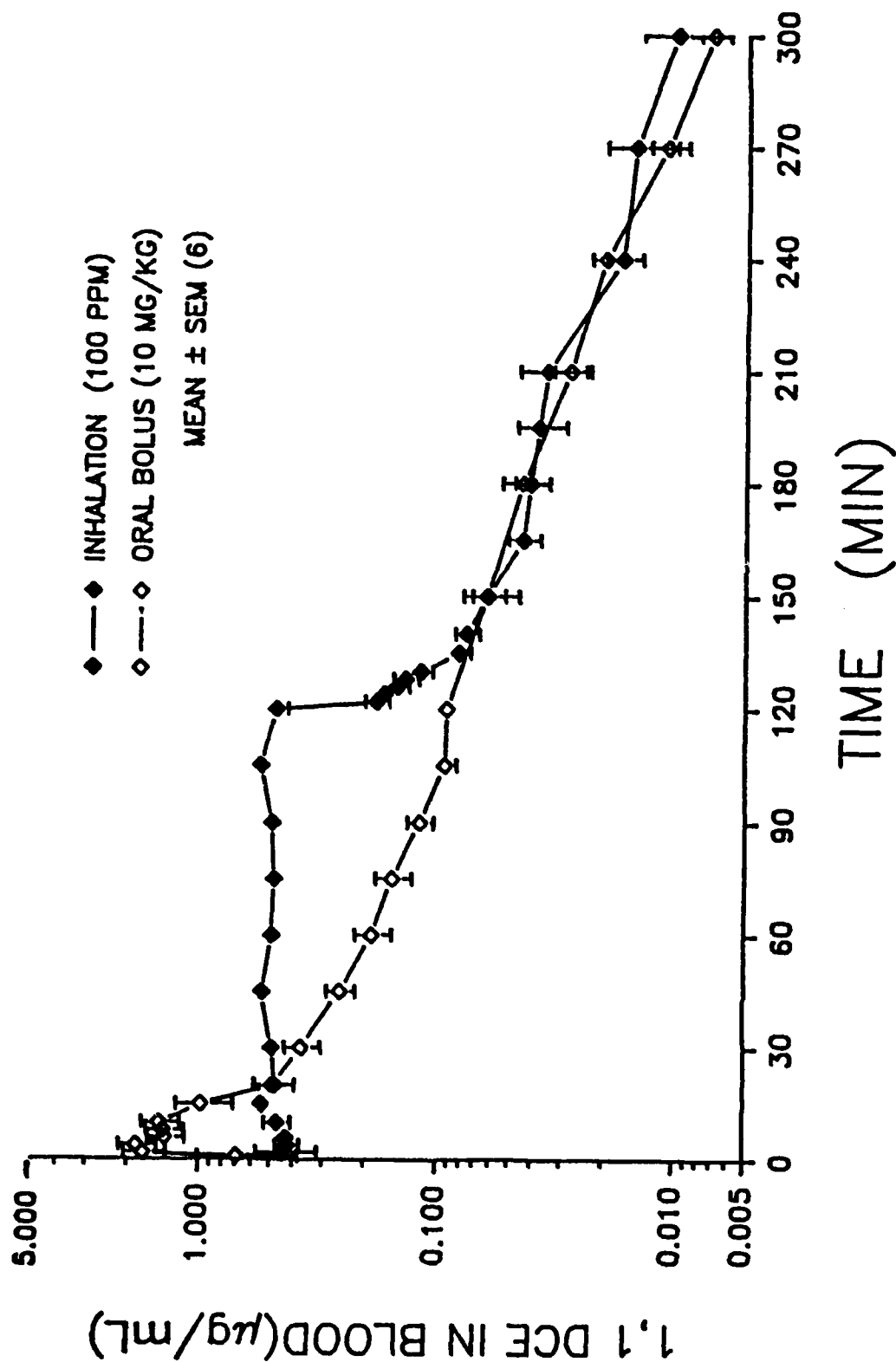


Fig. B-7a

1,1-DICHLOROETHYLENE: INHALATION VS. ORAL BOLUS
100 PPM VS. 10 MG/KG



1,1-DICHLOROETHYLENE: INHALATION VS. MULTIPLE BOLUS 100 PPM VS. 10 MG/KG

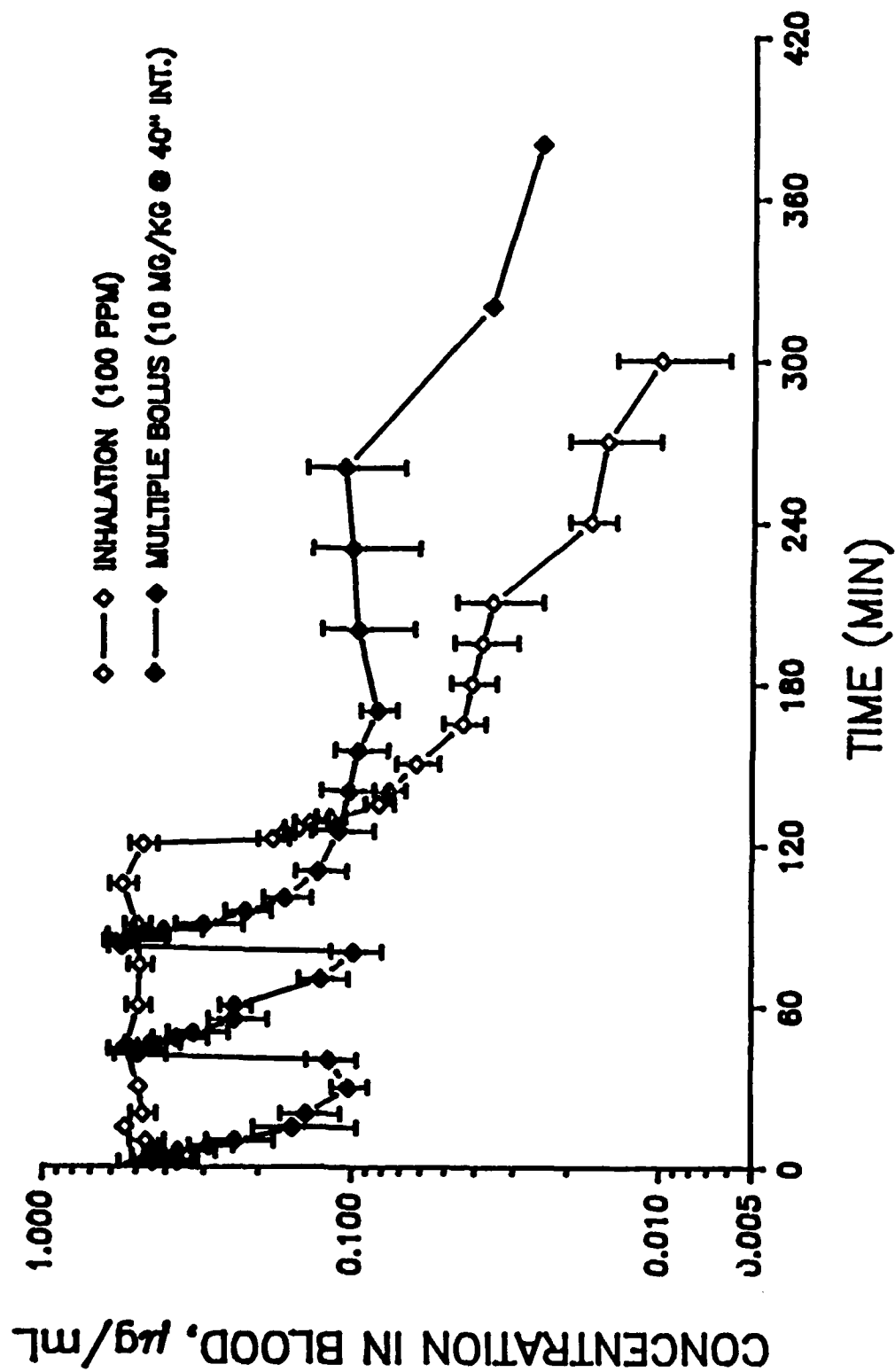


Fig. B-7b

1,1-DICHLOROETHYLENE: INHALATION VS. GASTRIC INFUSION

100 PPM VS. 10 MG/KG

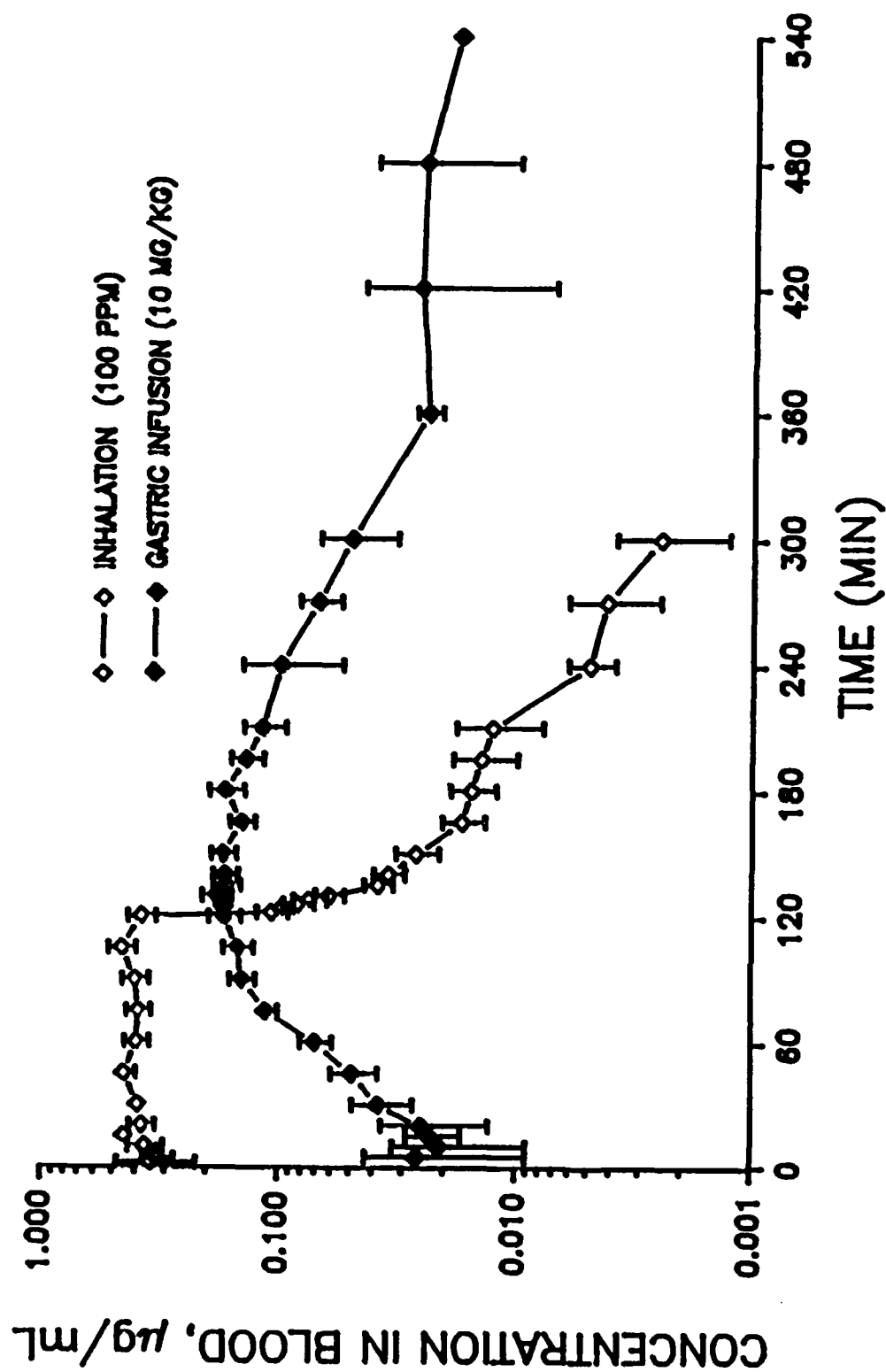


Fig. B-7c

APPENDIX C

STUDIES OF THE PHARMACOKINETICS OF PCE

- 1.) During and following inhalation exposures in rats
- 2.) Following oral administration in rats

Parameters for the Physiological Pharmacokinetic
Model for PER in the Rat

<u>Parameter</u>	<u>Value</u>
Alveolar Ventilation Rate (ml/min), V_a	115.3 (50 ppm exposure) 101.4 (500 ppm exposure)
Inhaled Gas Concentration (mg/ml)	0.351 (50 ppm exposure) 3.55 (500 ppm exposure)
Alveolar Mass Transfer Coefficient	500 ml/min
Blood Flows (ml/min)	
Cardiac output, Q_b	106.4
Fat, Q_f	9.4
Liver, Q_{li}	39.8
Muscle, Q_m	12.8
Richly Perfused, Q_r	44.4
Tissue Volumes (ml)	
Alveolar	2.0
Blood	25.4
Fat	30.5
Liver	13.1
Lung	3.97
Muscle	248.0
Richly Perfused	15.2
Partition Coefficients	
Lungs:Air	70.3
Fat:Blood	108.99
Liver:Blood	3.72
Muscle:Blood	1.058
Richly Perfused: Blood	3.72
Metabolism Constants	
V_{max} ($\mu\text{g}/\text{min}$)	5.86
K_m ($\mu\text{g}/\text{ml}$)	2.938

Alveolar ventilation rates and inhaled concentrations were directly measured in the laboratory. Compartmental volumes and organ blood flows were obtained from Ramsey and Andersen (Toxicol. Appl. Pharmacol. 73:159-175,1984) and scaled to 340 g, the mean bw of rats in the present study. Partition coefficients and metabolism constants were utilized from Chen and Blancato (Pharmacokinetics in Risk Assessment, Drinking Water and Health Vol. 8:369-390, 1987). The alveolar mass transfer coefficient was based on the alveolar permeability-area product for methylene chloride of Angelo and Pritchard (Pharmacokinetics in Risk Assessment, Drinking Water and Health Vol.8:254-264,1987).

TABLE C-1

INHALATION EXPOSURE SYSTEM

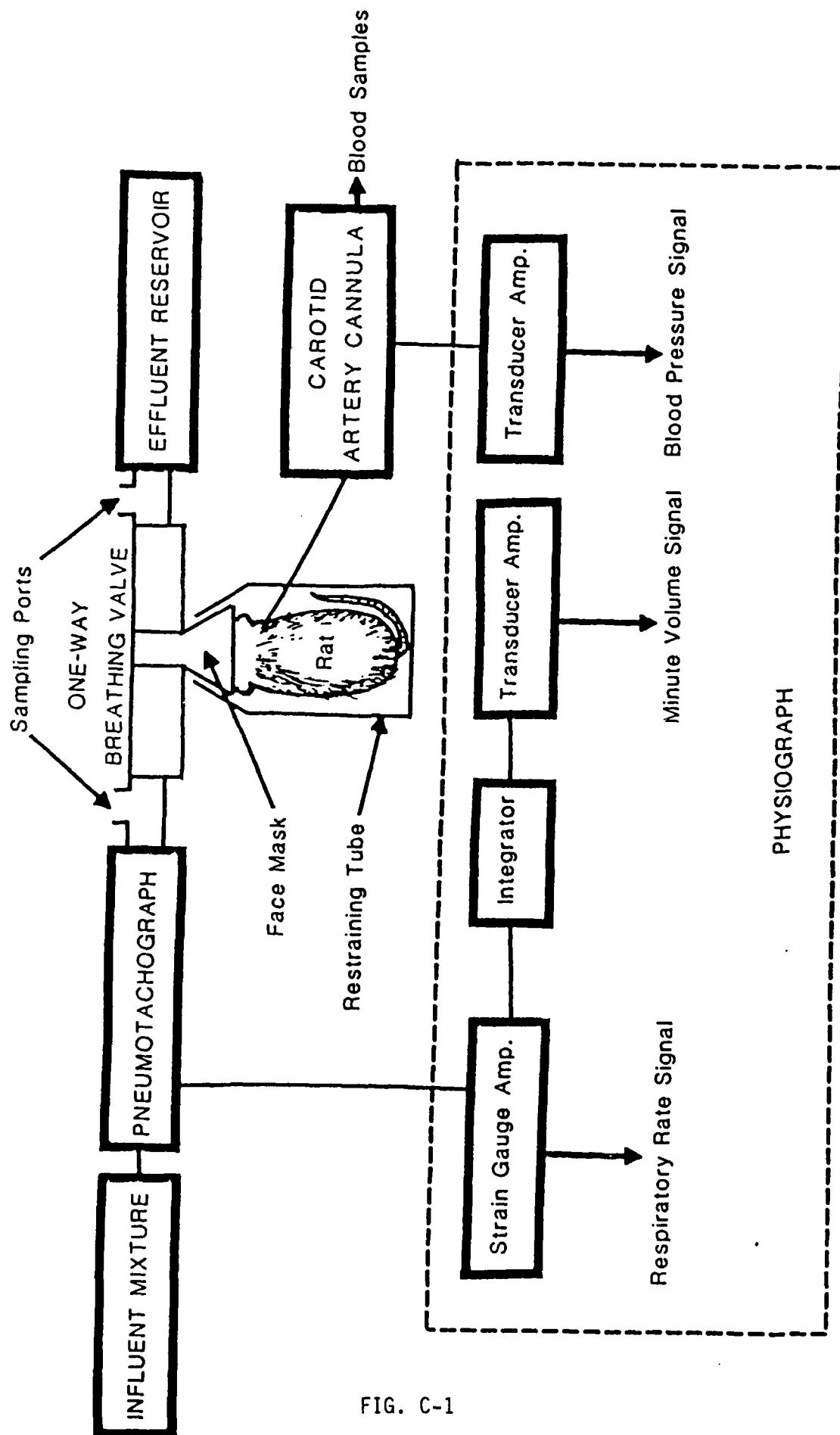


FIG. C-1

500 PPM PERCHLOROETHYLENE INHALATION EXPOSURE EXHALED BREATH CONCENTRATION

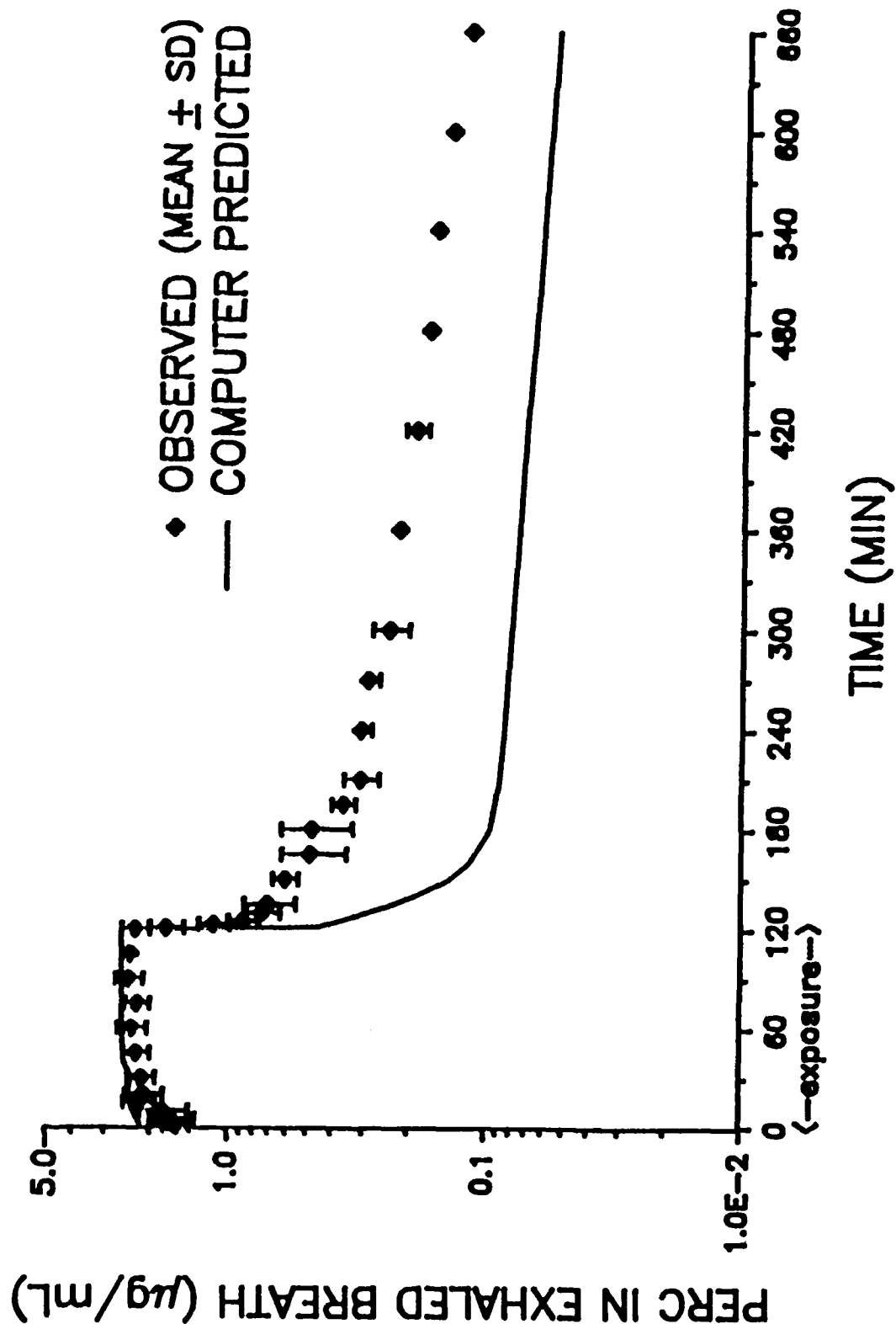


Fig. C-2

50 PPM PERCHLOROETHYLENE INHALATION EXPOSURE EXHALED BREATH CONCENTRATION

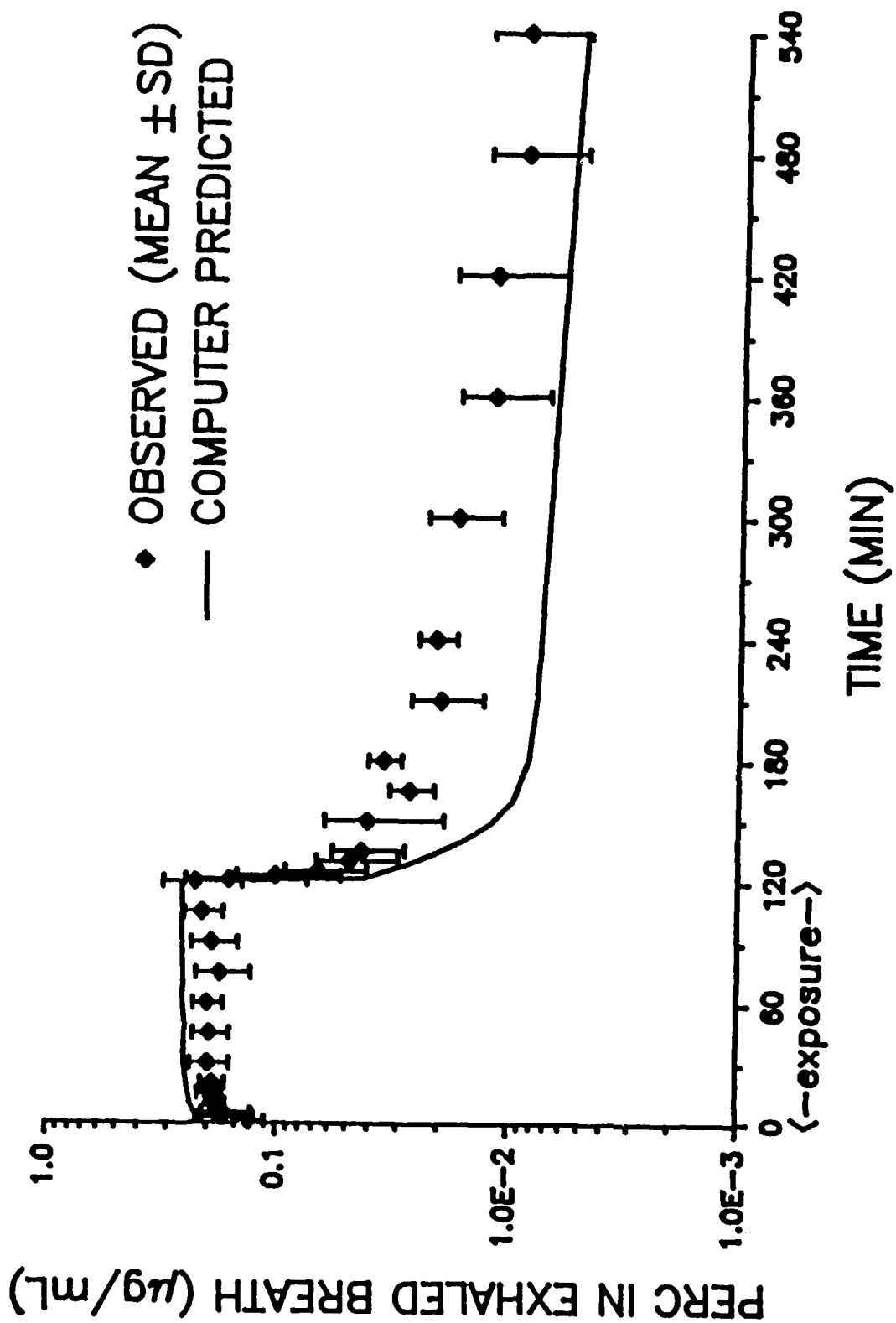


Fig. C-3

500 PPM PERCHLOROETHYLENE INHALATION EXPOSURE ARTERIAL BLOOD CONCENTRATION

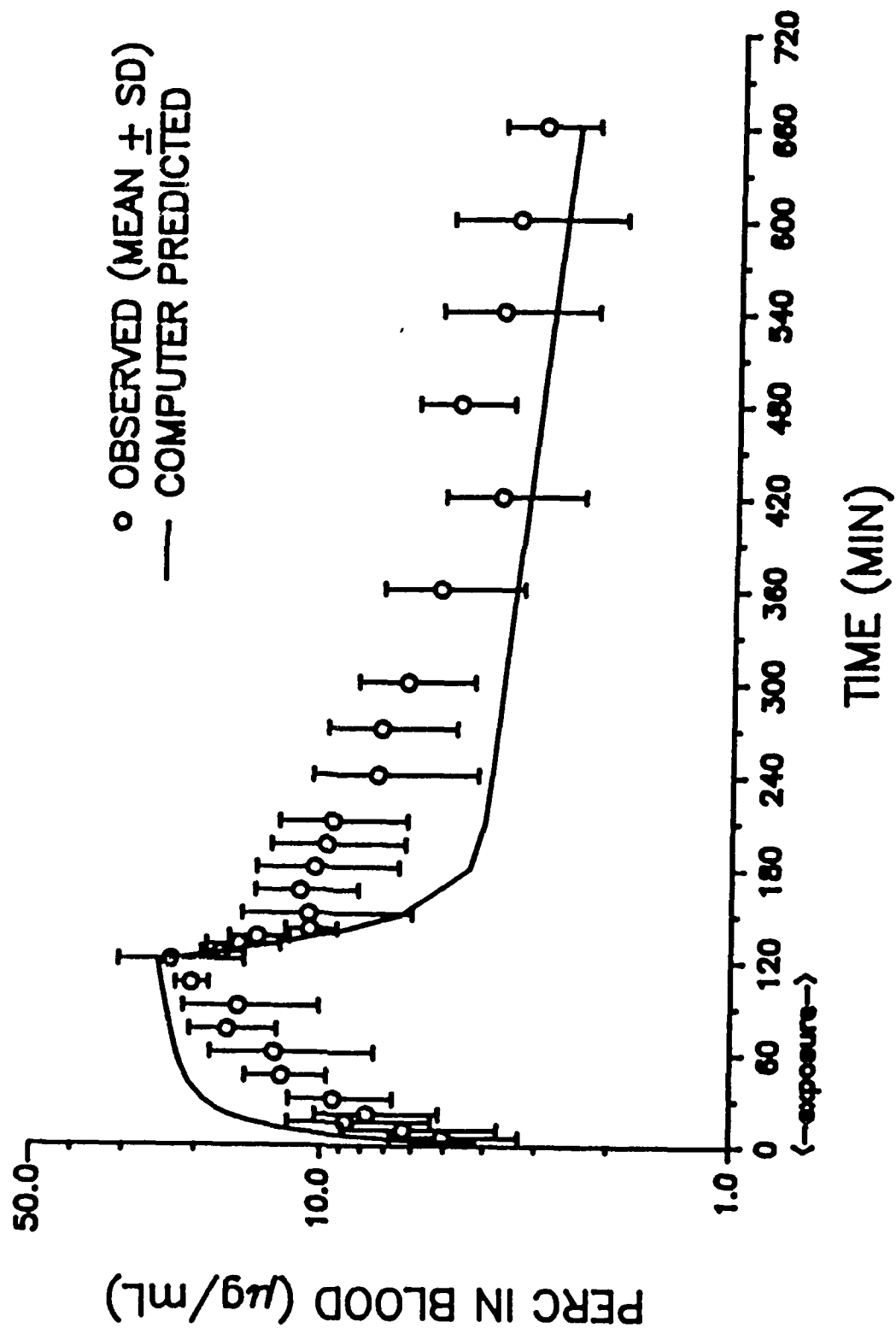
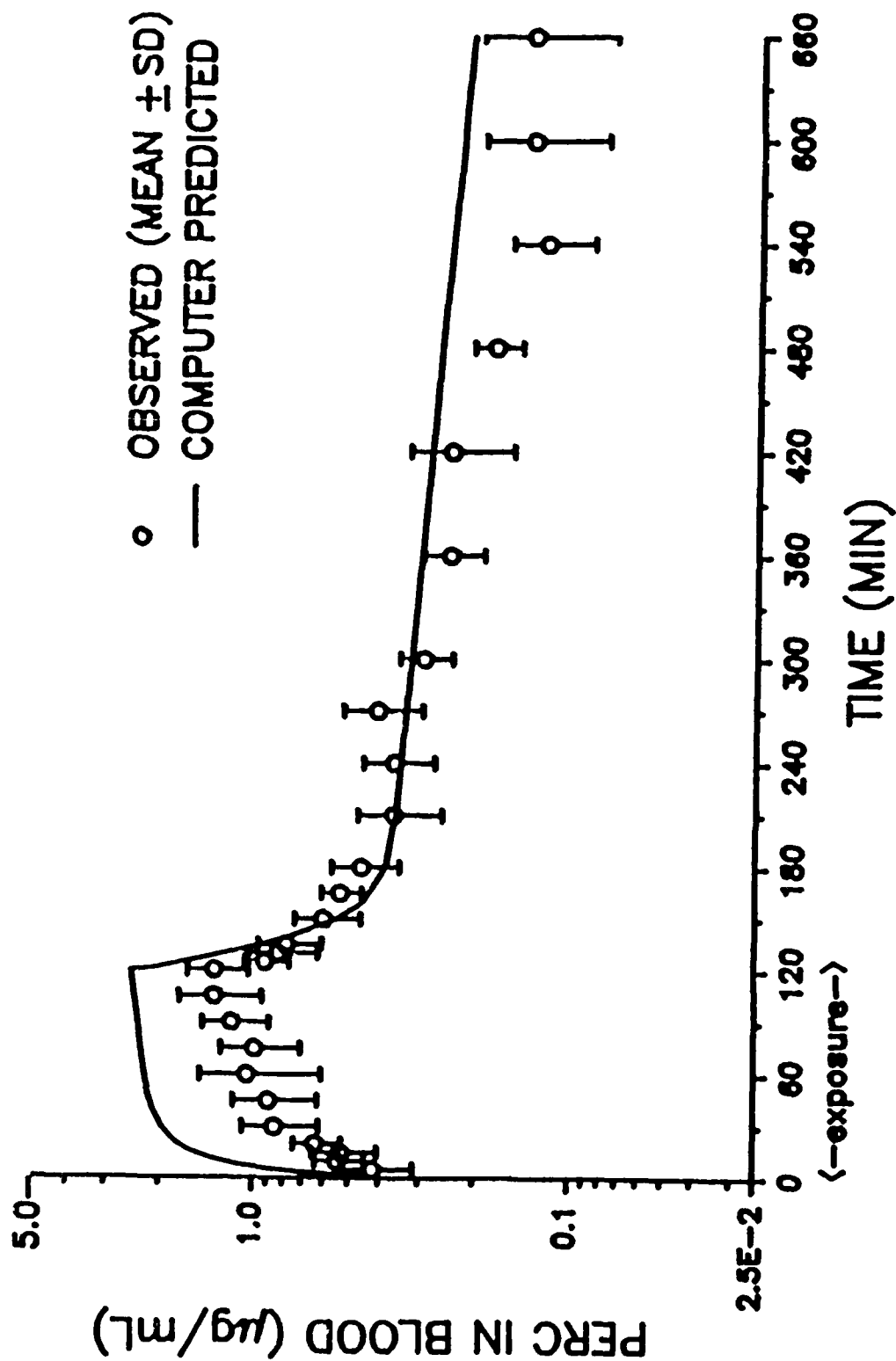


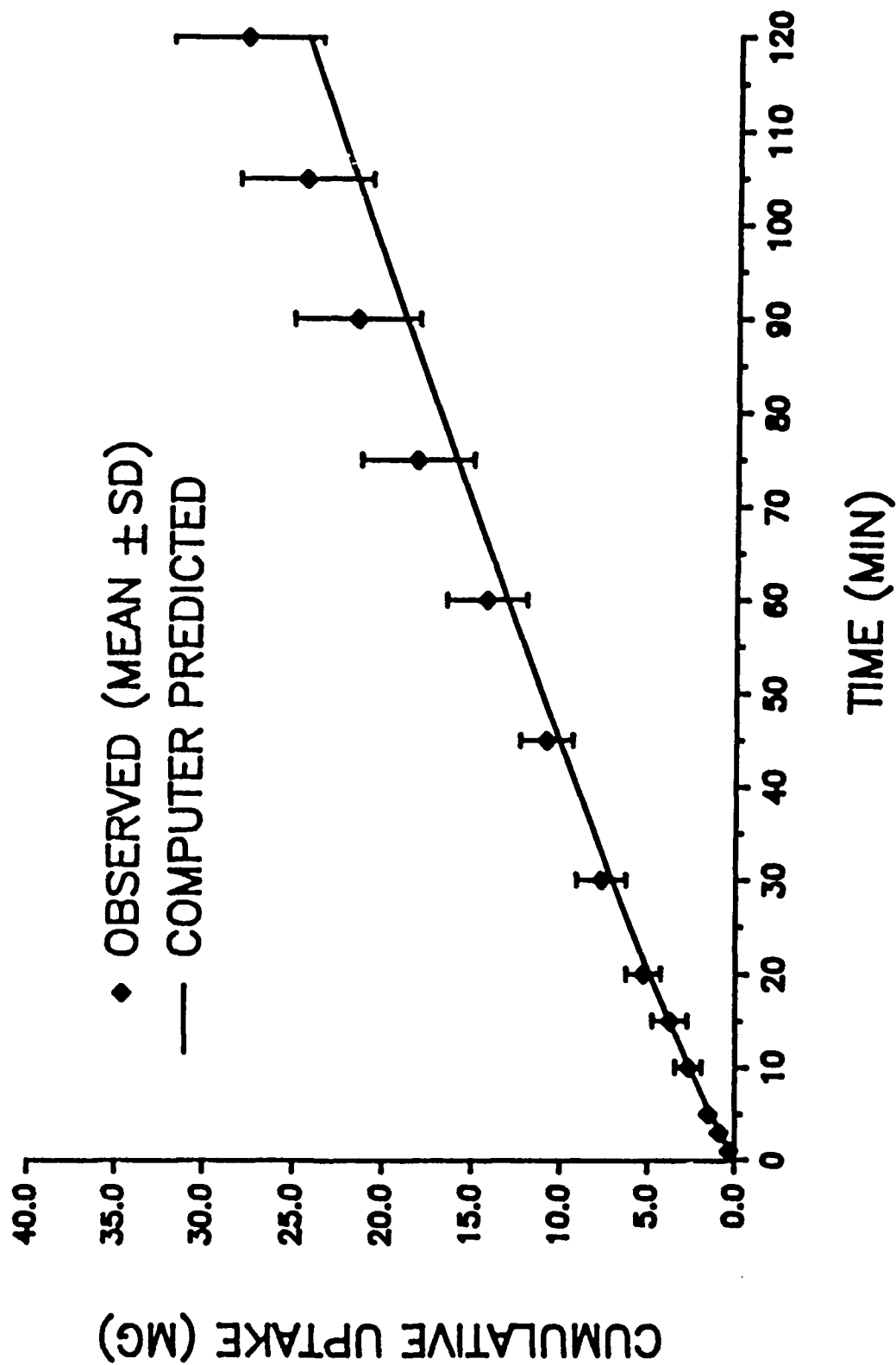
Fig. C-4

50 PPM PERCHLOROETHYLENE INHALATION EXPOSURE

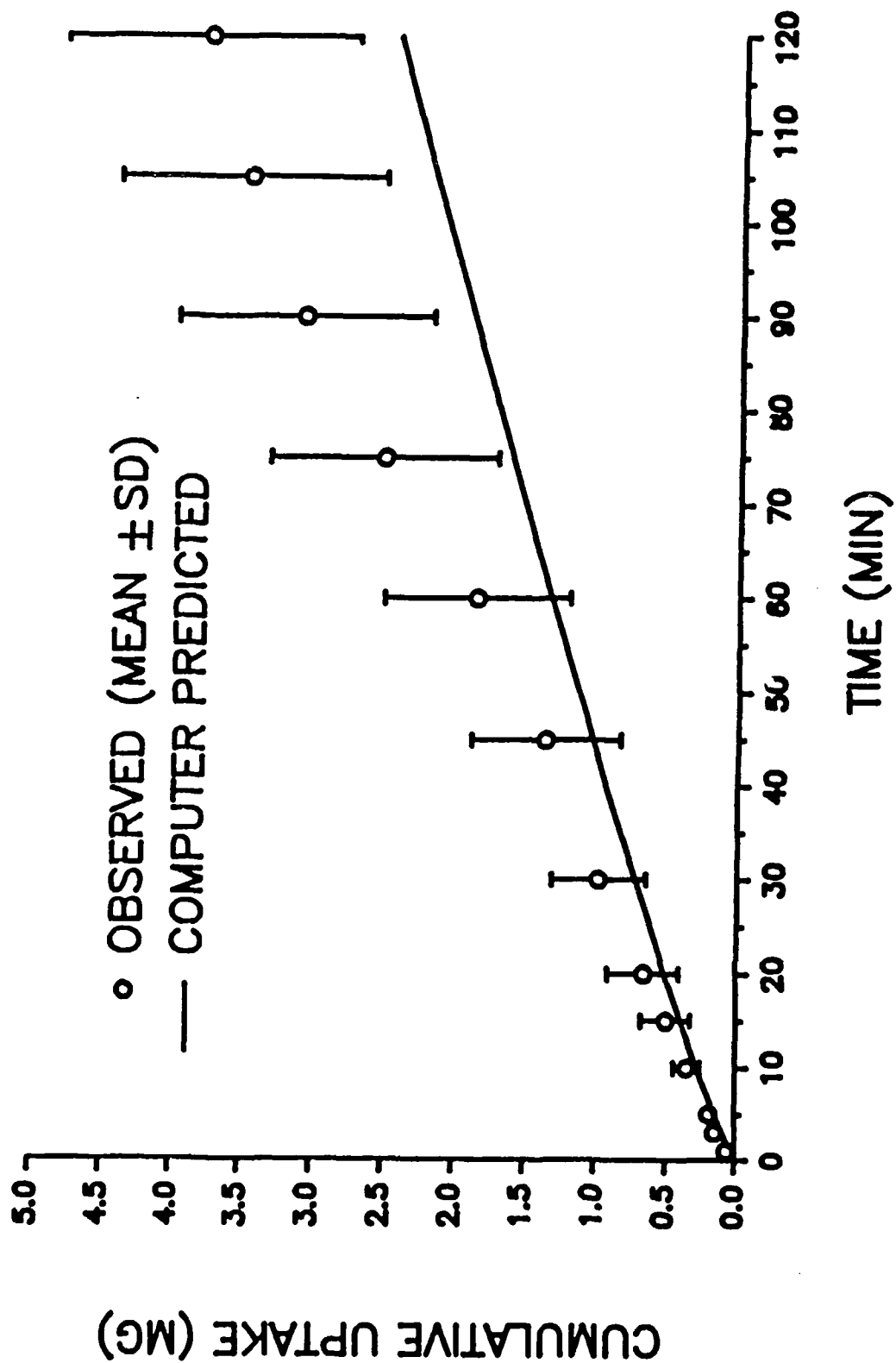
ARTERIAL BLOOD CONCENTRATION



500 PPM PERCHLOROETHYLENE INHALATION EXPOSURE CUMULATIVE UPTAKE



50 PPM PERCHLOROETHYLENE INHALATION EXPOSURE CUMULATIVE UPTAKE



PERCHLOROETHYLENE

ORAL BOLUS

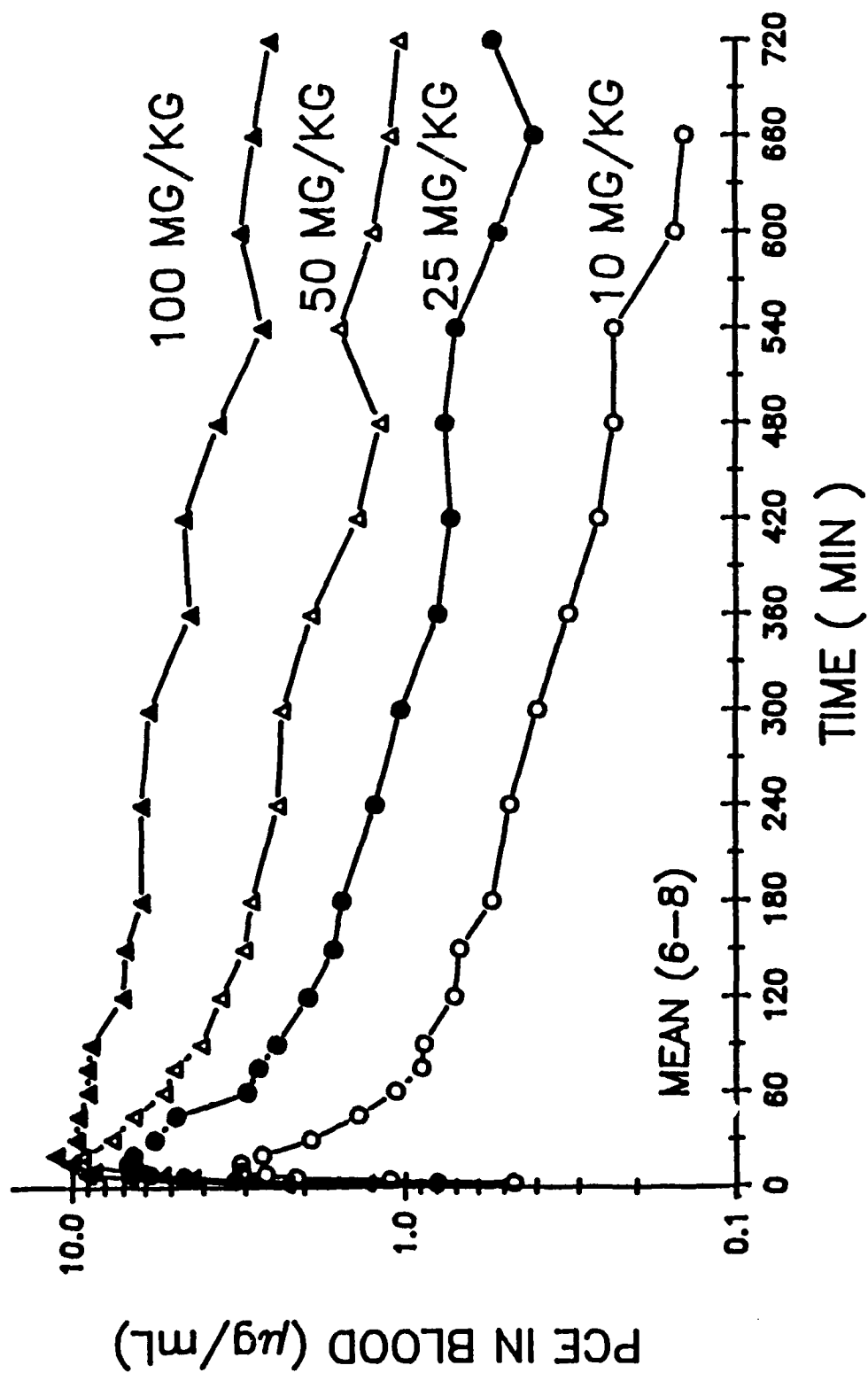
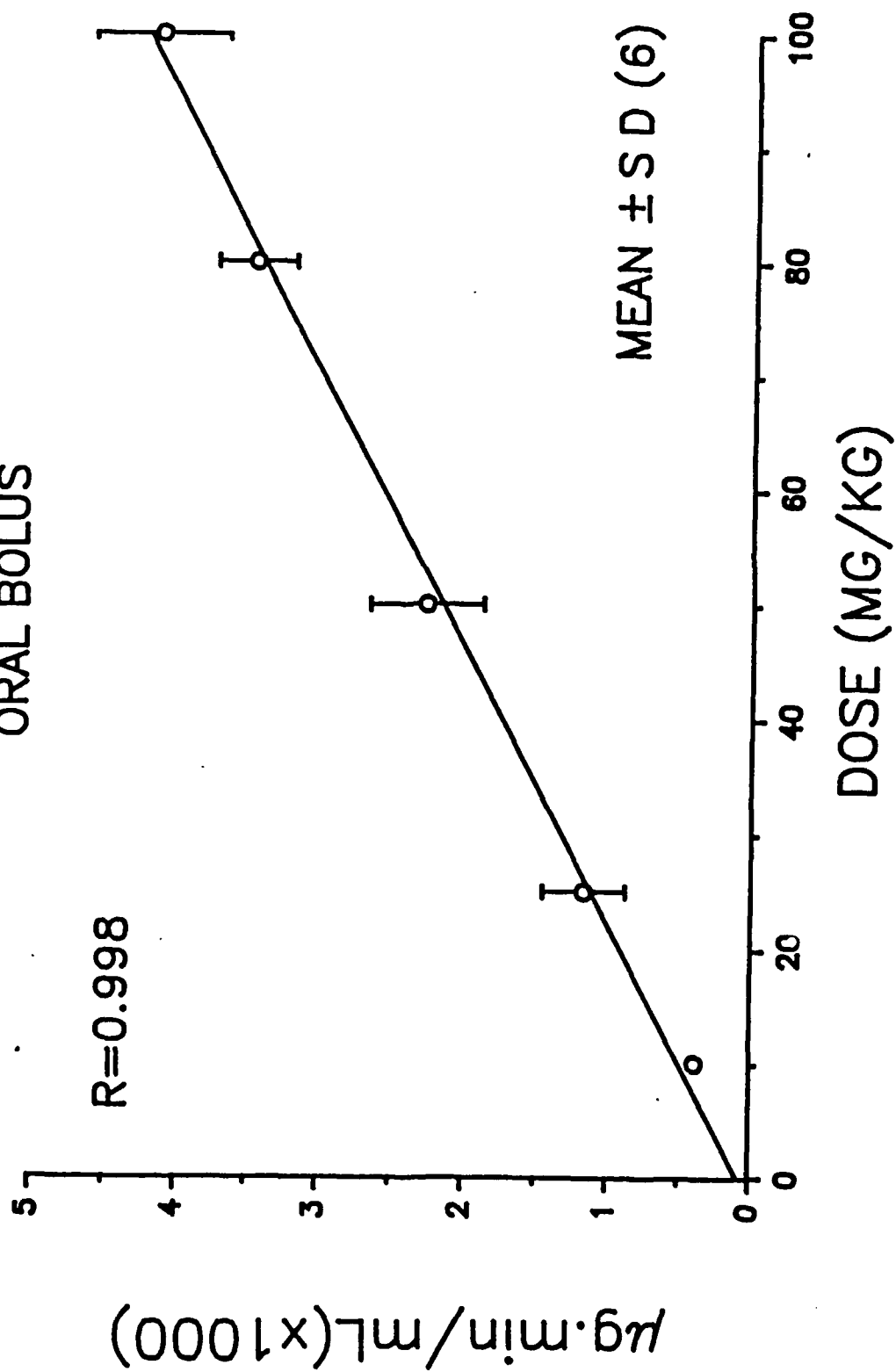


Fig. C-9

PERCHLOROETHYLENE

DOSE VS. AUC

ORAL BOLUS



APPENDIX D

STUDIES OF THE TISSUE UPTAKE, DISPOSITION, AND ELIMINATION OF PCE IN RATS

- 1.) Following intraarterial administration
- 2.) Following oral administration

PCE SACRIFICED AFTER 15 MIN(IA.)

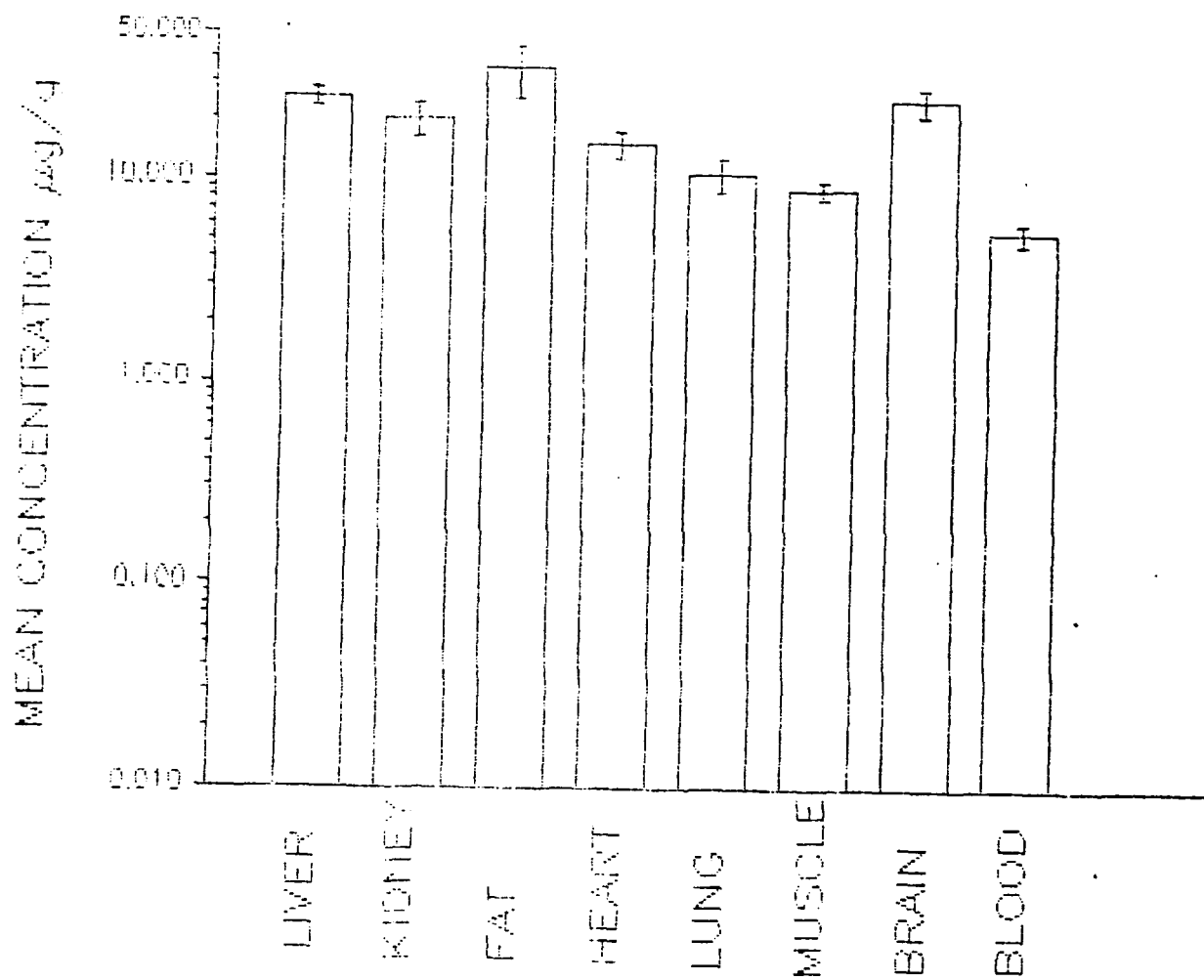


Fig. D-1

PCE SACRIFICED AFRET 30 MIN(IA.)

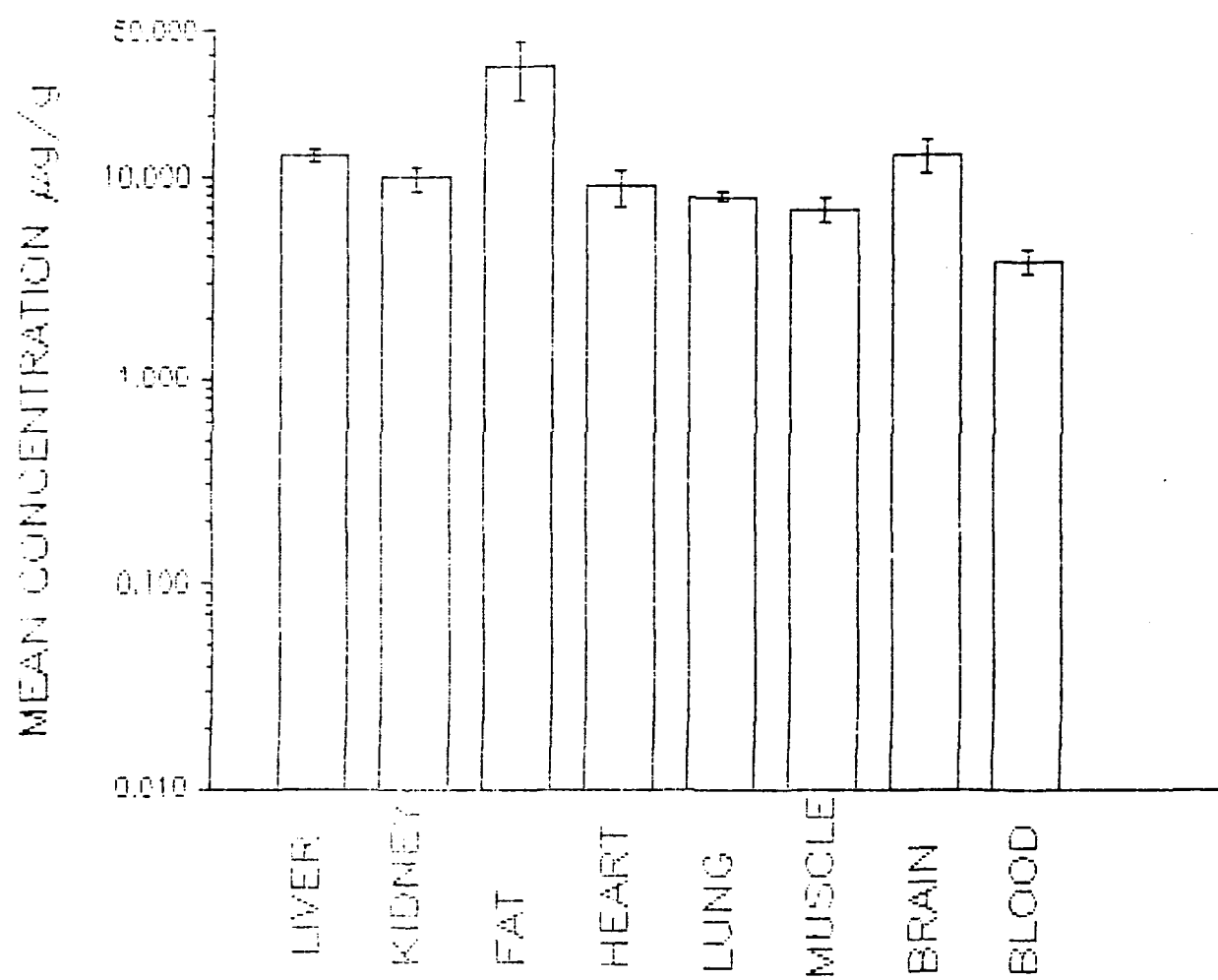


Fig. D-2

PCE SACRIFICED AFTER 1 HOUR(IA.)

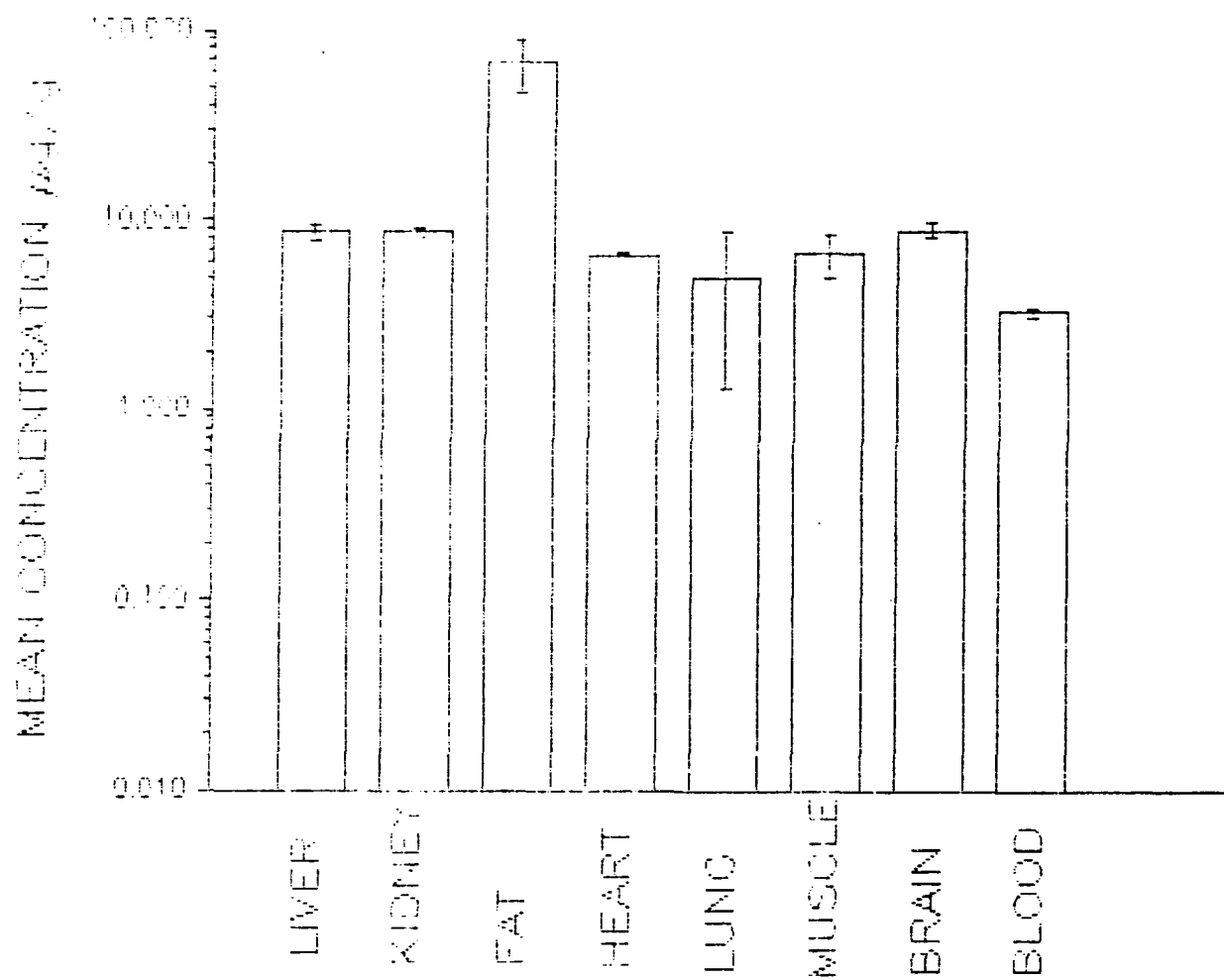


Fig. D-3

ACE SACRIFICED AFTER 2 HOURS (IA.)

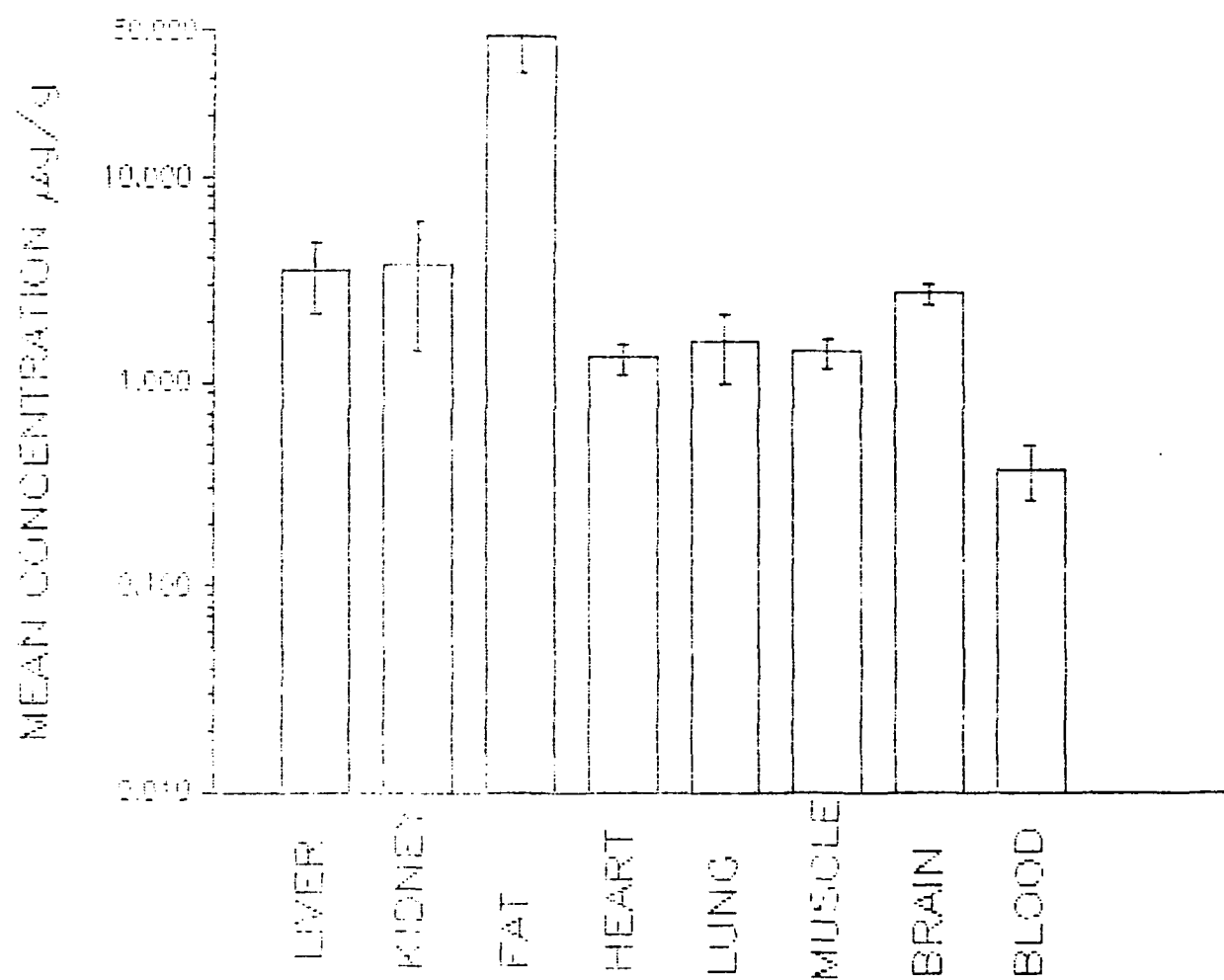


Fig. D-4

POE SACRIFICED AFTER 4 HOURS(IA.)

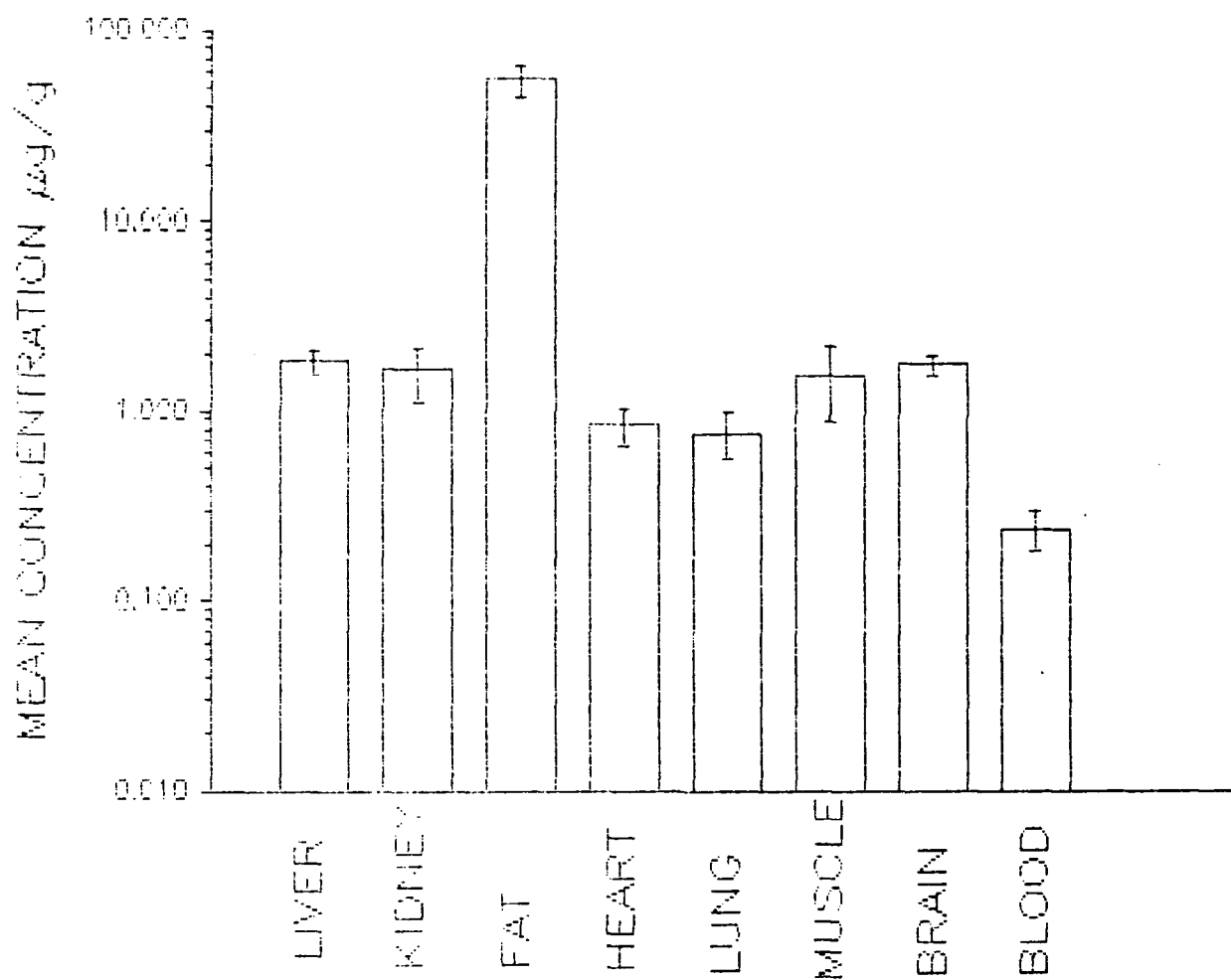


Fig. D-5

ACE SACRIFICED AFTER 6 HOURS (IA.)

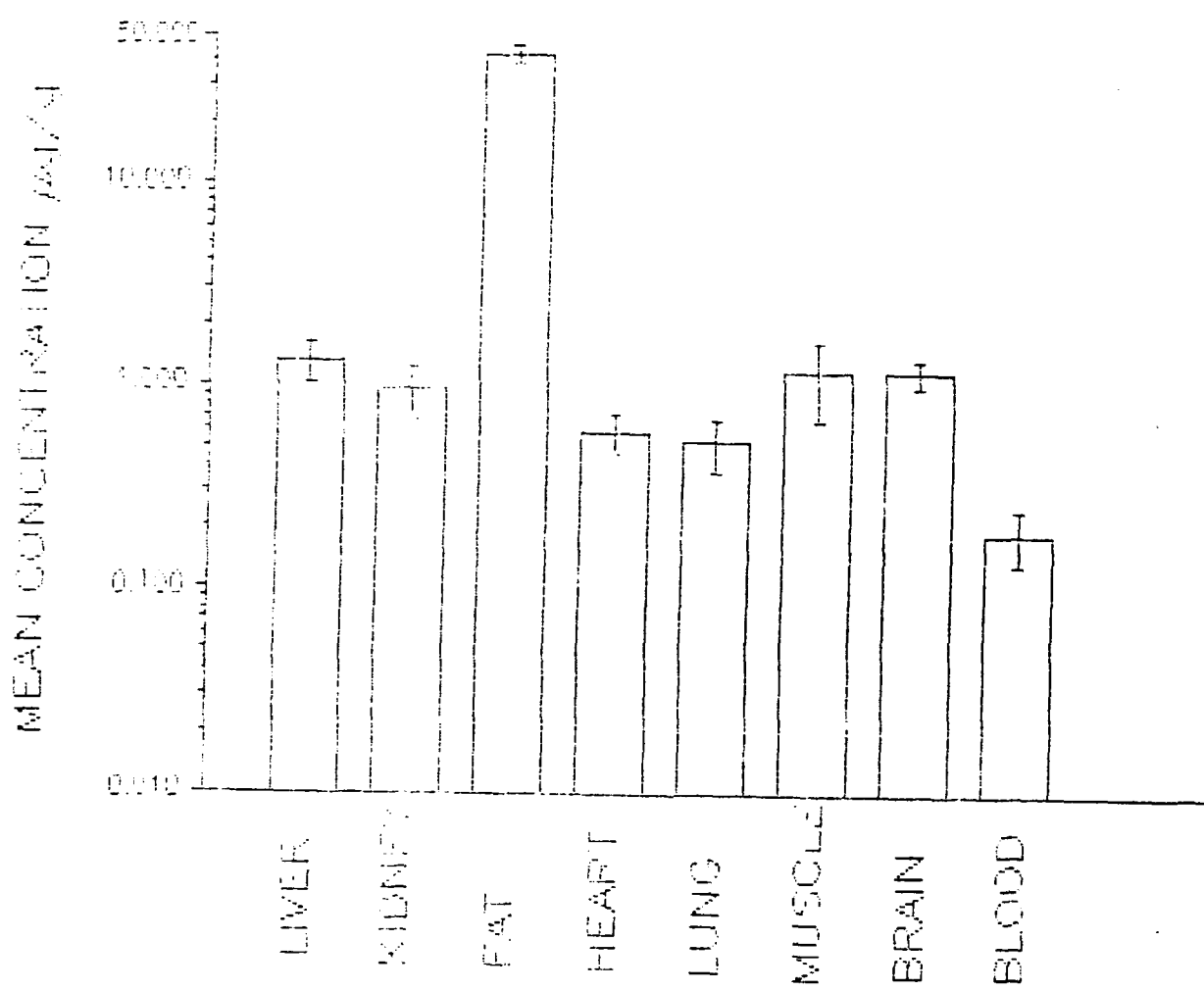


Fig. D-6

PIE SACRIFICED AFTER 12 HOURS(IA.)

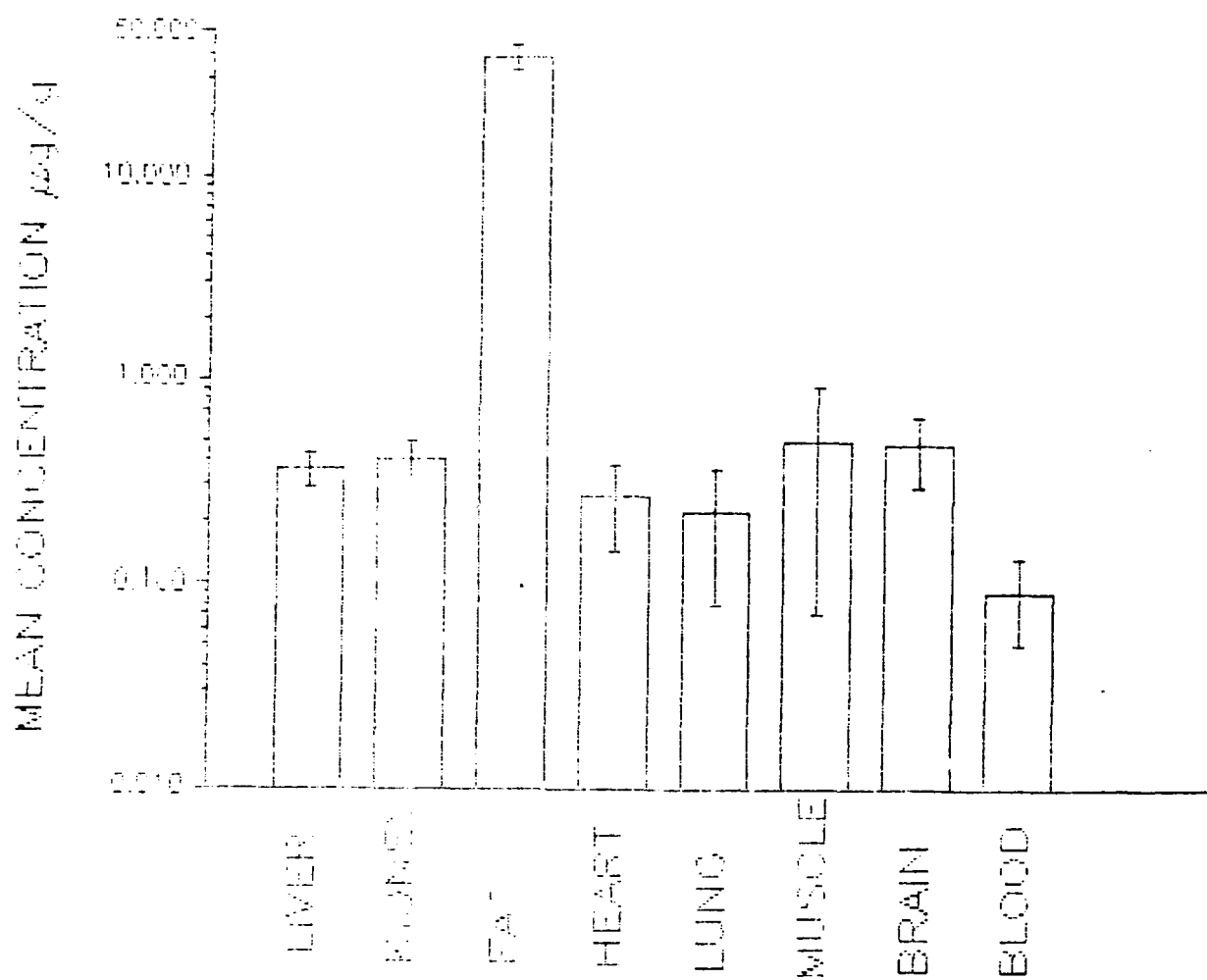


Fig. D-7

ROE SACRIFICED AFTER 18 HOURS (IA.)

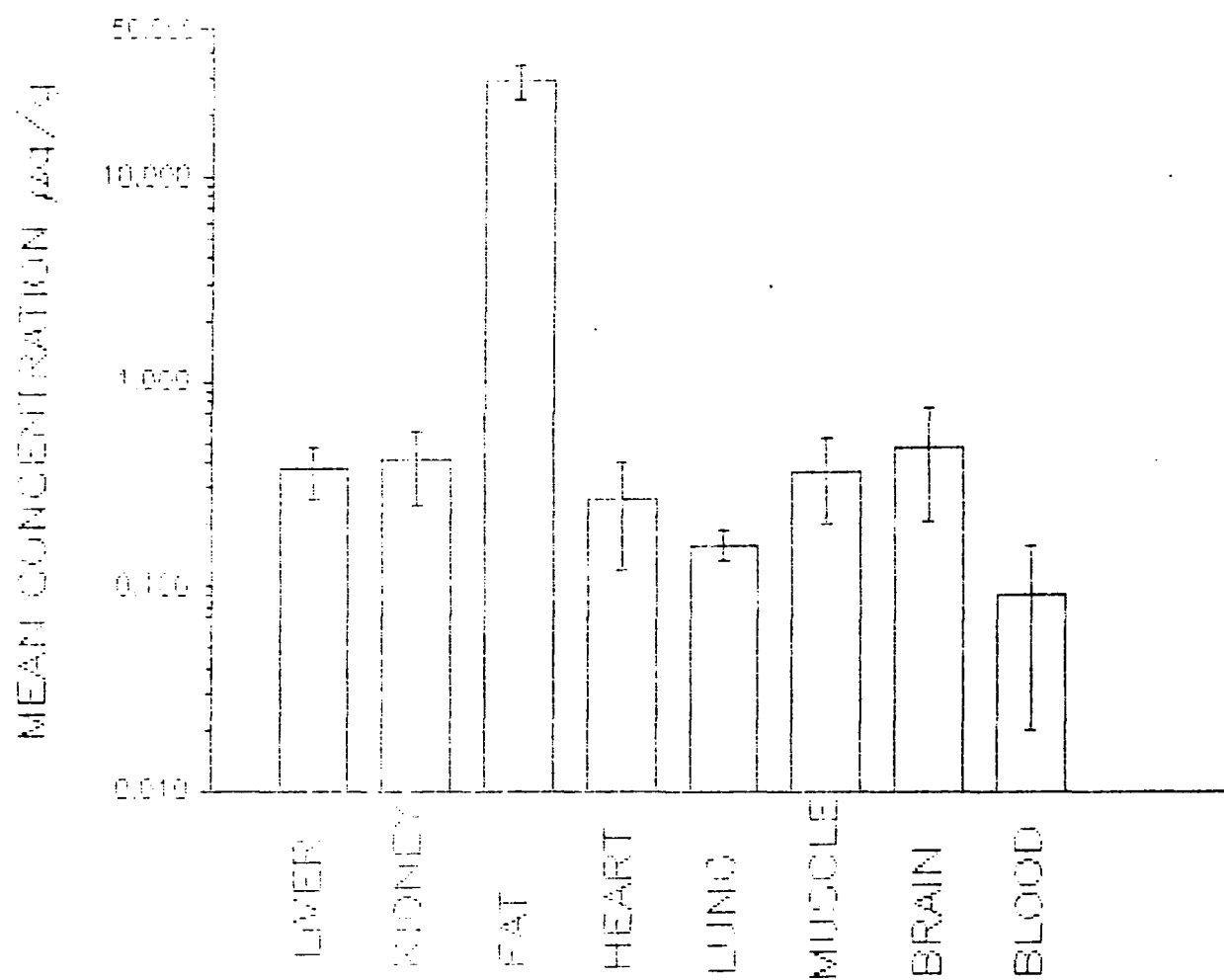


Fig. D-8

PCE SACRIFICED AFTER 24 HOURS (IA.)

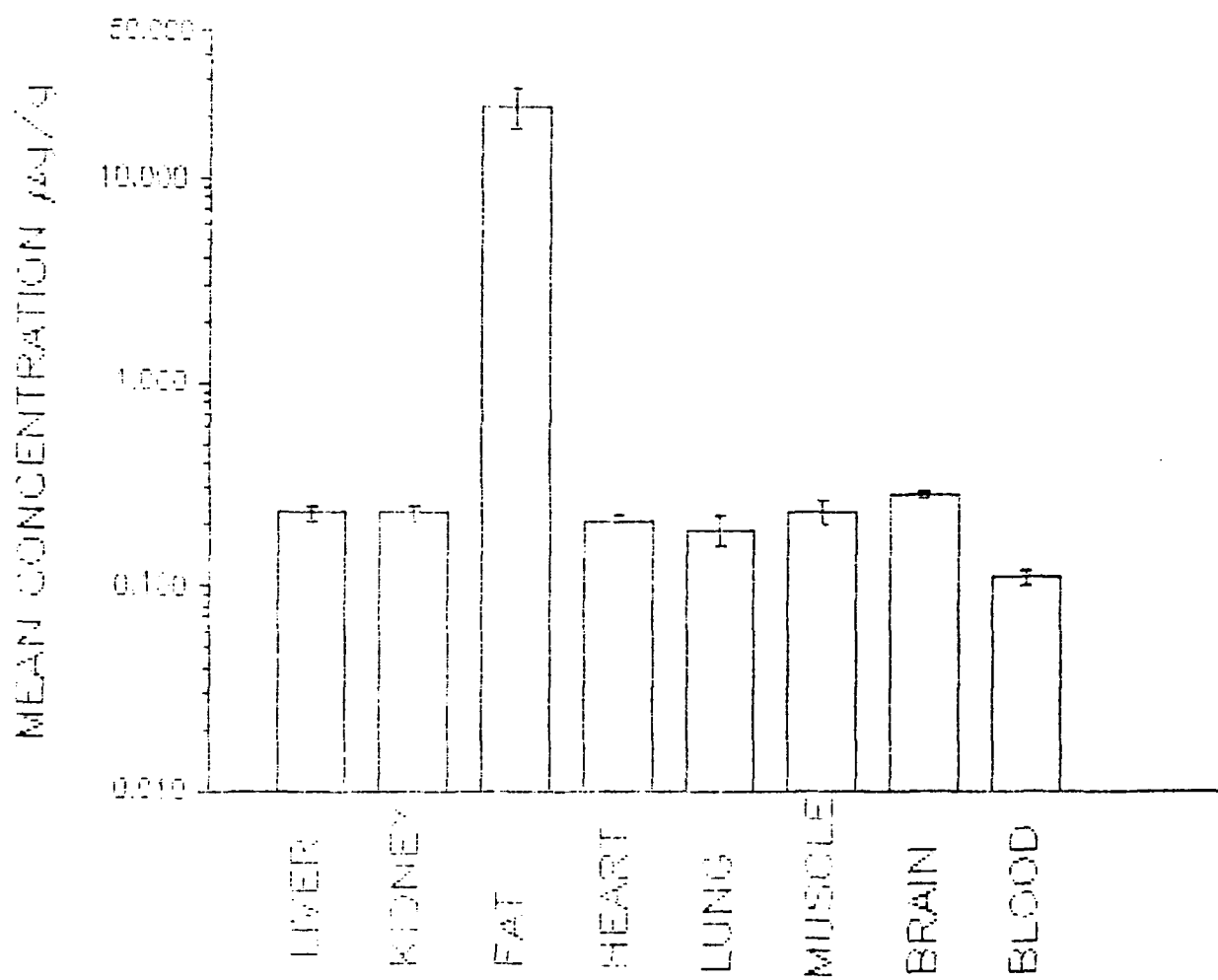


Fig. D-9

PCE SACRIFICED AFTER 48 HOURS(1A.)

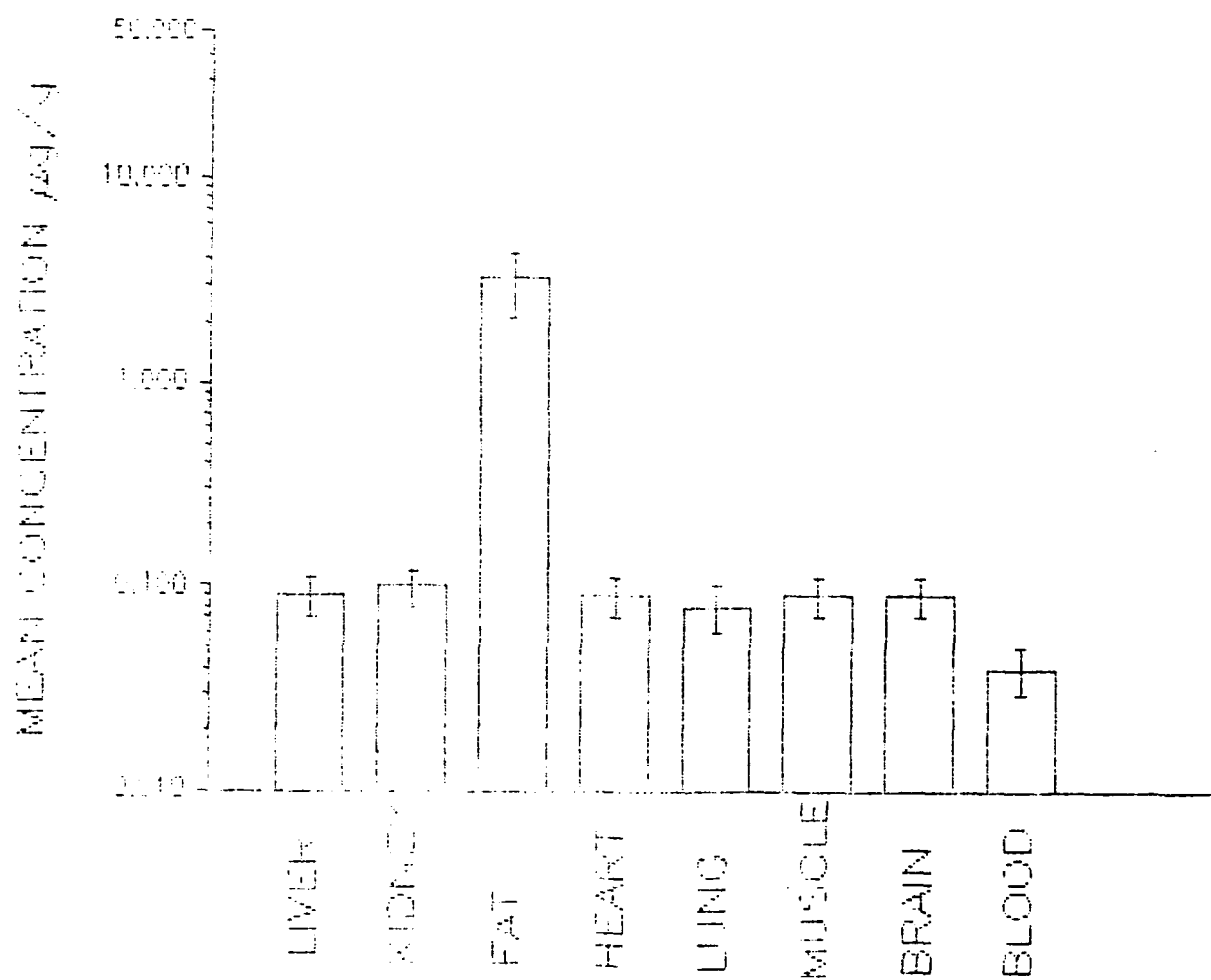


Fig. D-10

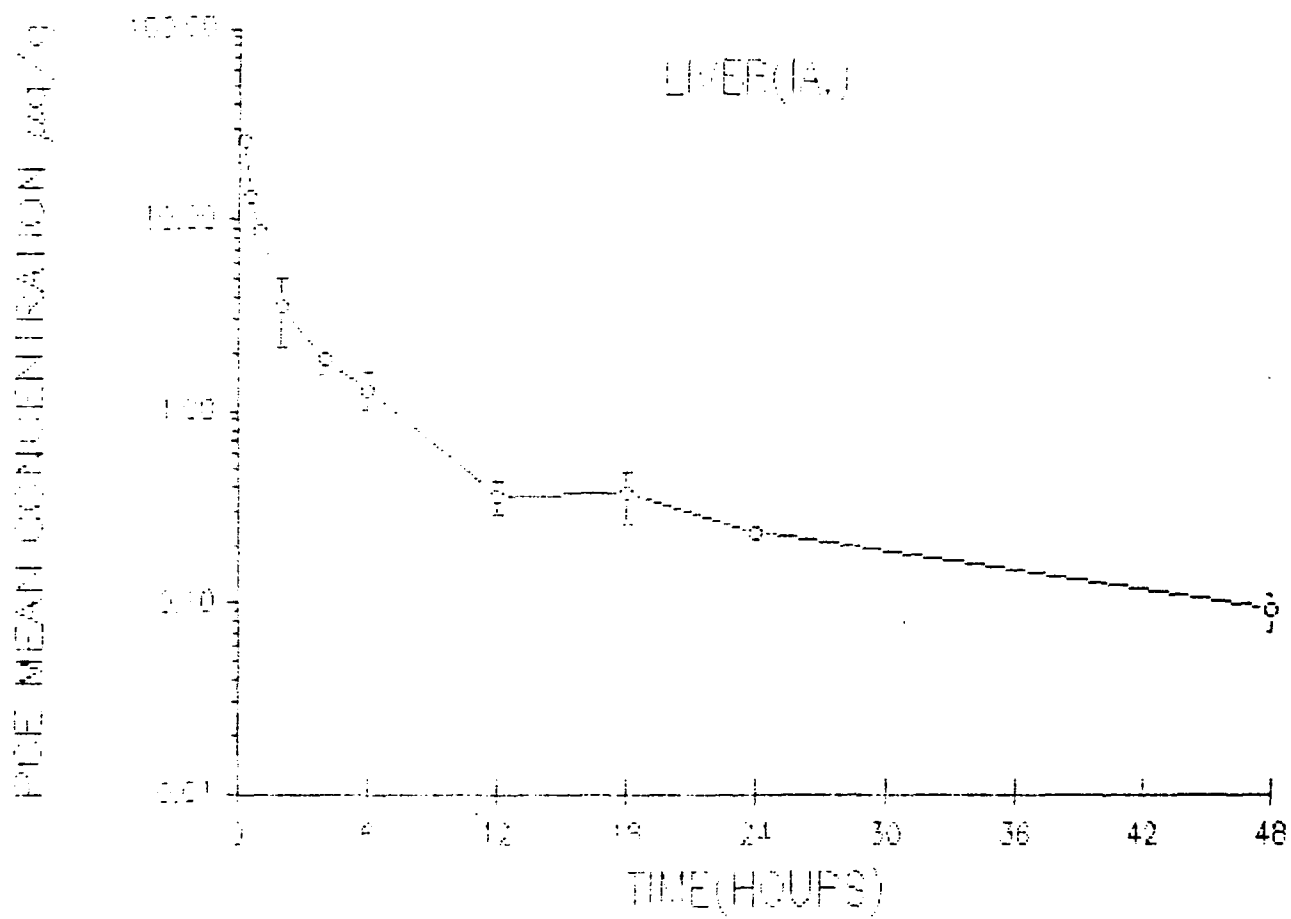


Fig. D-11

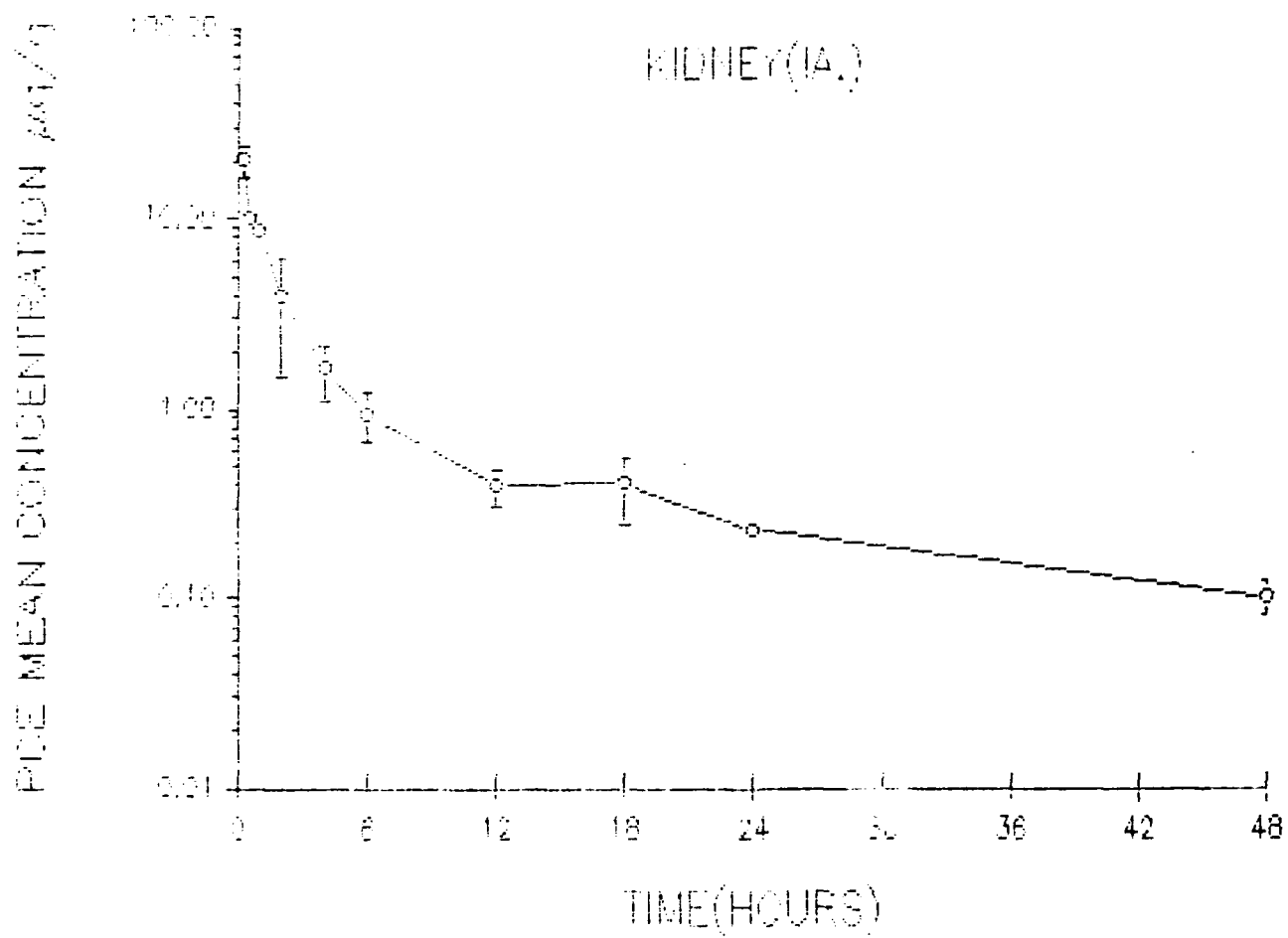


Fig. D-12

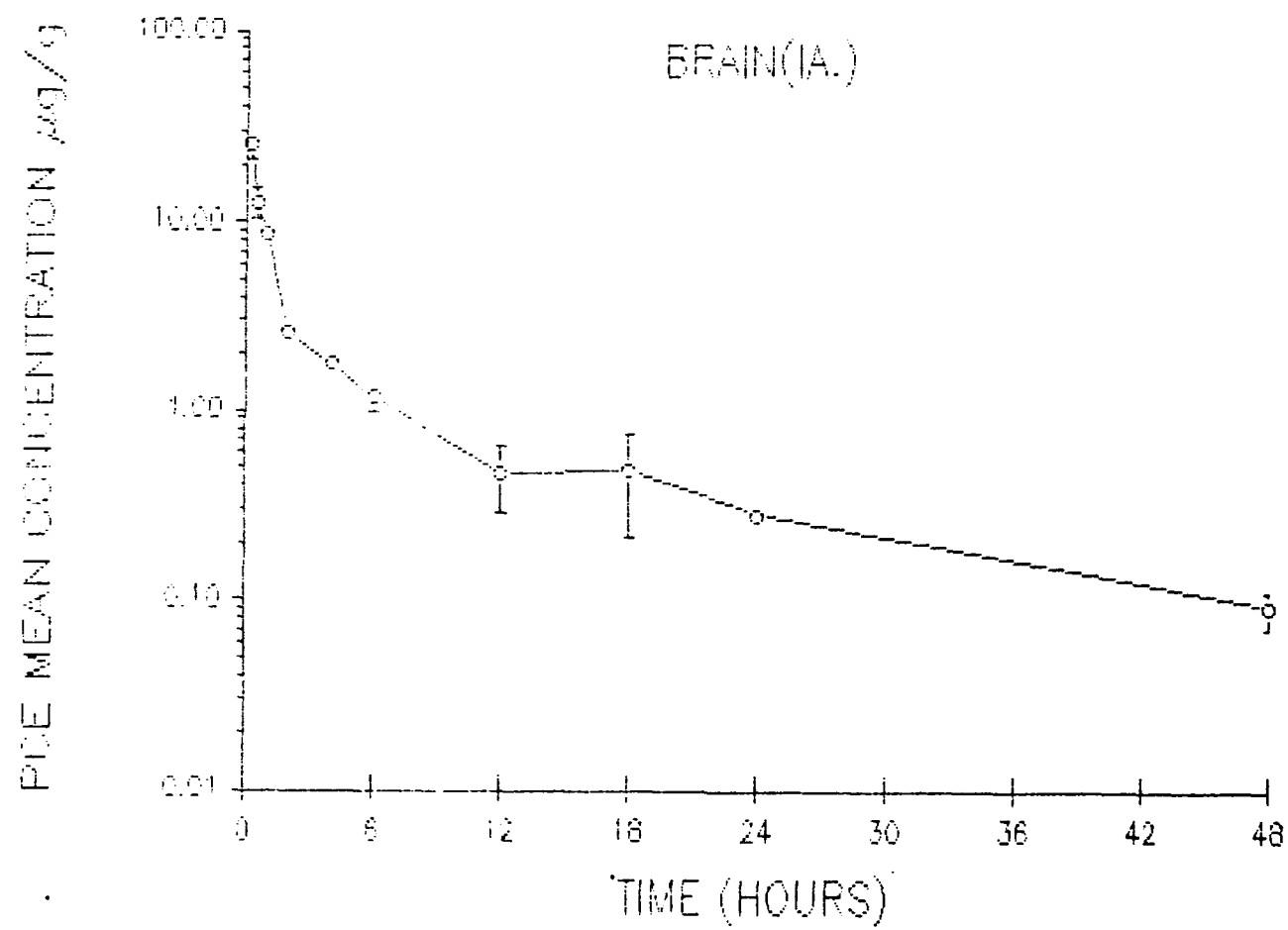


Fig. D-13

LUNG (IA.)

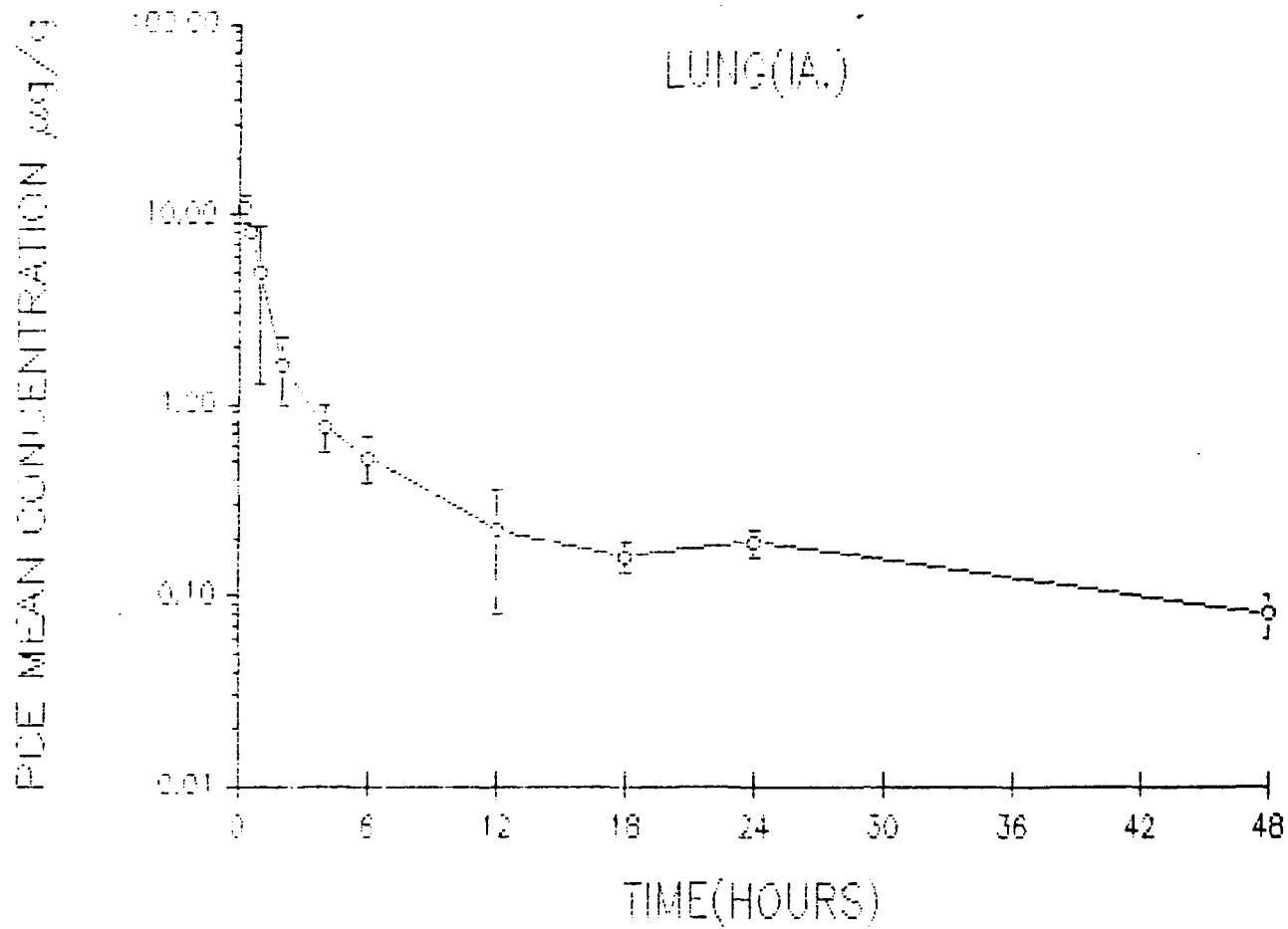


Fig. D-14

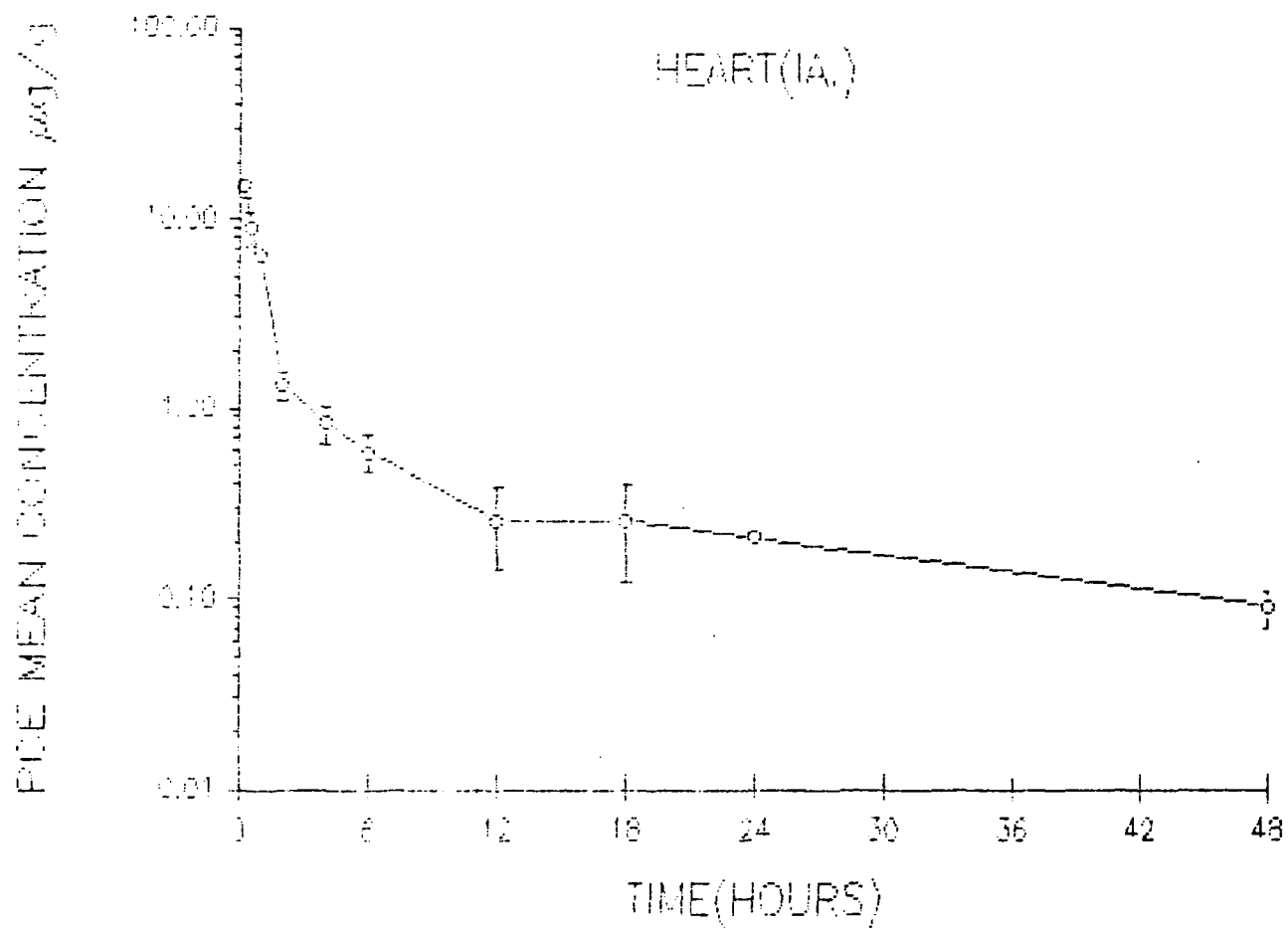


Fig. D-15

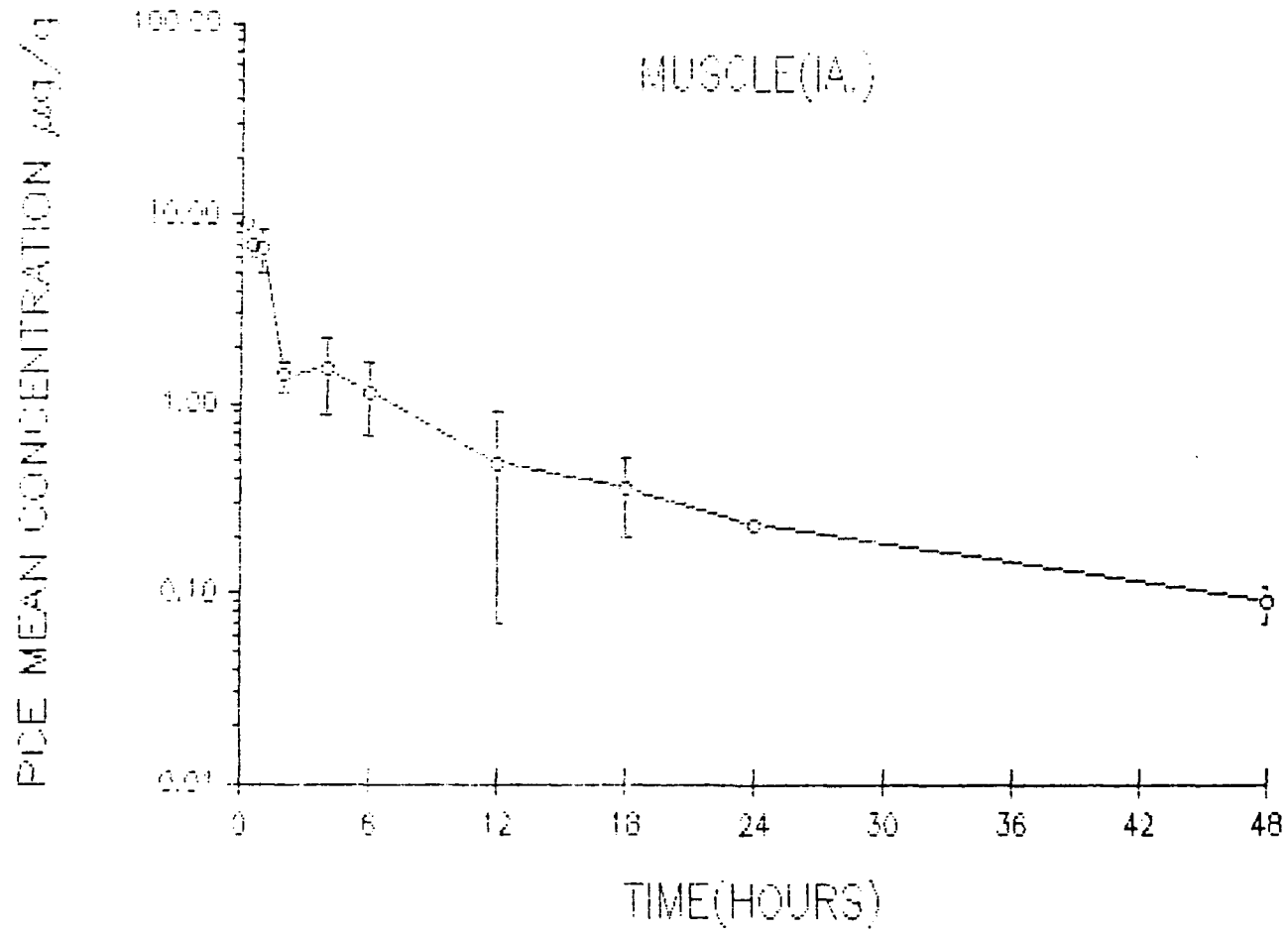


Fig. D-16

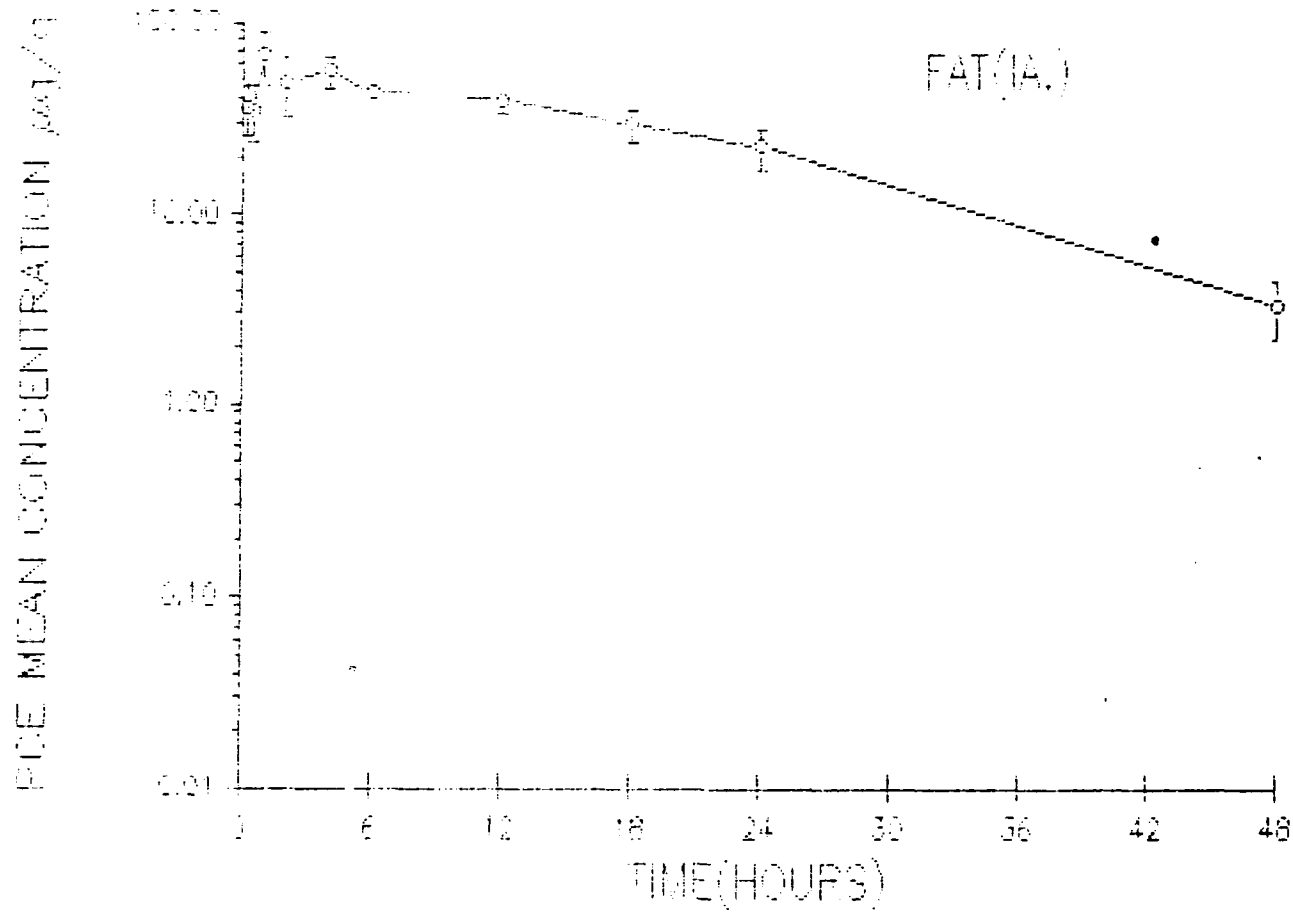


Fig D-17

POE SATURATED AFTER 15 MIN.(oral)

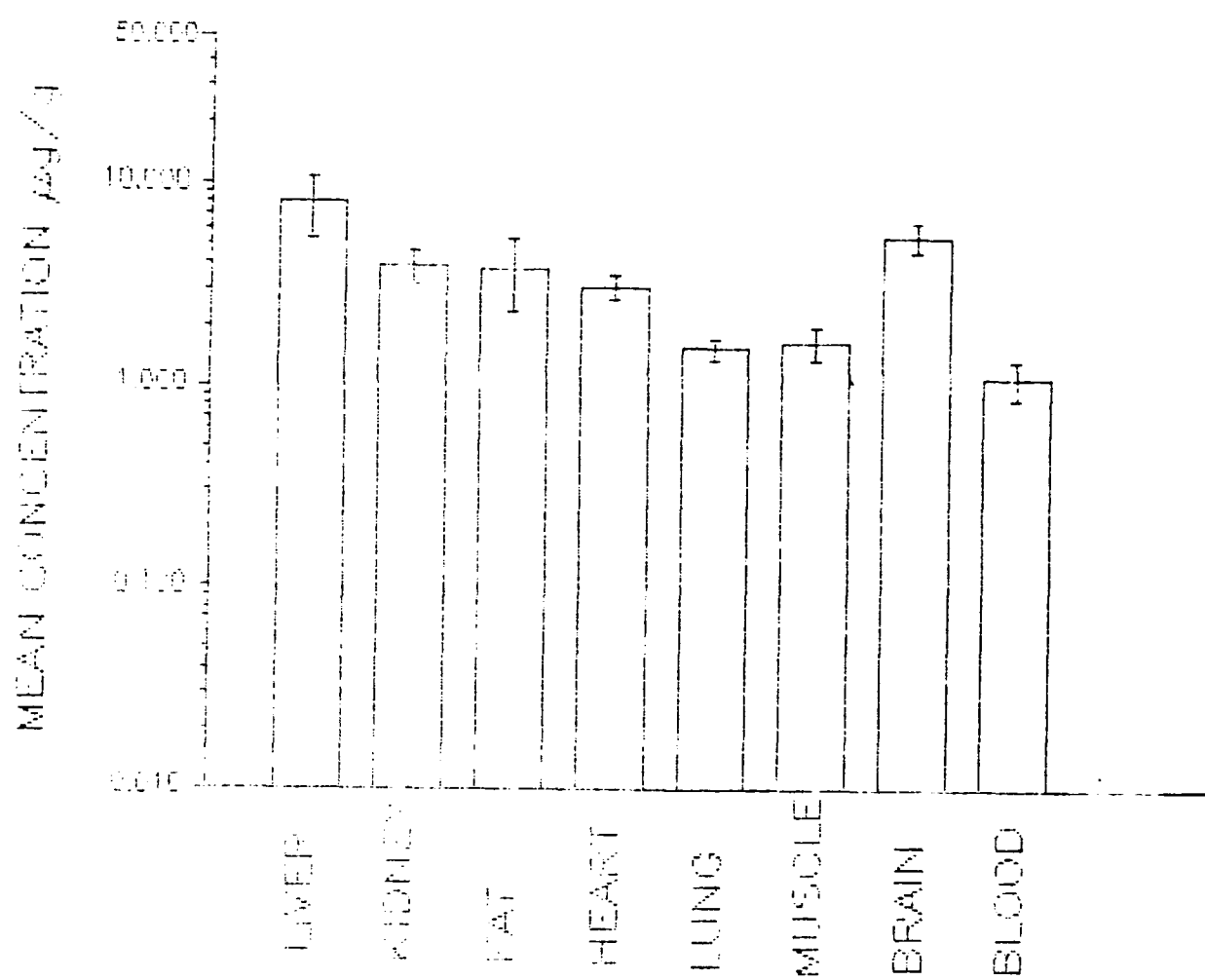


Fig. D-18

ALL SACRIFICED AFTER 30 MIN.(oral)

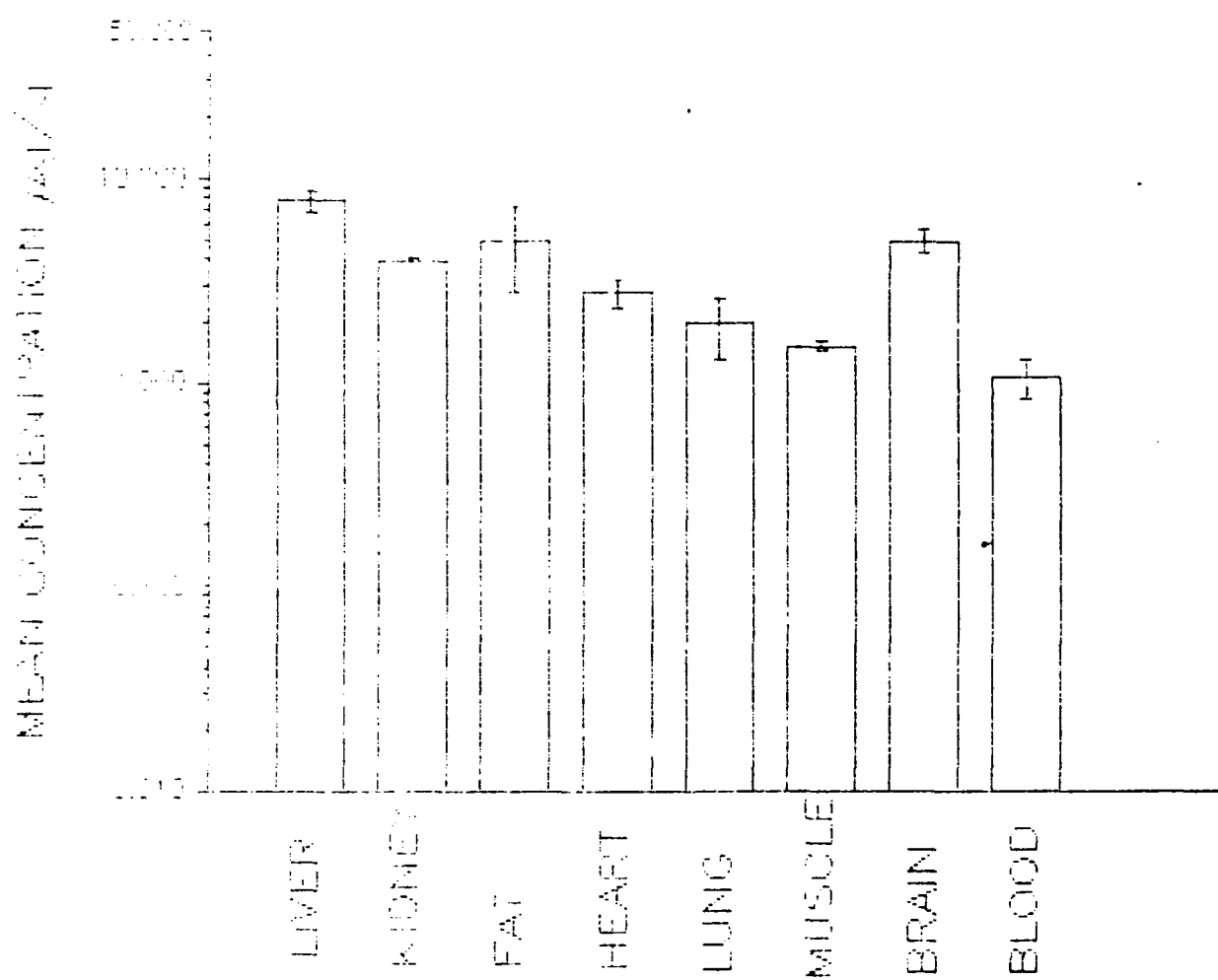


Fig. D-19

PIE SACRIFICED AFTER 1 HOUR(oral)

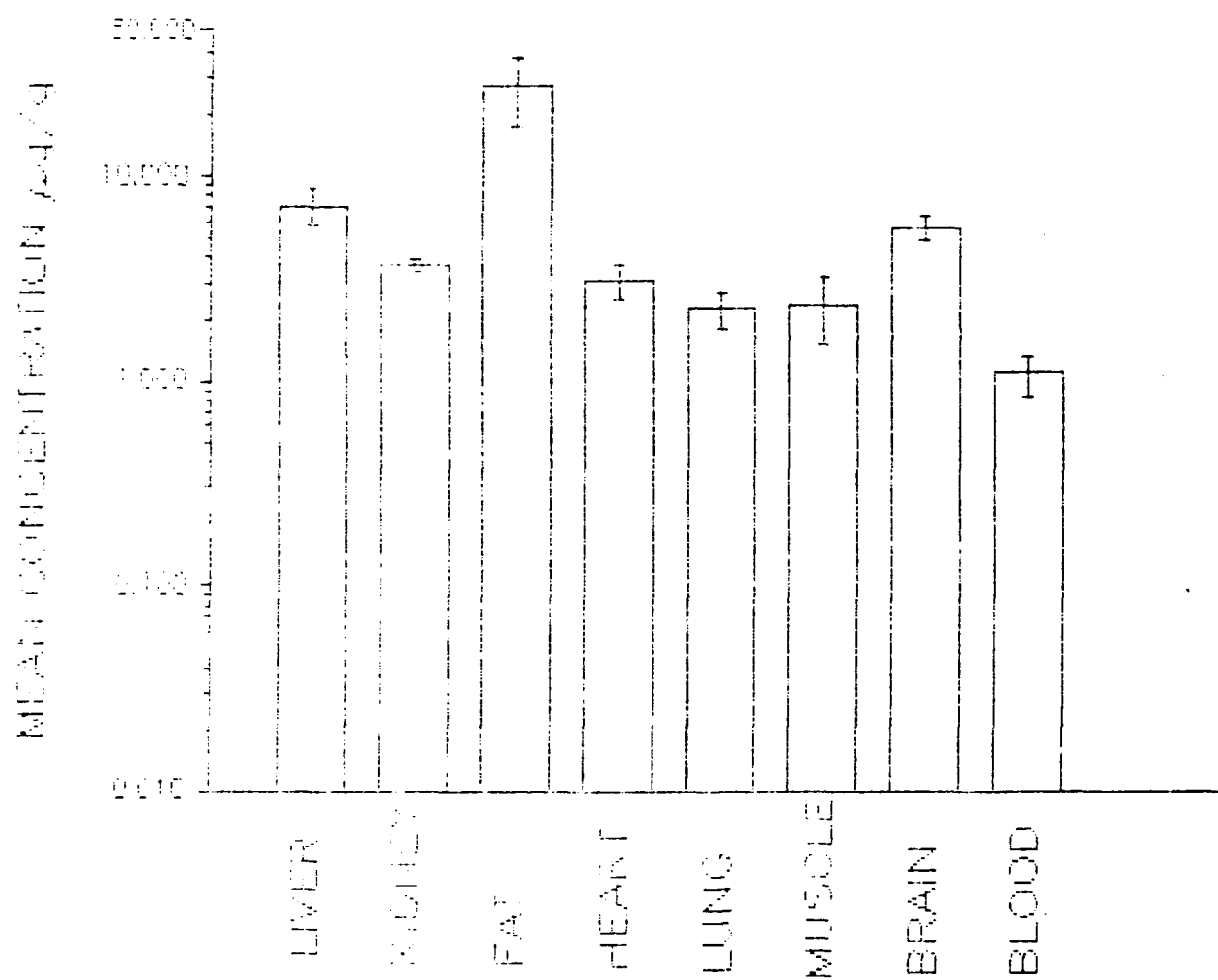


Fig. D-20

PCE SACRIFICED AFTER 2 HOURS(oral)

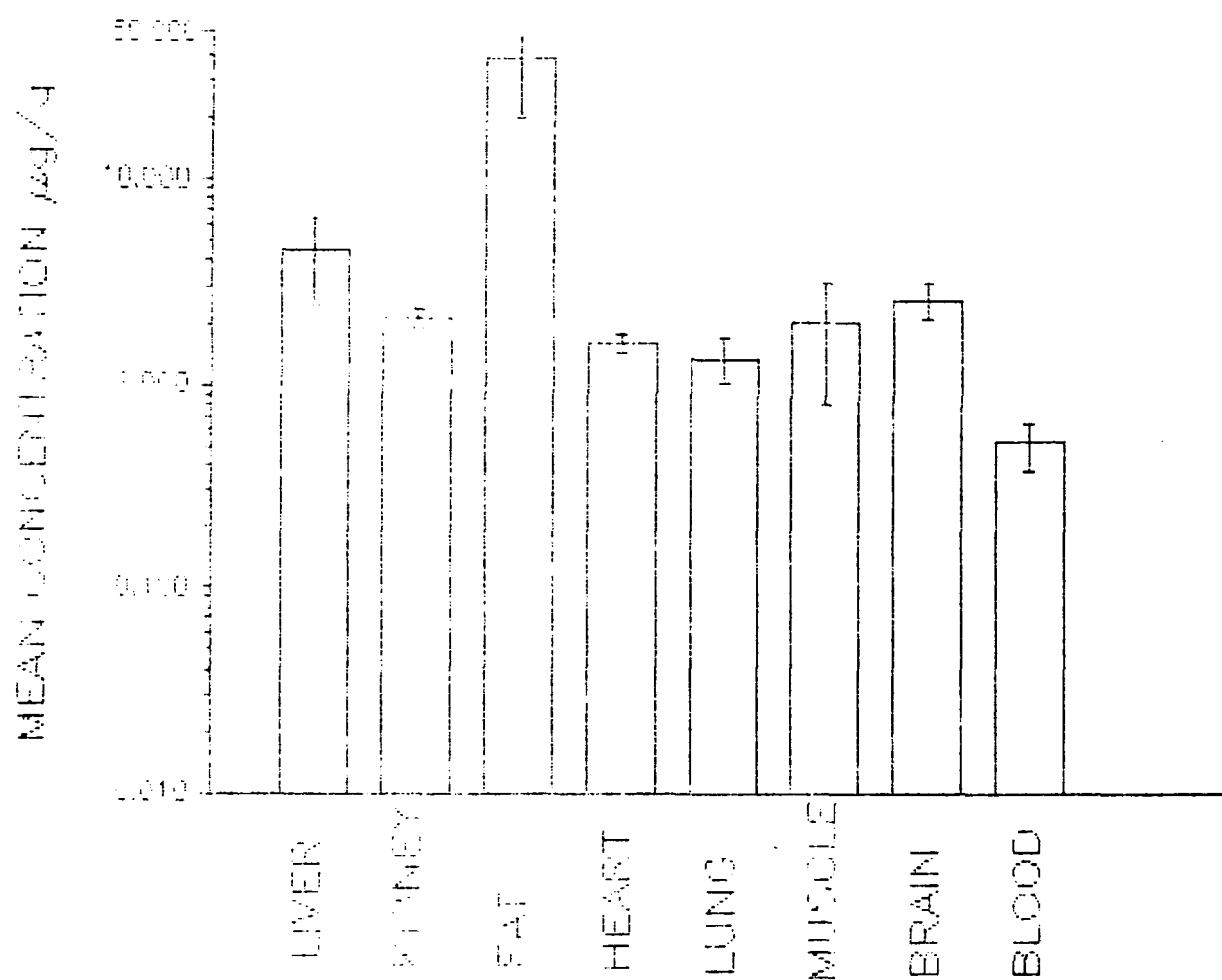


Fig. D-21

ROE SACRIFICED AFTER 4 HOURS(oral)

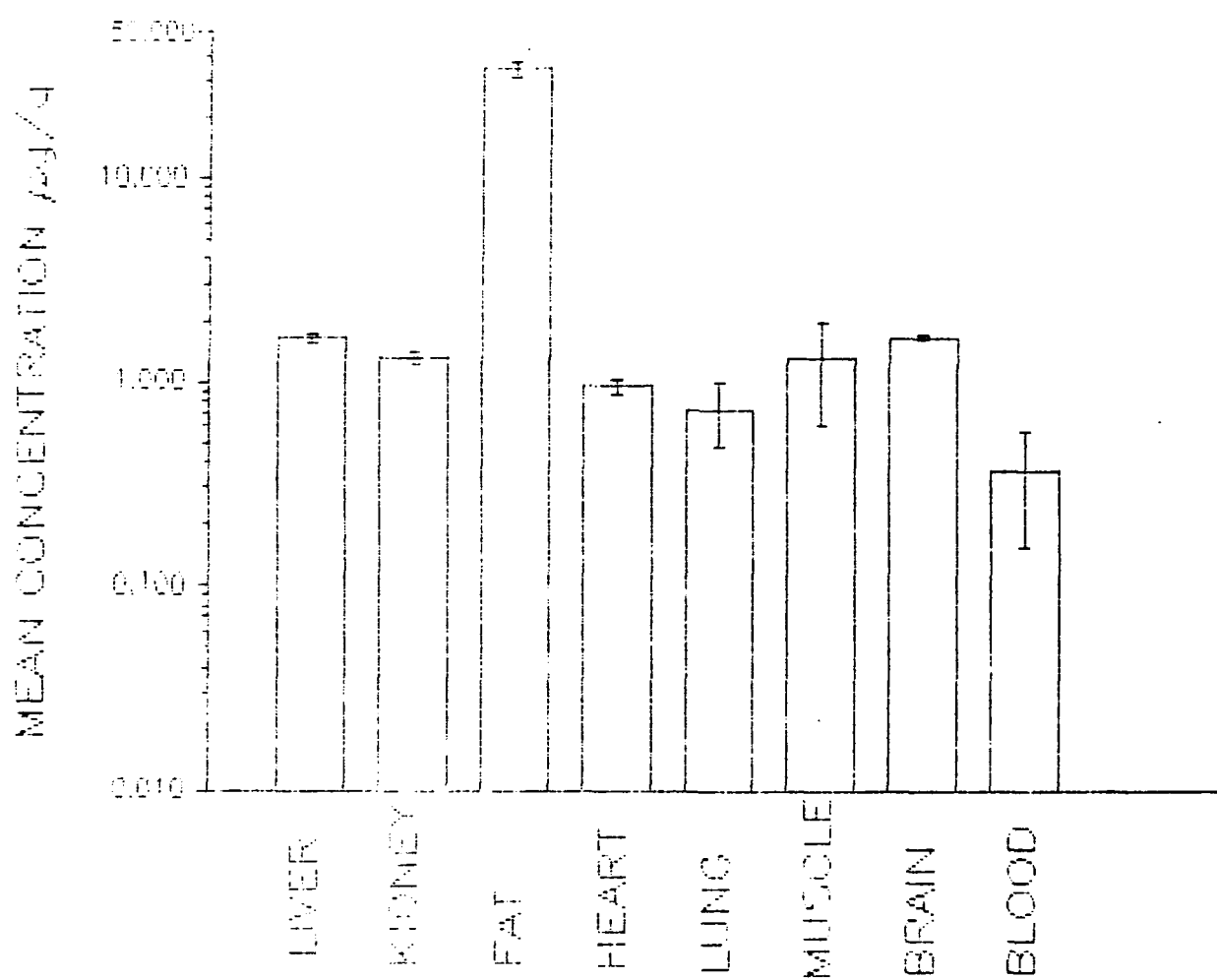


Fig. D-22

PCE SACRIFICED AFTER 6 HOURS(oral)

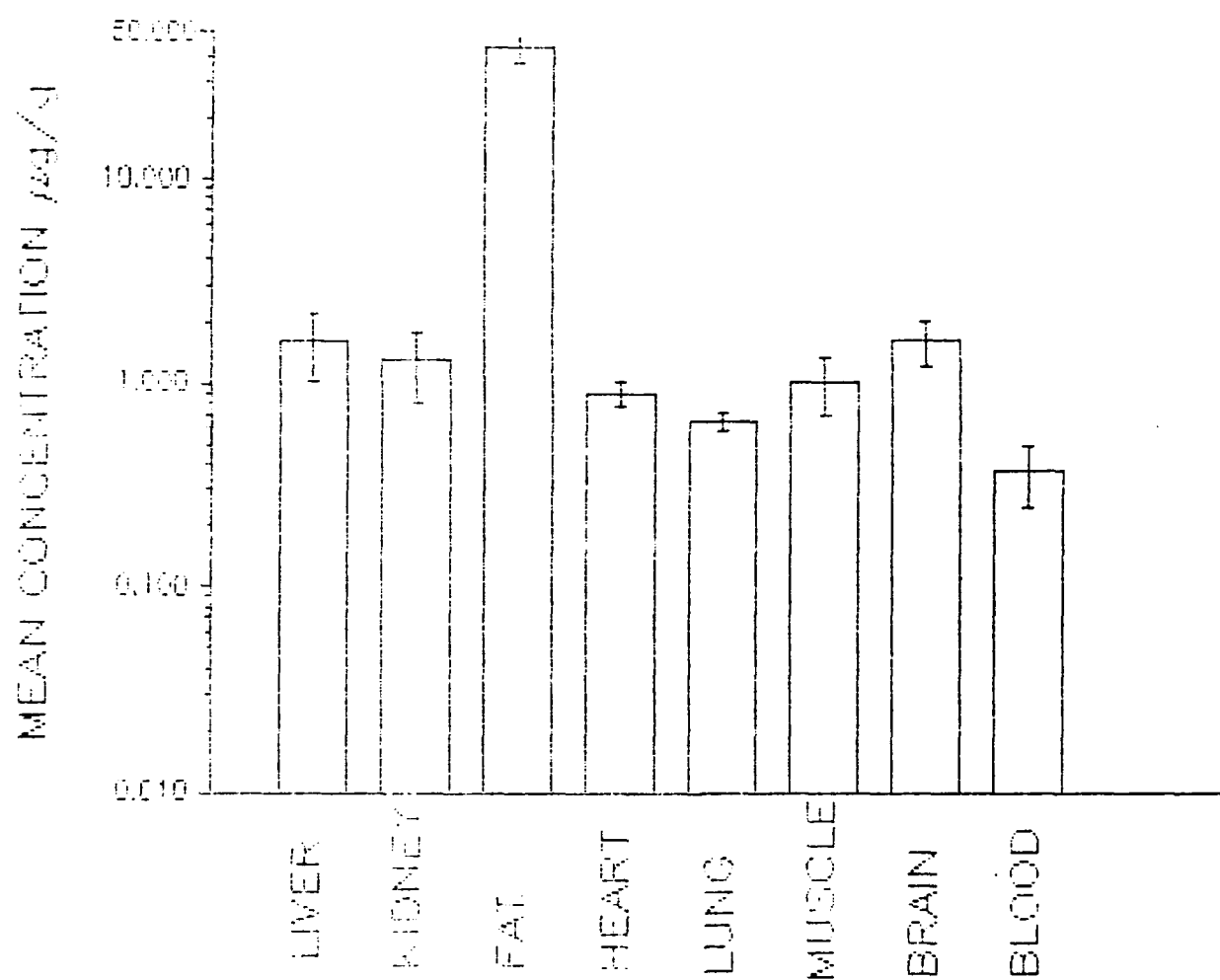


Fig. D-23

PCE SACRIFICED AFTER 12 HOURS(oral)

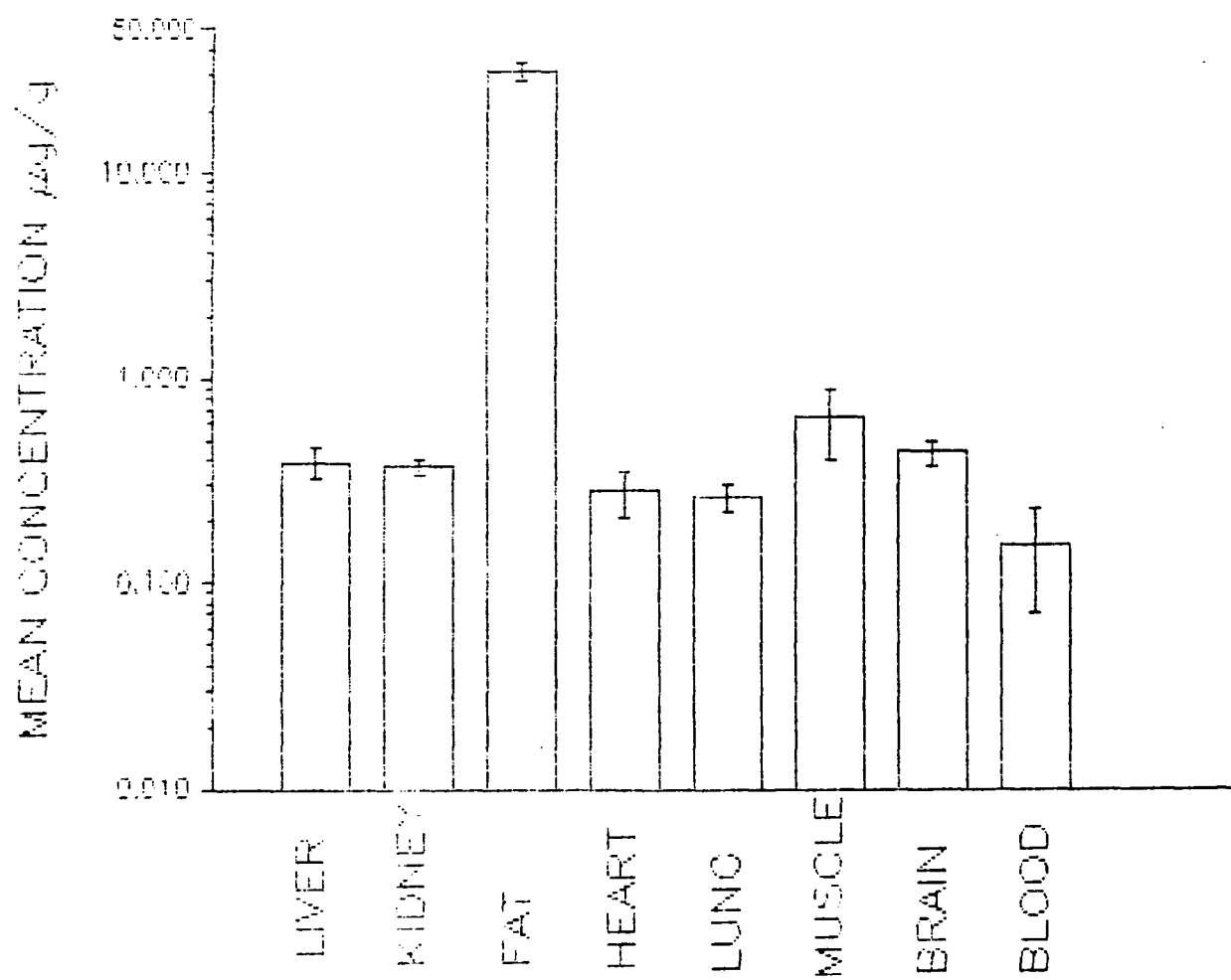


Fig. D-24

PCE SACRIFICED AFTER 18HOURS(oral)

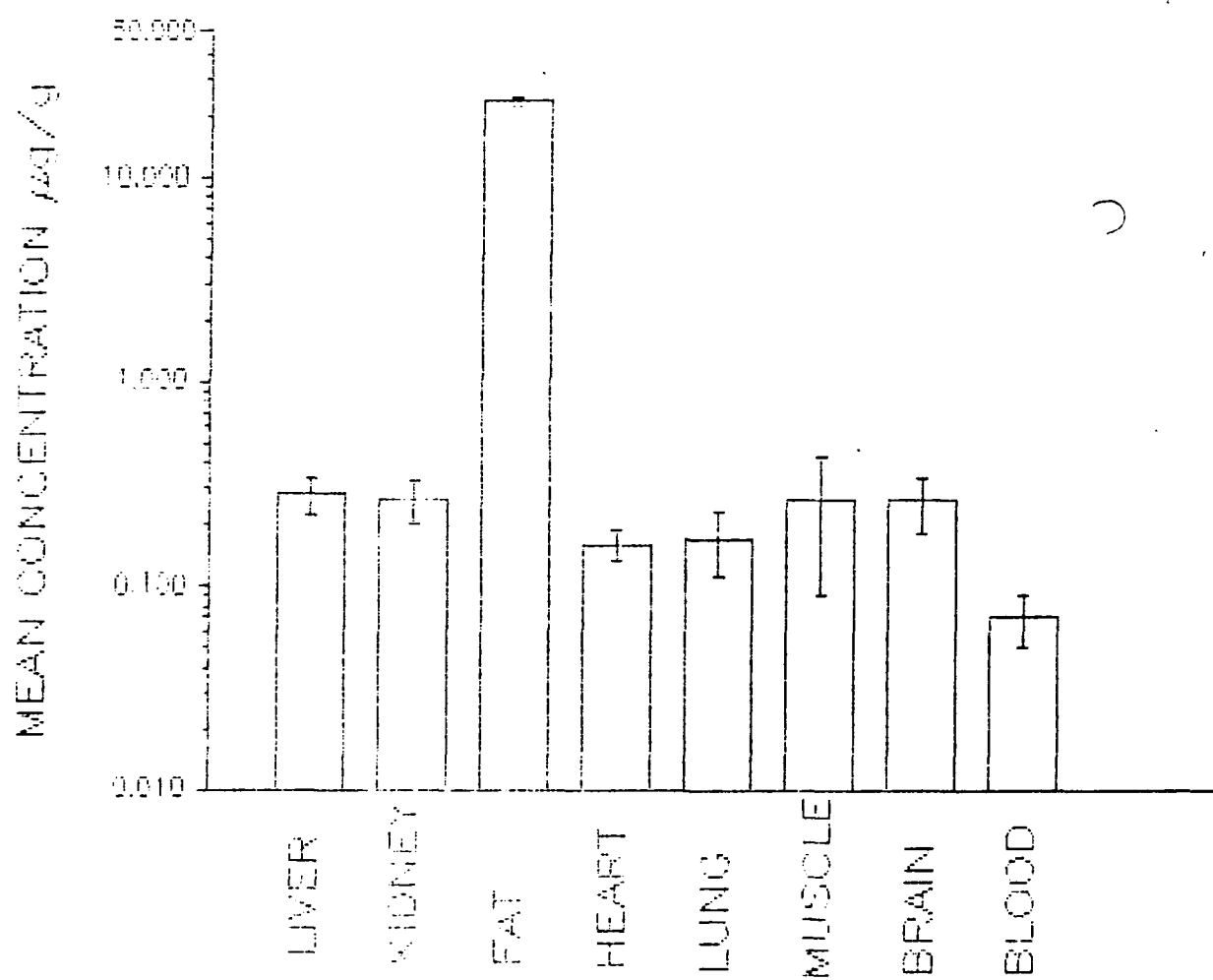


Fig. D-25

PCP SACRIFICED AFTER 24 HOURS (oral)

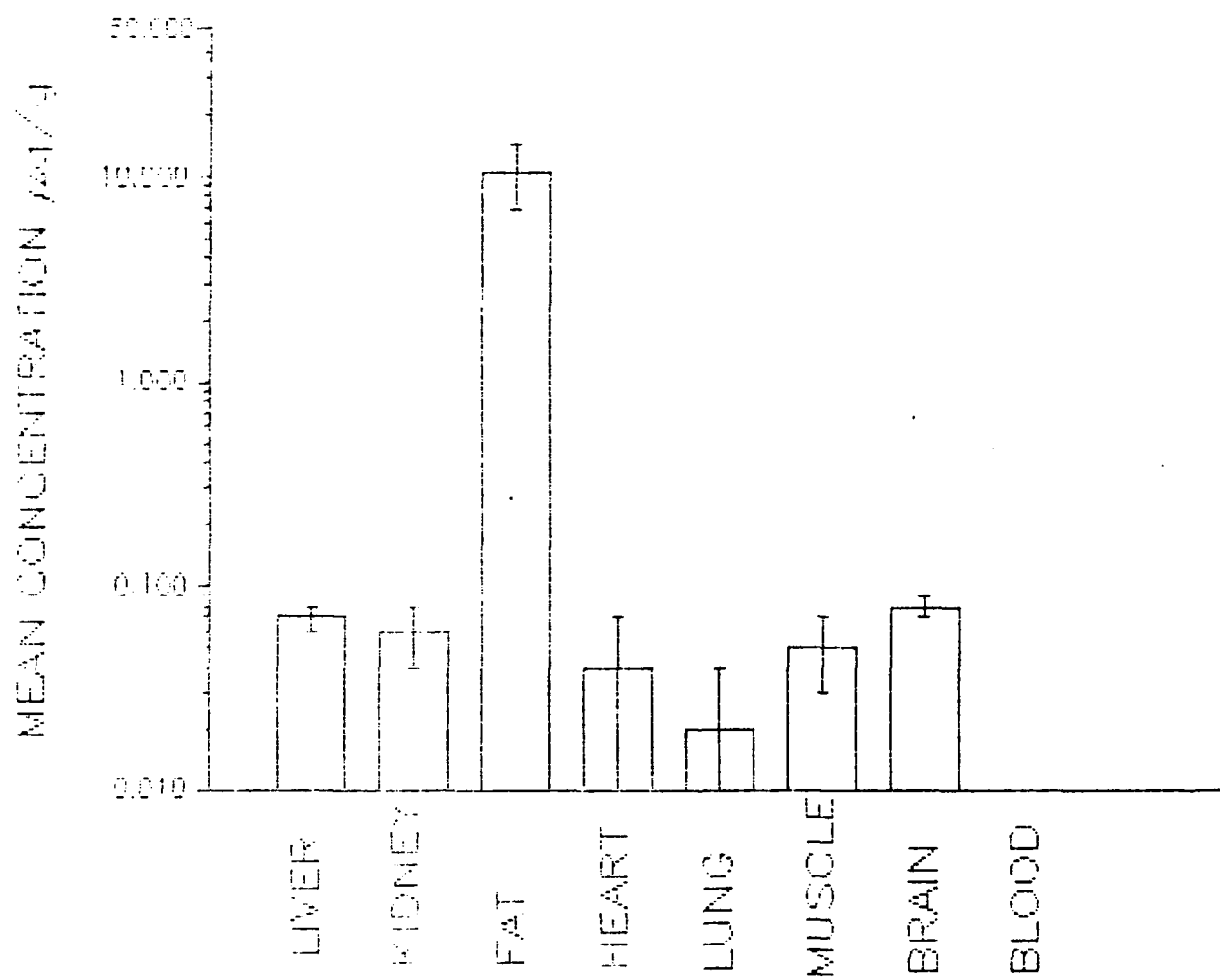


Fig. D-26

FILE SACRIFICED AFTER 48 HOURS(oral)

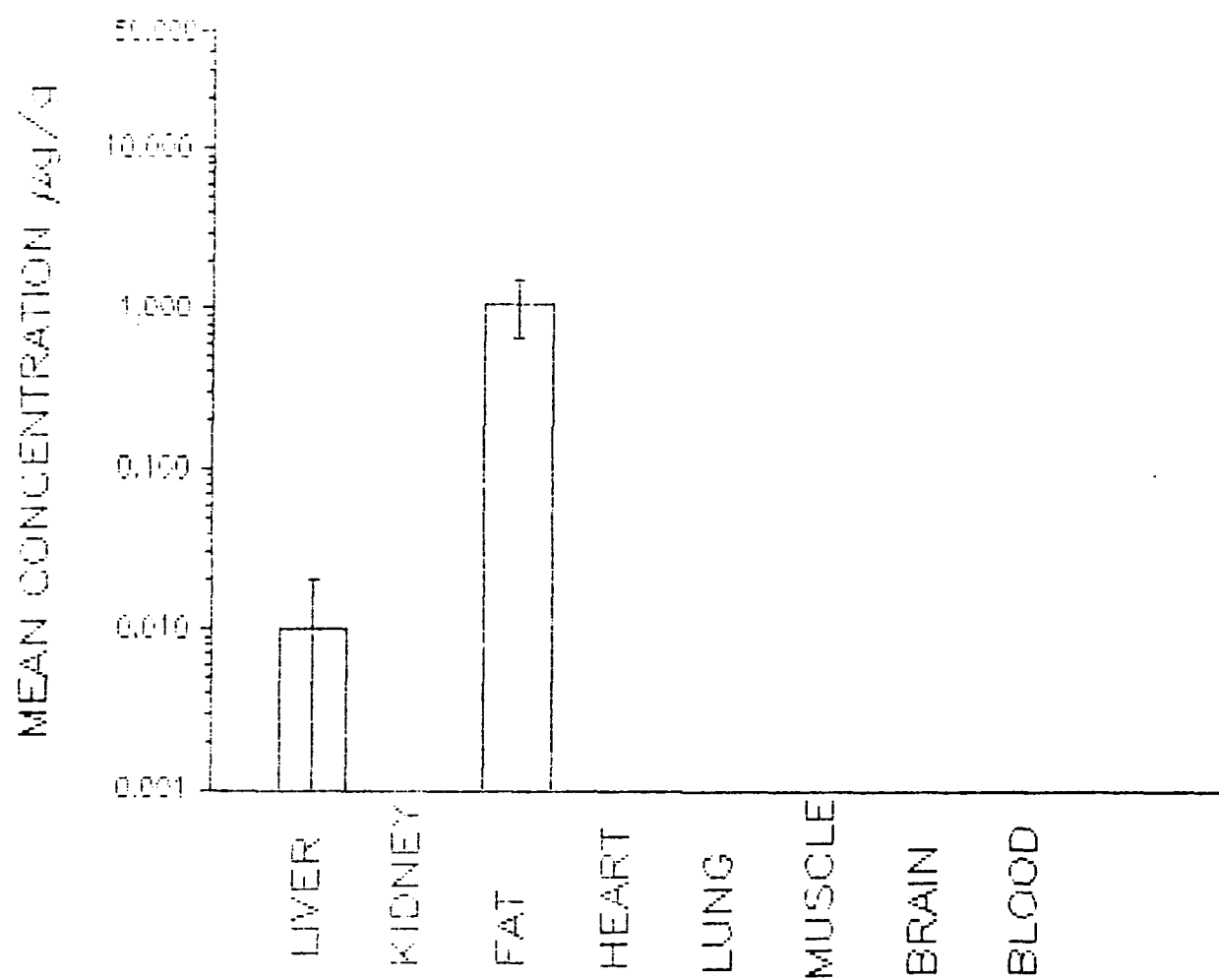


Fig. D-27

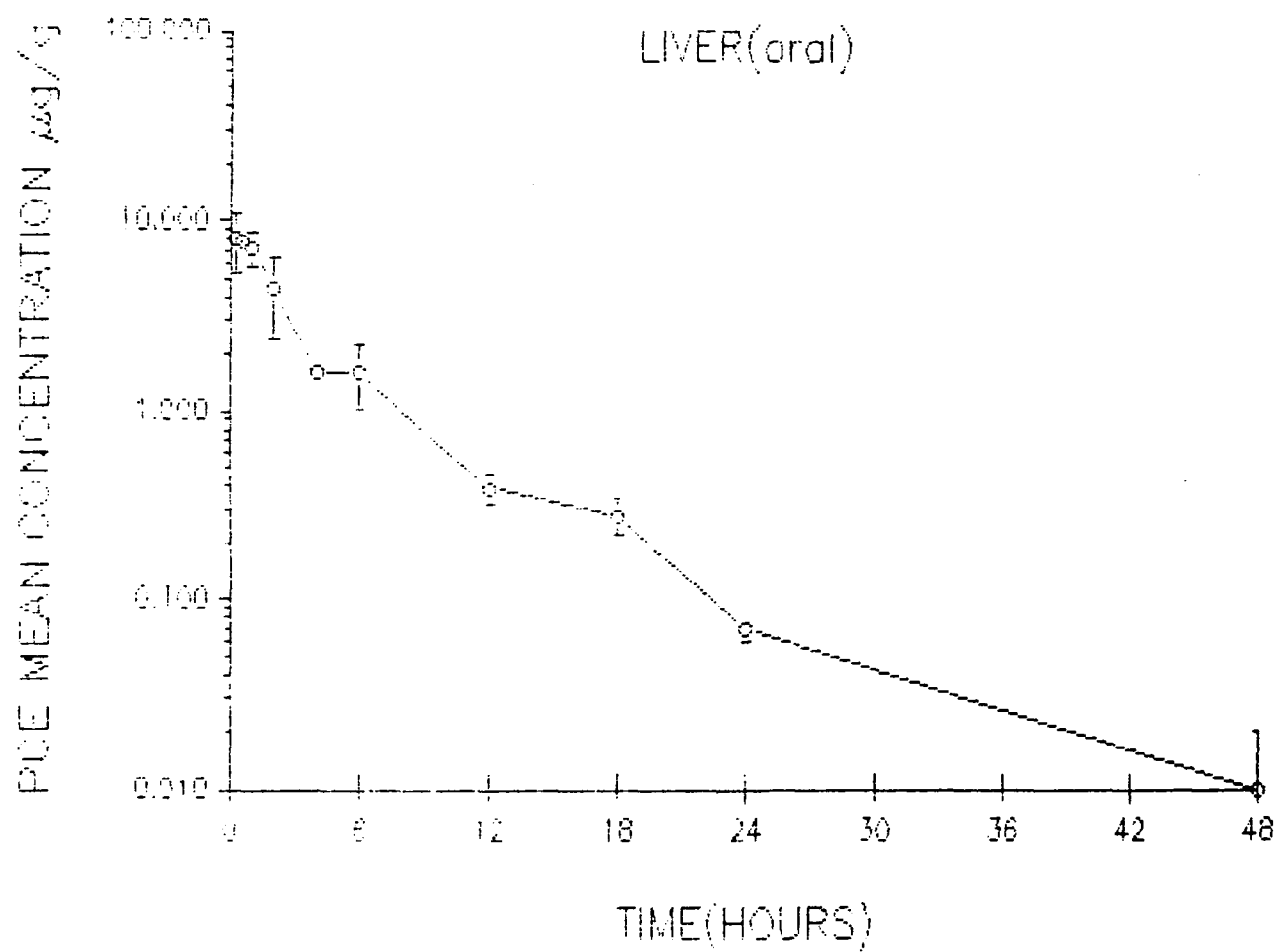


Fig. D-28

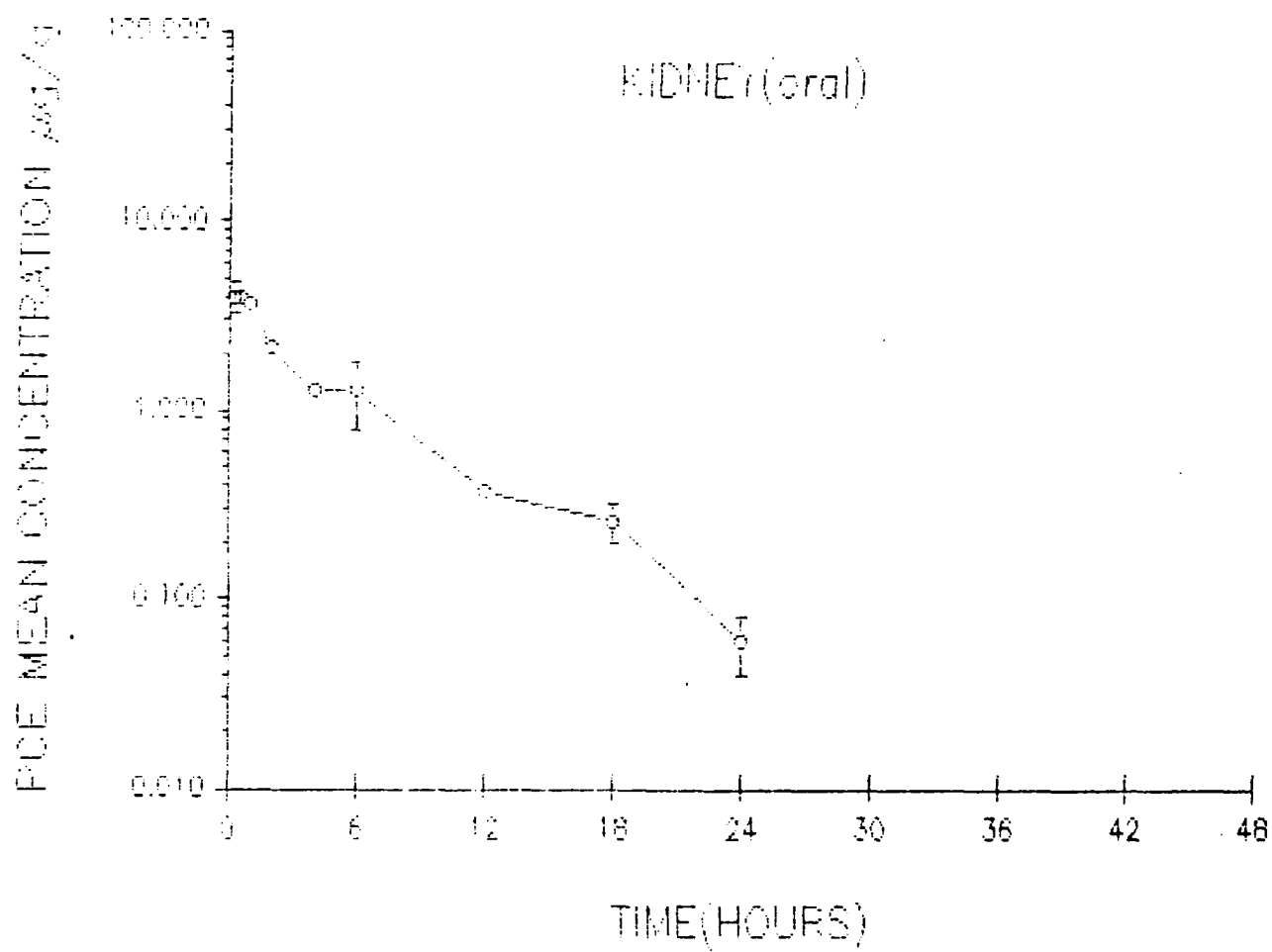


Fig. D-29

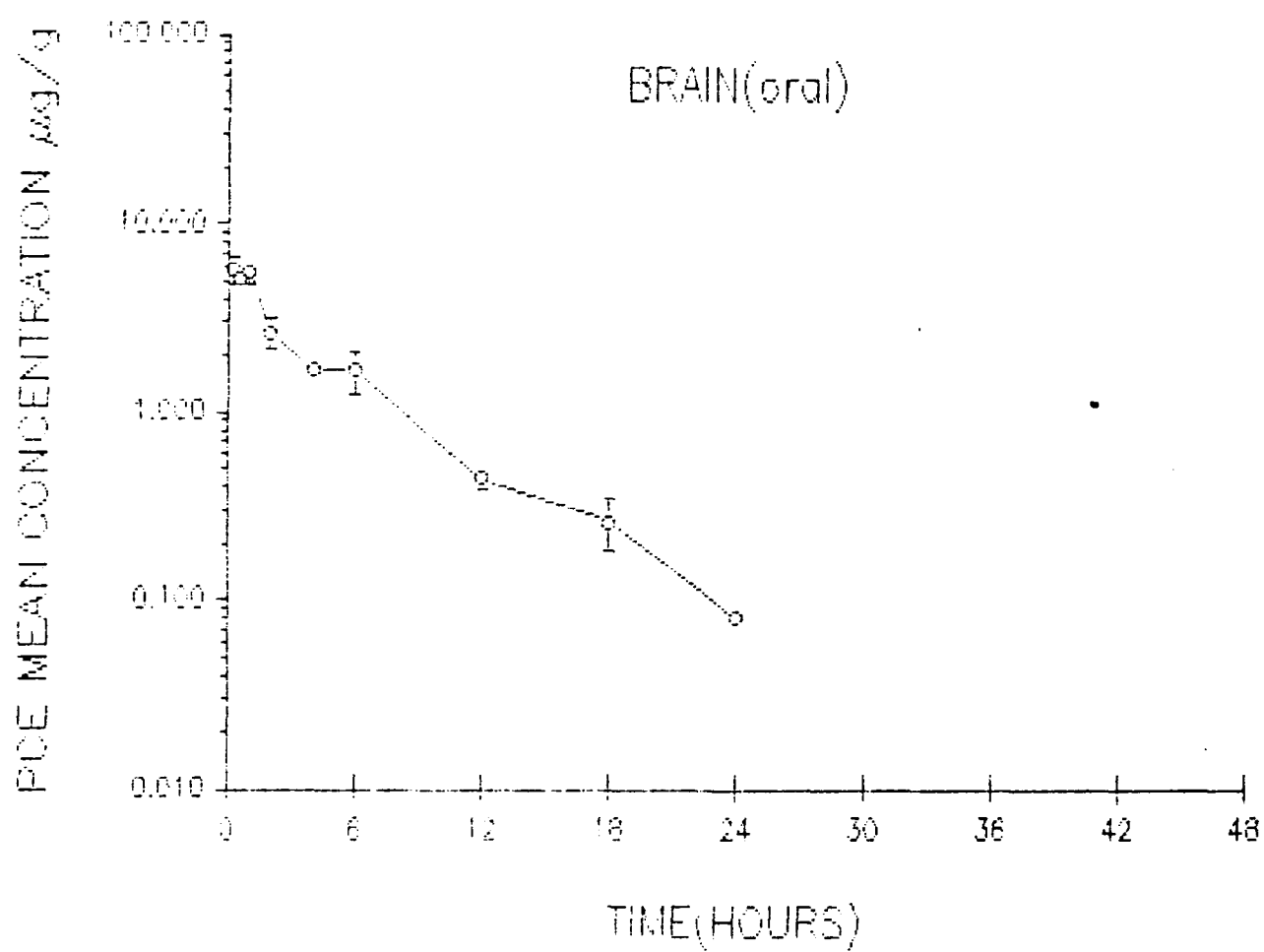


Fig. D-30

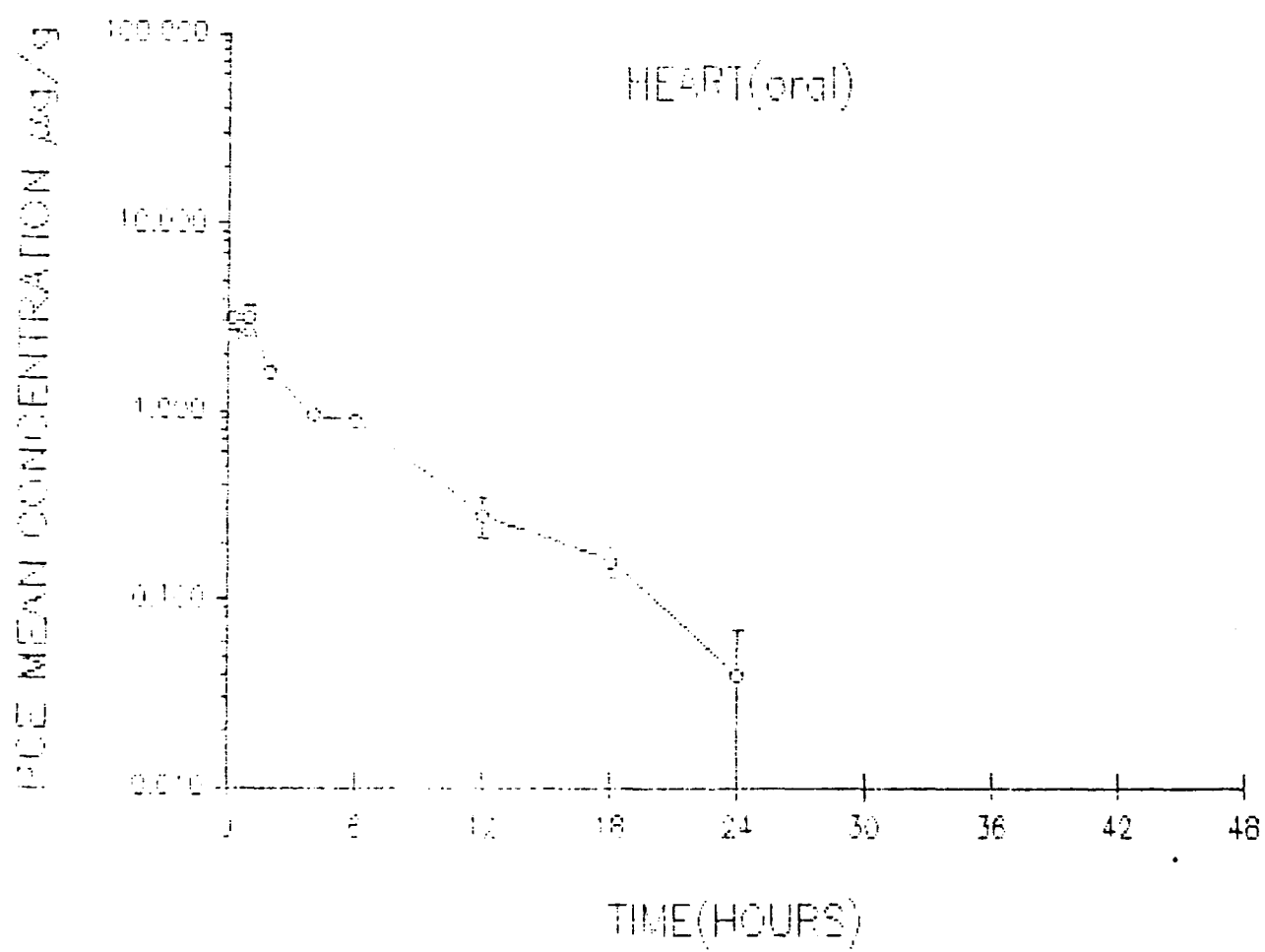


Fig. D-31

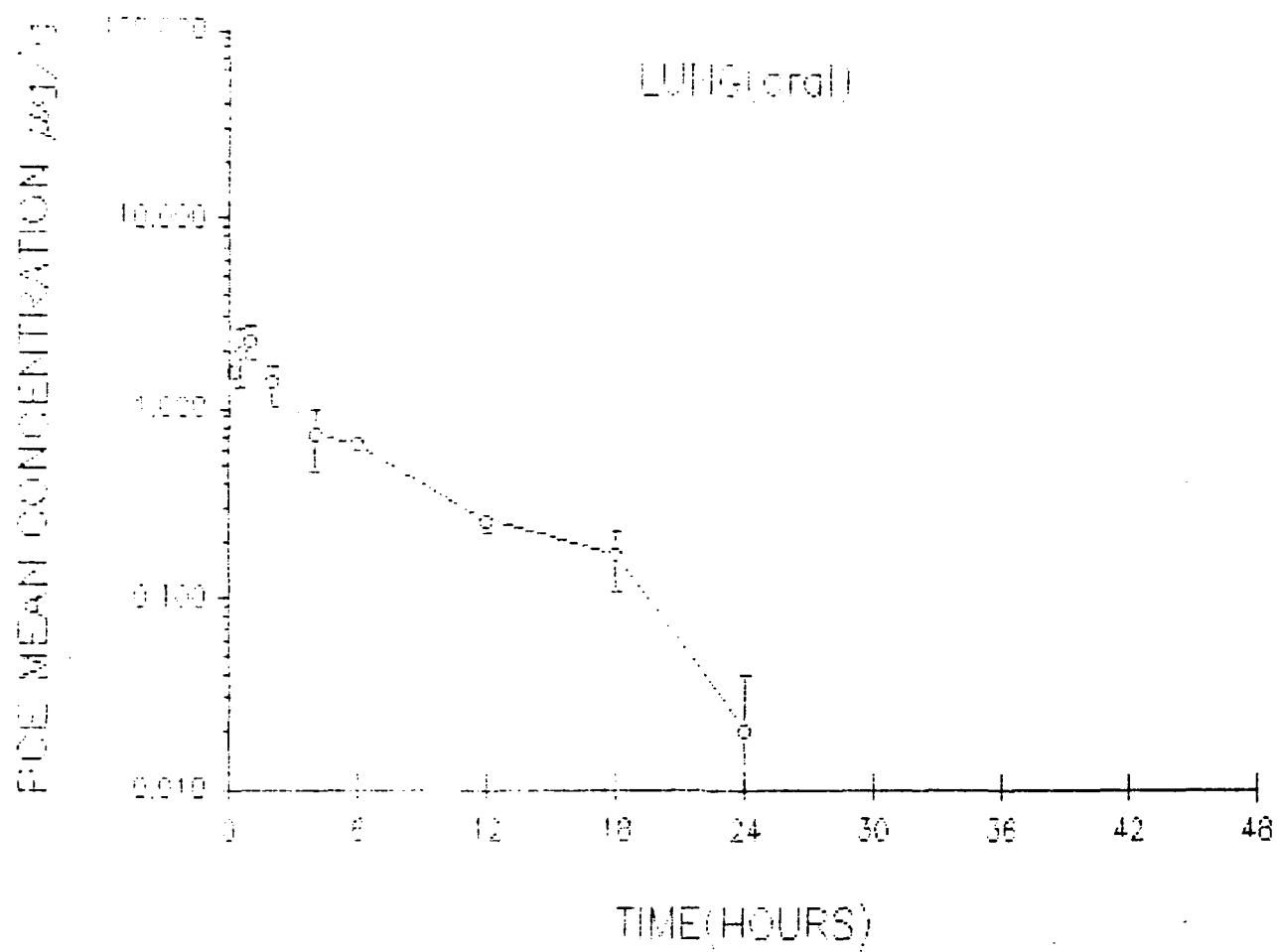


Fig. D-32

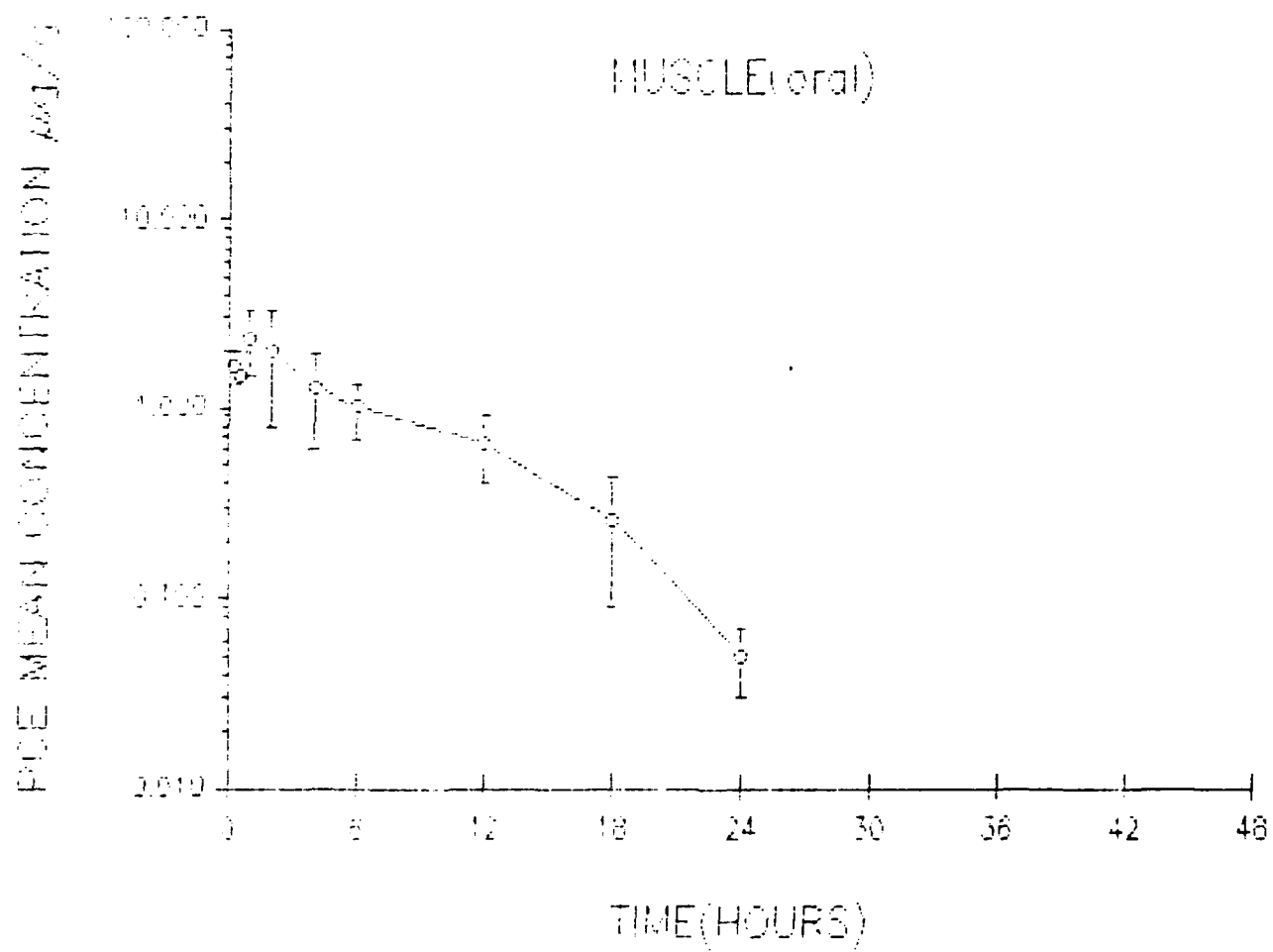


Fig. D-33

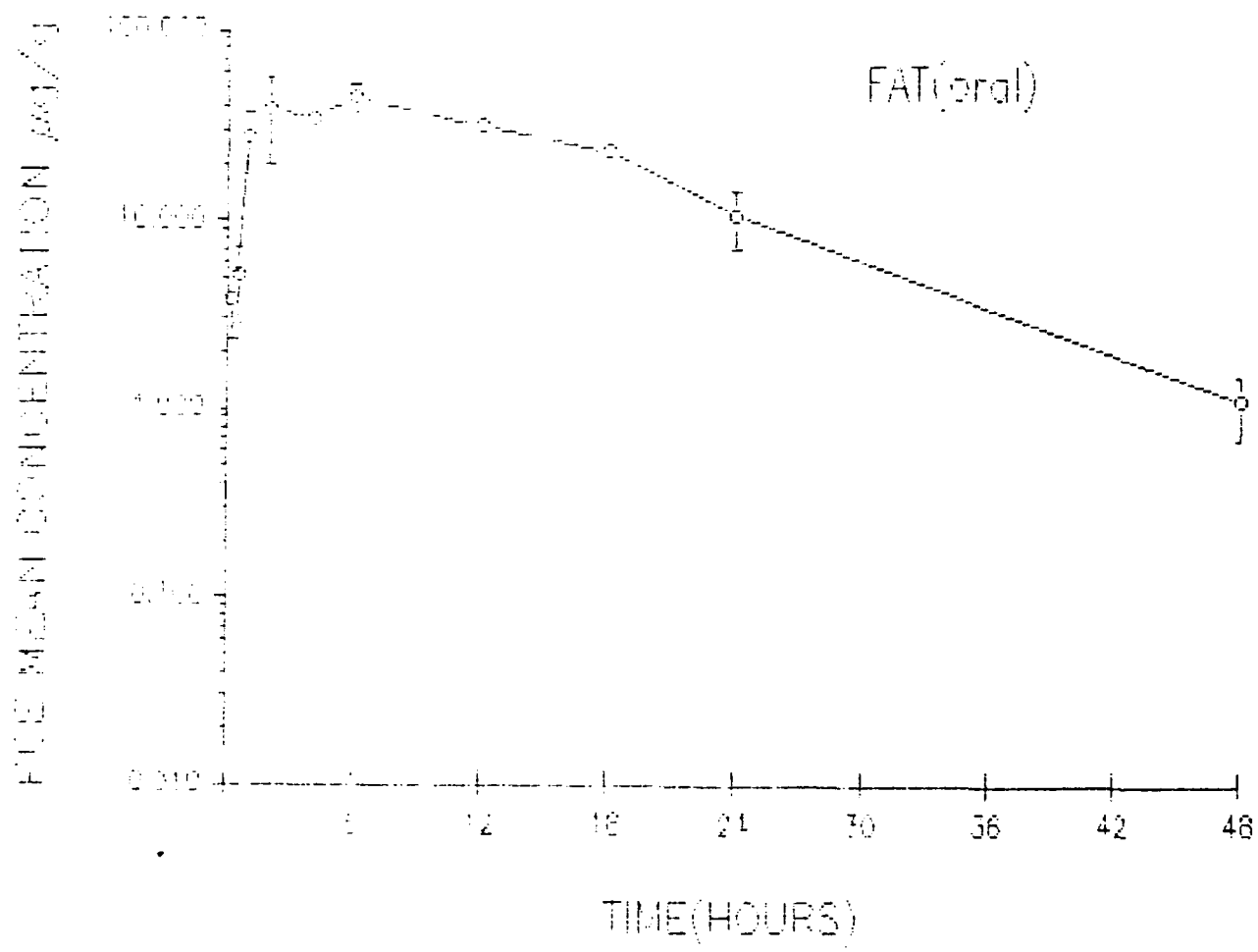


Fig. D-34

APPENDIX E

PHARMACOKINETIC STUDIES OF PCE IN DOGS FOLLOWING ORAL ADMINISTRATION

- 1.) Blood-concentration time profiles
- 2.) Tissue disposition

PCE 10 MG/KG IN BLOOD OF DOG#2(oral)

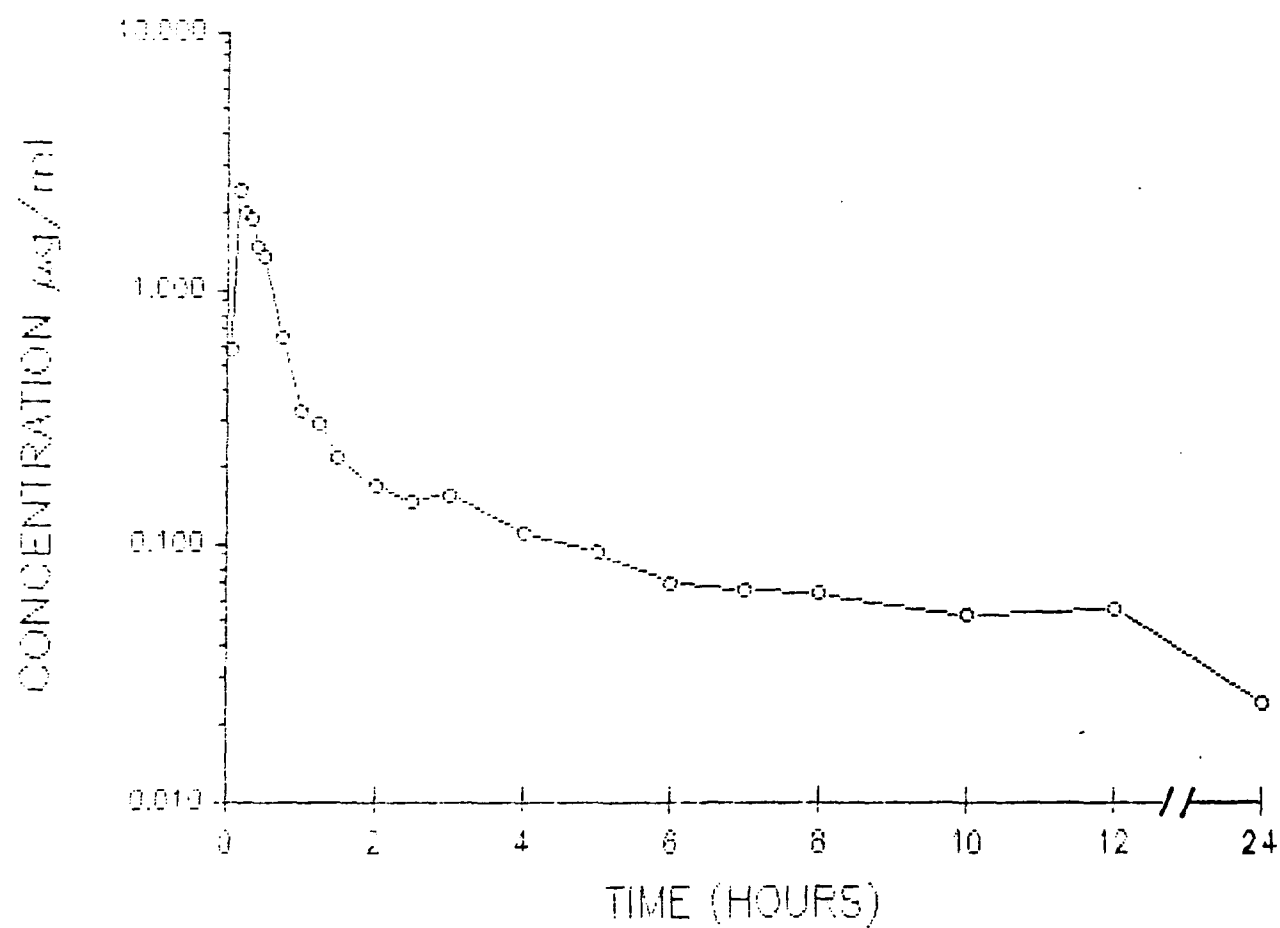


Fig. E-1

PCE 10 MG/KG IN BLOOD OF DOG#1(oral)

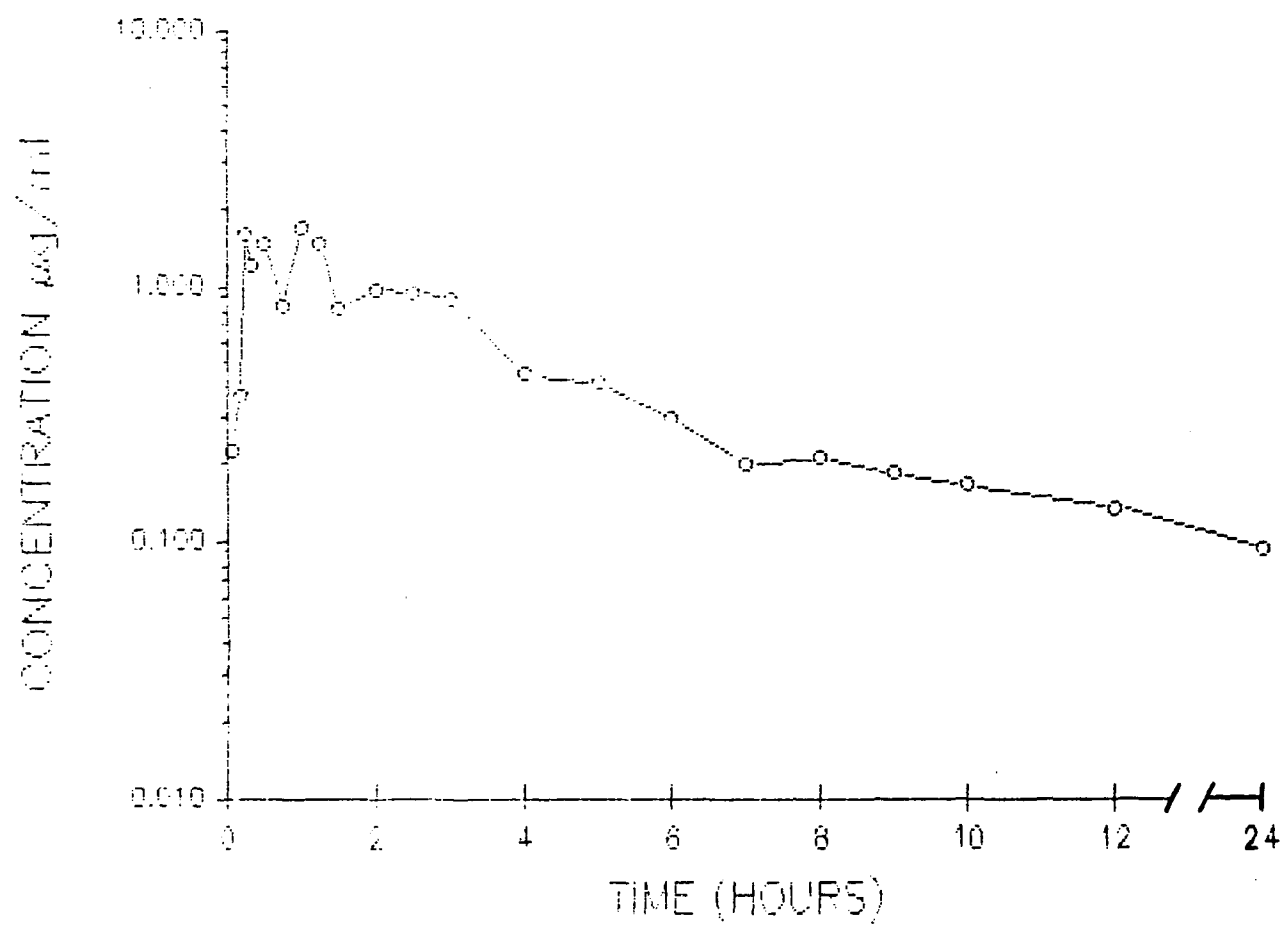


Fig. E-2

PCE III DOG#2 SACRIFICED AFTER 24hrs.(oral)

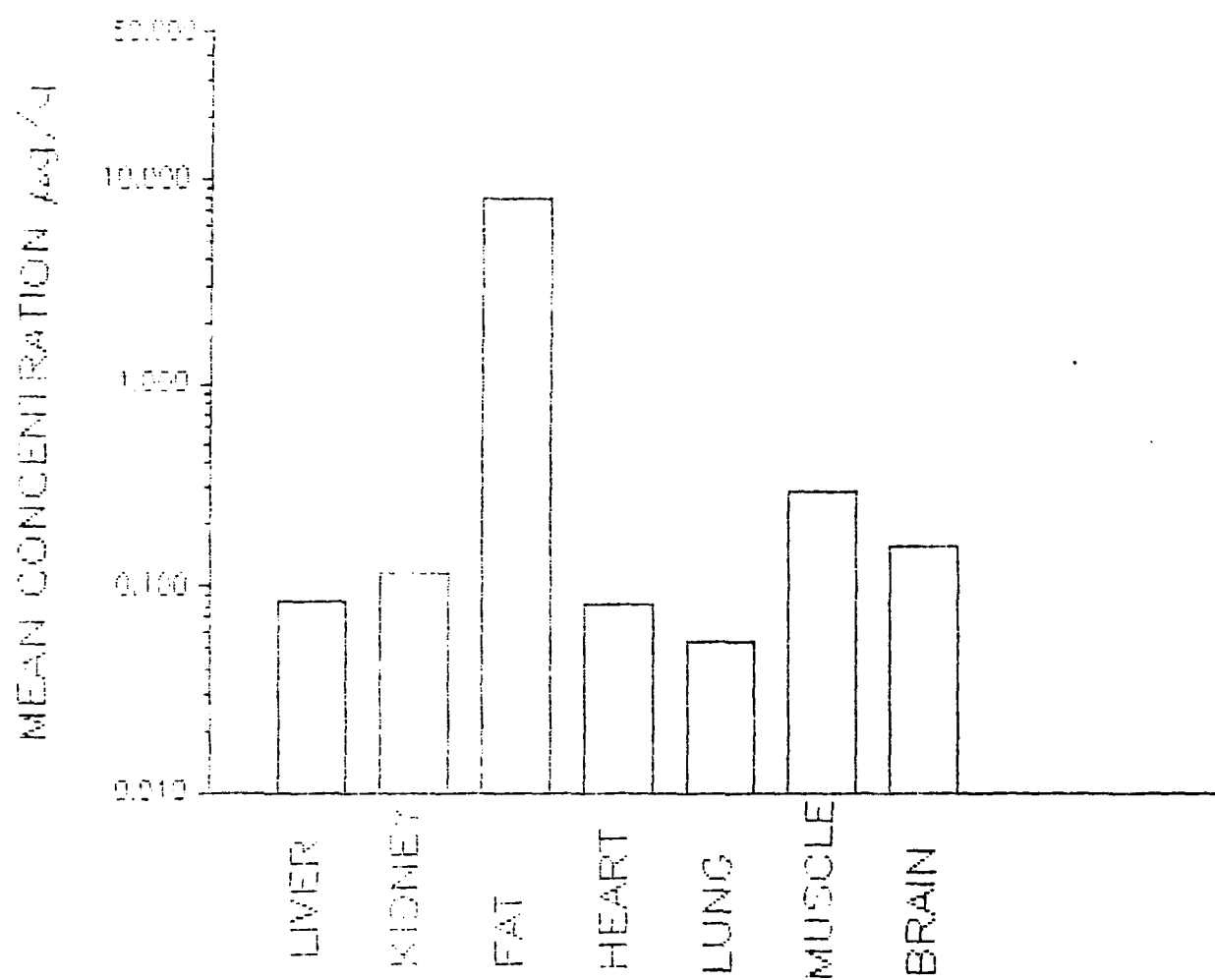


Fig. E-3

PCE III DOG#1 SACRIFICED AFTER 24 hrs.(oral)

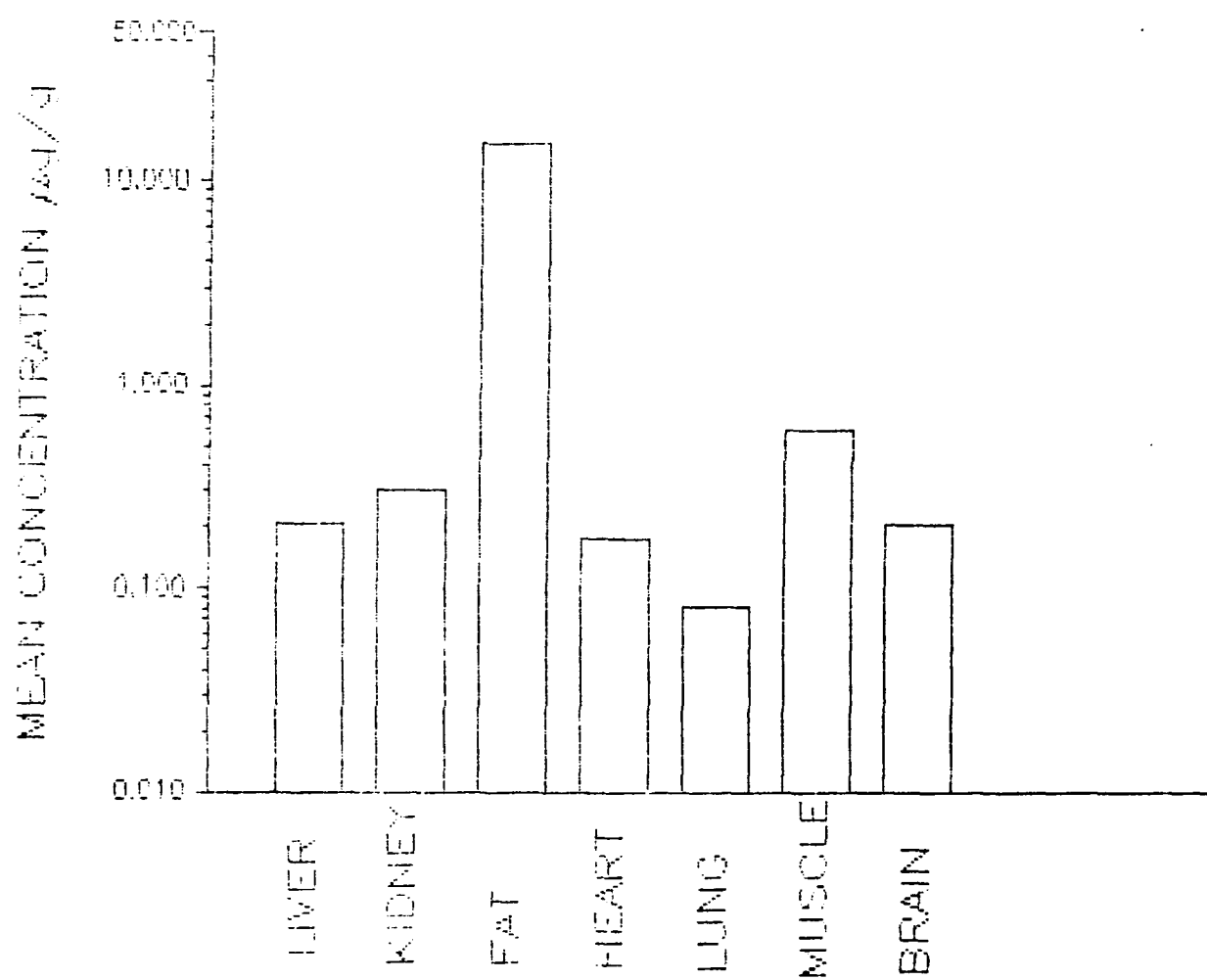


Fig. E-4

APPENDIX F

Cumulative List of Research Articles and Abstracts on Research Completed or Published in Year 2

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M. and Bruckner, J.V., "The uptake and elimination of 1,1,1-trichloroethane (TRI) during and following inhalation exposures in rats". Toxicology and Applied Pharmacology 98: 385-397 (1989).

Dallas, C. E., Ramanathan, R., Muralidhara, S., Gallo, J.M. and Bruckner, J.V., "Physiological pharmacokinetic model for trichloroethylene inhalation exposure in rats". (now being submitted to Toxicology and Applied Pharmacology, 1989).

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M. and Bruckner, J.V., "Comparative pharmacokinetics of inhaled and ingested 1,1-dichloroethylene in rats". (to be submitted to Toxicology and Applied Pharmacology, 1989).

Ramanathan, R., Muralidhara, S., Dallas, C.E., Gallo, J.M. and Bruckner, J.V., "Influence of the pattern of ingestion on the pharmacokinetics of perchloroethylene (PER) in rats". 28th Annual Meeting of the Society of Toxicology, Atlanta, GA; Toxicologist 9: 78 (1989).

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., Manning, R.O., and Bruckner, J.V., "Direct measurements of perchloroethylene in the blood and exhaled breath of rats during and following inhalation exposure". 28th Annual Meeting of the Society of Toxicology, Atlanta, GA; Toxicologist 9: 78 (1989).

Gallo, J.M., Dallas, C.E., and Bruckner, J.V., "Physiological pharmacokinetic models for 1,1,1-trichloroethane (TRI) and 1,1,1-trichloroethylene (TCE) in rats following inhalation and oral exposures". 28th Annual Meeting of the Society of Toxicology, Atlanta, GA; Toxicologist 9: 230 (1989).