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Interspecies Extrapolations of Halocarbon Respiratory and Tissue Kinetics:
Applications to Predicting Toxicity in Different Species

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Annual Report for Period 15 July 1993 - 14 July 1994

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A series of experiments have been conducted to provide a pharmacokinetic data base for interspecies comparisons and for formulation and validation of physiologically-based pharmacokinetic models. The basic experimental design has involved giving equal doses of halocarbons in different species, including mice, rats, and dogs. Perchloroethylene (PCE), tetrachloroethane (TET), trichloroethylene (TCE), and trichloroethane (TRI) have been employed as test chemicals, in order to evaluate the relative importance of the physicochemical property of volatility on the kinetics and toxicity of halocarbons. In order to determine the dose received in target organs and other tissues, serial samples of brain, liver, kidney, lung, heart, skeletal muscle, and adipose tissue have been taken and analyzed for halocarbon content after administration of PCE, TET, and TRI in rats, PCE and TET in dogs, and TRI in mice. For neurobehavioral studies, an operant testing system has been employed for monitoring the central nervous system effects of halocarbons. Neurobehavioral studies have been conducted following oral and inhalation exposure to PCE, and from inhalation exposure to TRI in rats. Neurobehavioral
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depression was compared with uptake of PCE and TRI in the brain and blood of rats, and with TRI in mice. The direct measurements of halocarbon concentrations in exhaled breath, blood and tissues have provided an extensive data base that has been used to formulate and validate the physiologically-based pharmacokinetic (PBPK) models for exposure to halocarbons. *In vivo*-derived partition coefficients for developing PBPK models for rats and dogs were determined from detailed tissue determinations in these species. Using the direct measurements data, the ability of the PBPK models to generate accurate predictions of halocarbon concentrations were evaluated in blood, exhaled breath, and seven tissues.

TECHNICAL SUMMARY

A series of experiments have been conducted to provide a pharmacokinetic data base for interspecies comparisons and for formulation and validation of physiologically-based pharmacokinetic models. The basic experimental design has involved giving equal doses of halocarbons to the rat and the dog, two species of widely different size. Perchloroethylene (PCE), tetrachloroethane (TET), trichloroethylene (TCE), and trichloroethane (TRI) have been employed as test chemicals, in order to evaluate the relative importance of the physicochemical property of volatility on the kinetics and toxicity of halocarbons. The respiratory elimination of TCE and systemic uptake of TCE and PCE has been measured in rats. In order to determine the dose received in target organs and other tissues, serial samples of brain, liver, kidney, lung, heart, skeletal muscle, and adipose tissue have been taken and analyzed for halocarbon content after administration of PCE, TET, and TRI in rats, and PCE and TET in dogs. A neurobehavioral operant testing system has been set up and a protocol established for monitoring the central nervous system effects of halocarbons. Neurobehavioral studies have been conducted following oral and inhalation exposure to PCE, and from inhalation exposure to TRI in rats. The direct measurements of halocarbon concentrations in exhaled breath and tissues have provided an extensive data base that will be used to formulate and validate the physiologically-based pharmacokinetic (PBPK) models for exposure to halocarbons. The blood and exhaled breath kinetics of PCE and TCE were accurately simulated by PBPK models. The physiological parameters for PBPK model formulation in the dog have also been procured in an extensive literature search. The partition coefficients for developing PBPK models for rats and dogs were determined from detailed tissue determinations in these species.

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I.   OVERALL OBJECTIVE AND SPECIFIC AIMS

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

SPECIFIC AIMS in the proposed studies are to:

1)   Determine the respiratory elimination of physicochemically disparate volatile organic compounds (VOCs) in two animal species. Rats and dogs will be administered selected halocarbons by inhalation and oral exposure Concentrations of expired parent compounds will be monitored in the exhaled breath for appropriate periods during and following exposure. Together with monitoring of the respiratory volumes of the test animals, this data will enable calculation of the cumulative uptake and elimination of the halocarbons. Data from both species will be compared to existing data sets for respiratory elimination in humans.

2)   Delineate the tissue disposition of inhaled halocarbons in two animal species. Rats and dogs will be exposed to halocarbons by inhalation. Concentrations of the parent compound in brain, liver, heart, lung, kidney, skeletal muscle, and adipose tissue will be measured over time, in order to provide an assessment of the actual target organ dose for correlation with neurobehavioral toxicity and for development and validation of physiologically-based pharmacokinetic and toxicodynamic models.

3)   Validate physiologically-based pharmacokinetic (PBPK) models for predicting the tissue pharmacokinetics of halocarbons in two animal species. PBPK models that have been initially developed in rats in our previous studies, with blood and expired air data for inhalation exposures and with tissue data for intraarterial and oral exposures, will be further validated for accuracy in interspecies extrapolations. Direct measurements of exhaled breath and tissue concentrations and associated parameters in rats and dogs in the initial two phases of the presently proposed project will be used to further test the accuracy of our PBPK models. Models will be developed each for the rat and the dog. The observed animal data and pharmacokinetic parameters will be used to formulate allometric relationships which can then be used to predict human disposition of halocarbons.

4)   Correlate the neurobehavioral toxicity of inhaled VOCs in two species with the target organ concentration. Rats and dogs will be exposed to selected

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halocarbons at defined inhaled concentrations and lengths of exposure. Neurobehavioral tests for operant performance will be performed periodically during and after exposures. The magnitude of central nervous system (CNS) effects of each solvent will be correlated with the target organ (i.e., brain) halocarbon concentration, as determined in (2), at each time-point. Thereby, it will be possible to determine whether equivalent target organ doses in the rats and dogs elicit CNS effects of comparable magnitude in each species.

5)   Develop and validate toxicodynamic models for inhaled halocarbons. Brain halocarbon concentrations will be correlated with the magnitude of neurobehavioral toxicity in an appropriate equation. These relationships will be used in conjunction with the PBPK model developed in (3). The CNS effects observed in (4) will be compared to predicted effects to assess the validity of the model in the two species tested. Validated models may allow the prediction of CNS effects over time of exposure using: a) extrapolations from pharmacokinetic data; b) simulations in the absence of experimental data.

II.   FUNDAMENTAL HYPOTHESIS TESTING

A very important question faced by scientists and administrators conducting risk assessments is the relevance of toxic effects seen in animals to anticipated adverse effects in humans. Pharmacokinetic studies are playing an increasingly important role in species to species extrapolations in toxicology. Blood concentration over time, as a measure of bioavailability, is routinely accepted as an index of the level of chemical in the entire system, and therefore a representative parameter 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 organ or tissue. It is now recognized that chemical toxicity is a dynamic process, in which the degree and duration of toxic effect in each tissue is dependent upon systemic absorption, tissue distribution, interactions with cellular components, and clearance from the tissue and body by metabolism and excretion. Estimation of the risk of toxic injury from pharmacokinetic data is based on the assumption that the intensity of the response from a given dose is dependent upon the magnitude of the dose received by a target tissue. A related assumption can be stated in the form of a HYPOTHESIS:

THE DOSE RECEIVED IN A PARTICULAR TARGET TISSUE IN ONE SPECIES WILL HAVE THE SAME DEGREE OF EFFECT AS AN EQUIVALENT TARGET TISSUE DOSE IN A SECOND SPECIES.

There are surprisingly few scientific data that are applicable to this basic assumption, although it is a very important premise in interspecies extrapolations in toxicology. If valid, it is a logical basis on which to evaluate the suitability of different species as predictors of toxicity in humans (i.e., the species in which target organ deposition is most similar to man would likely be an appropriate animal model for toxicity testing).

A series of parallel studies in different animal species is therefore being employed to test the foregoing hypothesis. The rat and dog have been subjected

to equivalent exposure to halocarbons. Similarities and differences in respiratory elimination, tissue disposition, and toxicity between the species are being determined. <u>Neurobehavioral alterations</u> will be used as a <u>toxic end-point</u> in the currently proposed work, since: a) OSSA and EPA commonly use neurobehavioral effects as the basis for deriving standards for exposure to VOCs; b) central nervous system (CNS) depression is caused by and can be directly correlated with the concentration of parent compound in the CNS (Bruckner and Peterson, 1981).

III. DEVELOPMENT OF A PBPK MODEL FOR PCE USING TISSUE-CONCENTRATION-TIME DATA

PBPK model development in recent years has primarily incorporated the various tissue compartments of the test subject, the partitioning of the test compound between the tissues and the blood stream, and various uptake and elimination processes of importance to the test chemical. For volatile organic chemicals, however, the lack of an accurate tissue assay has prevented the actual verification of the tissue compartments that are so central to the PBPK model structure. This has also led to the derivation of the partition coefficients almost exclusively from *in vitro* data.

I therefore developed an accurate assay for the short-chain aliphatic halocarbons in a variety of rodent tissues, and utilized this assay in evaluating these critical core components of PBPK models. Using direct measurements of the compounds in the tissues of test animals, tissue:blood partition coefficients were thus derived for the first time from *in vivo* data. These *in vivo* coefficients were then employed in a revised PBPK model, in which for the first time the accuracy of the models in predicting the tissue concentrations of the test compounds over time could be evaluated. Previous model validations for volatile organic chemicals were primarily restricted to using limited blood and exhaled breath data.

This work has led to a significant extension of the potential for validation of the PBPK models for short-chain aliphatic halocarbons. Documentation of the remarkable accuracy of the models to predict inhaled halocarbon tissue concentrations over time in a variety of tissues has far exceeded that of any previously published efforts.

A paper on the derivation and use of the *in vivo* derived PBPK model components is currently in press in the journal *Toxicology and Applied Pharmacology*. The galley proofs for this paper have been included as section A of the Appendix and the reference is as follows:

Dallas, C.E., Chen, X.M., O'Barr, K., Muralidhara, S., Varkonyi, P., and Bruckner, J.V. "Development of a physiologically based pharmacokinetic model for perchloroethylene using tissue concentration-time data." In Press, *Toxicology and Applied Pharmacology*, (1994).

The tissue disposition of perchloroethylene (PCE) was characterized experimentally in rats in order to (1) obtain input parameters from *in vivo* data for the development of a physiologically based pharmacokinetic (PBPK) model, and

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(2) use the PBPK model to predict the deposition of PCE in a variety of tissue
s following inhalation exposure. For the derivation of model input parameters,
male Sprague-Dawley rats received a single bolus of 10 mg PCE/kg body wt in
polyethylene glycol 400 by ia injection through an indwelling carotid arterial
cannula. Other male Sprague-Dawley rats inhaled 500 ppm PCE for 2 hr in dynamic
exposure inhalation chambers. Serial samples of brain, liver, kidney, lung,
heart, skeletal muscle, perirenal fat, and blood were taken for up to 72 hr
following ia injection, during the 2-hr inhalation exposure, and for up to 72 hr
postexposure. Blood and tissue PCE concentrations were analyzed using a gas
chromatography headspace technique. Following ia administration, the tissues
exhibited similar terminal elimination half-lives ($t\frac{1}{2}$). As comparable tissues
$t\frac{1}{2}s$ are consistent with a blood-flow-limited model, tissue:blood partition
coefficients were calculated for noneliminating compartments by division of the
area under the tissue concentration-time curve (AUC) by the blood AUC. Liver PCE
concentration versus time data were employed in the calculation of *in vivo*
metabolic rate constants. A PBPK model was developed using these parameters
derived from the ia data set and used to predict tissue PCE concentrations during
and following PCE inhalation. Predicted tissue levels were in close agreement
with the levels measured over time in the seven tissues and in blood. Tissue
concentration-time data can thus provide valuable input for parameter estimation
and for validation of PBPK model simulations, as long as independent *in vivo* data
sets are used for each step.

IV. UTILIZATION OF THE PBPK MODEL FOR PREDICTION OF SYSTEMIC UPTAKE AND
     RESPIRATORY ELIMINATION OF PCE

Using the *in vivo* derived partition coefficients in the PBPK model, the
accuracy of model predictions of PCE uptake and respiratory elimination were
evaluated. The pharmacokinetic studies conducted in the project provided a
unique data base from which to test the model. Data from the direct measurements
of exhaled breath and blood concentrations were compared to simulated values
calculated from mass-balance differential equations comprising the model.
Thereby, the accuracy of the model was tested and adjustments made where
necessary to improve the model simulations.

A companion publication to the paper described in section III is also in
press in the journal *Toxicology and Applied Pharmacology*, and describes this
evaluation of PBPK model predictions using the *in vivo* derived model components.
The galley proofs for this second paper have been included as section B of the
Appendix and the reference is as follows:

Dallas, C.E., Chen, X.M., Ramanathan, R., Varkonyi, P., Gallo, J.M.,
and Bruckner, J.V. "Use of physiologically-based model to predict
the systemic uptake and respiratory elimination of
perchloroethylene." In Press, *Toxicology and Applied Pharmacology*,
(1994).

The pharmacokinetics of inhaled perchloroethylene (PCE) were studied in
male Sprague-Dawley rats to characterize the pulmonary absorption and elimination
of the volatile organic chemical (VOC). The direct measurements of the time
course of PCE In the blood and breath were used to evaluate the ability of a

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physiologically based pharmacokinetic (PBPK) model to predict systemic uptake and elimination of PCE. Fifty or 500 ppm PCE was inhaled for 2 hr through a miniaturized one-way breathing valve by unanesthetized male Sprague-Dawley rats of 325-375 g. Serial samples of the inhaled and exhaled breath streams, as well as arterial blood, were collected during and following PCE inhalation and analyzed by headspace gas chromatography. PCE-exhaled breath concentrations increased rapidly to near steady state (i.e., within 20 min) and were directly proportional to the inhaled concentration. Uptake of PCE into the blood was also rapid, but blood levels continued to increase progressively over the course of the 2-hr exposure at both exposure levels. Cumulative uptake, or total absorbed dose, was not proportional to the exposure level. A PBPK model was developed form *in vivo* parameters determined from tissue concentration-time data in a companion ia study (Section III). PCE concentrations in the blood and exhaled breath during the following PCE inhalation were well predicted by the PBPK model. Despite species differences in blood:air and lung:air partition coefficients, the model was used to account for similar levels of PCE and other VOCs in the expired air of rats and humans. The model also accurately simulated percentage uptake and cumulative uptake of PCE over time. The model's ability to predict systemically absorbed doses of PCE under a variety of exposure scenarios should be useful in assessment of risks in occupational and environmental settings.

V.   EXTENSION OF PBPK MODEL DEVELOPMENT TO INTERSPECIES COMPARISONS IN RATS AND DOGS

The use of *in vivo*-derived partition coefficients in PBPK models has also been extended to the dog, enabling interspecies comparison of these model predictions with the previous model developed for the rat (Section III and IV). Models developed in a series of species may be scaled based on allometric relations to allow prediction of chemical concentrations in other species without the collection of additional experimental data. Model parameters such as tissue volumes and blood flows can be scaled based on allometric relationships which are functions of animal weight. The ability to scale-up animal physiological models to humans is a powerful tool to obtain predictions of tissue chemical concentrations in humans.

There have not been any PBPK models for PCE or other VOCs published in which the dog was a test species. Therefore, partition coefficients were derived from a time course series of measurements in seven tissues of dogs after PCE administration. In order to test the validity of the model thus constructed from *in vivo*-derived model components, the time-course of uptake, deposition and elimination of PCE in blood and seven tissues was determined in additional experiments in two species, and the data utilized to test the accuracy of model predictions. The rat and the dog were selected, in order to utilize dissimilar species commonly employed in toxicological and pharmacological testing. A PBPK model was used to account for differences in the kinetics of PCE in two species, by simulating metabolism and exhalation of PCE in each animal.

A paper has been accepted for publication on this work, and is now in press in the journal *Environmental Research*. A copy of this paper is included as section C of the Appendix and the reference is as follows:

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Tackett, R.L., and Bruckner, J.V. "Use of tissue disposition data form rats and dogs to determine species differences in input parameters for physiological model for perchloroethylene." In Press, *Environmental Research*, 19°4.

Tissue disposition of perchloroethylene (PCE) was determined experimentally in two mammalian species of markedly different size, in order to derive input parameters for the development of a physiologically-based pharmacokinetic (PBPK) model, which could forecast the disposition of PCE in each species. Male Sprague-Dawley rats and male beagle dogs received a single bolus of 10 mg PCE/kg bw in polyethylene glycol 400 by gavage. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, perirenal fat and blood were taken for up to 72 hr following PCE administration. Blood and tissue PCE concentrations were analyzed using a gas chromatography headspace technique. Dogs exhibited considerably longer tissue and blood half-lives than did rats. The dogs also exhibited larger area under tissue concentration versus time curves (AUC) for all tissues except the liver. Whole body clearance of PCE in the rat was greater than in the dog. Model simulations indicated this could be attributed to more rapid and extensive PCE exhalation and metabolism by the rat. The *in vivo* blood:air partition coefficient determined for rats was similar to an *in vitro* value reported by Gargas *et al.* (1989). *In vivo* tissue:blood partition coefficients, however, were 1.4 to 2.8 times greater than published *in vitro* values. The PCE *in vivo* blood:air partition coefficient for the dog was twice that of the rat, but tissue:blood partition coefficients were 1.5 to 3.0 times greater in the rat than the dog. These results demonstrated the existence of significant differences in partition coefficients in two species commonly used in toxicity testing. The PBPK model was shown to have utility in predicting the impact of metabolism and exhalation on pharmacokinetics of PCE in different species of widely differing size.

VI. APPLICATION OF PBPK MODEL PREDICTIONS ACROSS SPECIES, DOSE, AND EXPOSURE ROUTE

There has been a lack of direct measurements data necessary to evaluate the influence of different species, dose levels, and routes of administration on the accuracy of PBPK model predictions for VOCs. Similarities in the anatomy and physiology of mammalian species make interspecies extrapolations (i.e., animal scale-up or -down) possible. Model input parameters such as alveolar ventilation, tissue volumes and blood flows, and metabolism can be scaled as from one species to another. Changing the amount of chemical entering the animal allows for extrapolations between doses. Altering the point of entry of the chemical into the model enables pharmacokinetic comparisons between routes of administration. Therefore, the accuracy of the PBPK model for PCE to forecast the kinetics of PCE in the rat and dog was evaluated, in terms of the ability of the model to predict blood PCE levels in animals given different doses of different routes of exposure, by comparison of experimental blood concentration time courses with model simulations.

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A paper on these studies has been accepted for publication in the *Journal of Toxicology and Environmental Health.* A copy of the paper has been included as section D of the Appendix, and the reference is as follows:

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Tackett, R.L., and Bruckner, J.V. "A physiologically-based pharmacokinetic model useful in prediction of the influence of species, dose and exposure route on perchloroethylene pharmacokinetics." In Press, *Journal of Toxicology and Environmental Health,* (1994).

The ability of a physiologically-based pharmacokinetic (PBPK) model to predict the uptake and elimination of perchloroethylene (PCE) in venous blood was evaluated by comparison of model simulations with experimental data for two species, two routes of exposure and three dosage levels. Unanesthetized male Sprague-Dawley rats and beagle dogs were administered 1, 3, or 10 mg PCE/kg bw in polyethylene glycol 400 as a single bolus, either by gavage or by intraarterial (ia) injection. Serial blood samples were obtained from a jugular vein cannula for up to 96 h following dosing. PCE concentrations were analyzed by headspace gas chromatography. For each dose and route of administration, terminal elimination half-lives in rats were shorter than in dogs, and area under the blood concentration -time curves were smaller in rats than in dogs. Over a 10-fold range of doses, PCE blood levels in the rat were well predicted by the PBPK model following ia administration, and slightly underpredicted following oral administration. PCE concentrations in dog blood were generally overpredicted, except for fairly precise predictions for the 3 mg/kg oral dose. These studies provide experimental evidence of the utility of the PBPK model for PCE in interspecies, route-to-route and dose extrapolations.

VII.   COMPARISON OF TRI TOXICOKINETICS WITH NEUROBEHAVIORAL TOXICITY DURING INHALATION EXPOSURE

In the studies on the CNS effects of VOCs, schedule-controlled behavior has been frequently employed as a quantitative endpoint to measure neurobehavioral toxicity. The inhalation of TRI by test animals has been associated with reductions in the responding rates of operant behavior using various reinforcing schedules. However, there is a surprising paucity of data relating the disposition of VOCs in the blood and brain (the target organ) and these neurobehavioral effects. This data is of particular important to the testing of the hypothesis in this grant of evaluating relative toxic effects between species with equivalent VOC concentration in the target organ. Therefore, correlated studies were conducted to compare neurobehavioral effects in mice inhaling TRI with the blood and brain disposition of the compound.

These studies are included in a paper that is currently in press in the journal *Toxicology.* The galley proofs for this paper have been included as Appendix E, and the reference is as follows:

You, L., Muralidhara, S., and Dallas, C.E. "Comparisons between operant responding and 1,1,1-trichloroethane toxicokinetics in mouse blood and brain." In Press, *Toxicology,* (1994).

The effect of 1,1,1-trichloroethane (TRI) inhalation on operant response was evaluated in relation to the concentration of TRI in blood and brain tissue in mice during exposure. Male CD-1 mice were trained to lever-press for an evaporated milk reinforcer on a variable interval (VI 60) schedule for 2 h. Trained mice were then exposed to either 3500 or 5000 ppm TRI for up to 100 min, and the changes in the schedule-controlled performance were measured. Additional groups of mice were exposed under the same conditions as those used in the behavioral study and sacrificed at various times during exposure, and the blood and brain samples were collected and subsequently analyzed for TRI content by headspace gas chromatography. Uptake of TRI into blood and brain was rapid, with near steady-state levels reached after approximately 40-60 min of exposure. Inhalation of 5000 ppm, but not 3500 ppm TRI was seen to cause inhibition of operant response, starting ~30 min following the initiation of inhalation exposure and beginning to recover after 80 min of exposure. The threshold concentrations for the maximal behavioral inhibition were ~110 $\mu g/g$ and 130 $\mu g/ml$ in mouse brain and blood, respectively. It appears that in addition to TRI concentrations in blood and brain tissue, the time it takes to reach the apparent threshold TRI concentration was also a determinant for the onset of TRI neurobehavioral depression.

In order to appropriately correlate the toxicokinetics of these agents with the neurotoxicity response, one additional factor of potential importance to these lipophilic chemicals had to be addressed. It has been recognized that diet is one of the factors that can alter the pharmacokinetics and toxicity of a compound. The pattern, quantity, and content of dietary intake is able to change the responsiveness of a biological system to a toxicant, as well as to influence the bioavailability of the chemical in the system. However, the consequence of high milk intake on VOC pharmacokinetics that would occur during the behavioral sessions of neurobehavioral toxicity studies has not been examined previously. Since an effect on the pharmacokinetics of a toxicant often results in an alteration in toxicity, this study was undertaken to determine the potential impact of high milk intake during operant training on the pharmacokinetics of inhaled VOCs.

The paper on this study has been accepted for publication in the journal *Drug and Chemical Toxicology*. A copy of this paper has been included as Appendix F, and the reference is as follows:

You, L., Muralidhara, S., and Dallas, C.E. "The pharmacokinetics of inhaled 1,1,1-trichloroethane following high milk intake in mice." In Press, *Drug and Chemical Toxicology*, (1994).

In the evaluation of lipophilic halocarbons for neurobehavioral toxicity in operant testing, animals often receive large amounts of milk as a behavioral reinforcer over time. If this increase of fat in the diet sufficiently impacted the lipid depots of the animal, the pharmacokinetics of lipophilic test compounds might be significantly affected and thus obscure the accompanying neurobehavioral effects. The effects of milk intake, comparable to what was consumed as behavioral reinforcer during operant behavioral sessions, on the pharmacokinetics of inhaled 1,1,1-trichloroethane (TRI) were therefore examined in the blood and nine organ tissues of mice. Male CD-1 mice were food restricted so that their body weights would be reduced to an maintained at 80% of their original, and

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received a single gavage dose of 1.0 ml evaporated milk daily for three weeks. A control group with similar food restrictions was dosed with the same volume of water. inhalation exposures to 3500 ppm TRI for 100 minutes were conducted at the end of the treatment period. Blood and nine organ tissues were sampled at a series of time points, and their TRI contents were analyzed by headspace gas chromatography. The uptake of TRI was rapid, with near steady state approached in blood and most tissues after 40-60 minutes of exposure. All of the tissues except fat had similar TRI time-concentration profiles, while TRI concentrations in fat tissue were about 20-30 times higher than in other tissues. There was no statistically significant difference in the tissue concentrations between the milk-dosed group and water-dosed group at all of the time points for all tissues measured. Therefore, it appears unlikely that this level of milk intake as a reinforcer in behavioral studies will affect the results of operant testing evaluations by altering the pharmacokinetics of lipophilic halocarbons such as TRI.

Both of these studies in Section VII were presented at the most recent meeting of the Society of Toxicology in Dallas, TX (March, 1994). The reference for the abstract of this presentation is as follows:

You, L., Muralidhara, S., and Dallas, C.E. "Neurobehavioral effects of 1,1,1-trichloroethane and its relationship with brain concentration in mice," *Toxicologist* 14: 352, 1994.

VIII. CORRELATION OF PCE TOXICOKINETICS AND BEHAVIORAL EFFECTS FOLLOWING ORAL EXPOSURE IN RATS

The majority of reports of PCE-induced human toxicity has focused upon the neurological effects among the occupationally or experimentally exposed. Acute exposure in the 100 to 200 ppm range have typically resulted in reversible mood and behavioral changes and impaired coordination. Major EEG changes suggestive of cerebral cortical depression have been found among volunteers repeatedly exposed to 100 ppm. Subchronic exposure to even lower PCE concentrations has reportedly caused memory loss and insomnia as well as perceptual, attention, and intellectual deficits.

Acute neurological effects in humans after ingesting PCE appear to parallel those seen after inhalation. The oral administration of PCE to hundreds of thousands of patients as an anthelminthic agent at doses of 60 to 85 mg/kg (assuming a 70 kg body weight with doses of 4.2 to 6 g) typically produced inebriation, perceptual distortion, and exhilaration. The accidental ingestion of 600 to 800 mg/kg (assuming a 20 kg body weight with a dose of 12 to 16 g) by a 6-year-old boy was followed by drowsiness, vertigo, agitation, and hallucinations, prior to somnolence and coma.

The neurological effects of PCE exposure may be expressed as subtle behavioral changes that jeopardize one's state of well-being. It is important that such changes be assessed in an animal model in a valid, quantitative manner. Tests employing schedule-controlled operant behavior (SCOB) have demonstrated chemically related behavioral effects prior to irreversible neuropathological

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changes for a number of solvents, including toluene, trichloroethylene, and trichloroethane. While PCE's effects on open-field behavior motor activity and neuromuscular ability have been investigated, PCE's effect on the SCOB of laboratory animals has not been reported.

It has been suggested that the biological effects of solvents may be more closely related to blood or target tissue (i.e., brain) concentrations than administered dose (references). Unfortunately, efforts to integrate the pharmacokinetics of solvents with neurobehavioral effects have been few. It has been demonstrated that brain and blood toluene levels were highly correlated with the degree of CNS depression in mice, as measured in tests of unconditioned performance and reflexes. More recently, it has been shown that shock avoidance performance decrements were closely related to trichloroethylene levels in the blood of rats. It has also been reported that a correlation exists between blood trichloroethane levels and performance decrements in human volunteers. To date, however, no parallel investigations of the pharmacokinetics and neurobehavioral effects of PCE have been reported. Thus, the present study was designed to evaluate the relationship between the pharmacokinetic distribution or orally administered PCE and its effects on SCOB of rats.

1,1,2,2-tetrachloroethylene (PCE) of 99%+ purity was obtained from Aldrich Chemical Company, Inc. Burdick and Jackson Brand, High Purity Solvent iso-octane was obtained from Baxter Healthcare Corporation. Chemically naive, male Sprague-Dawley rats weighing 300 to 350 g were used in all experiments. Rats were housed two per cage in suspended wire bottom cages (36 x 20 x 20 cm) in a temperature- (22°C) and humidity- (45%) controlled room with a 12-hr light-dark cycle (light: 0700-1900 hr; dark: 1900-0700 hr). Rats were allowed to acclimate for a minimum of 7 days prior to use, during which time food and tap water were provided ad libitum. All experiments were conducted during the light cycle.

Twelve rats were transferred to individual polypropylene cages (48 x 25 x 20 cm) with corn cobb bedding and stainless steel wire lids. Rats were food restricted (10 ± 0.25 g/day) for 72 hr prior to being surgically implanted with an indwelling carotid artery cannula. Rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg body wt. of a mixture of ketamine HCL (100 mg/ml): acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in the proportion 3:2:1 (v:v:v). The cannula exited the skin at the nape of the neck and was protected from manipulation by surgical tape. The cannulated animals were placed in individual polypropylene cages (30 x 20 x 15 cm) with wire mesh tops for surgical recovery and subsequent blood sampling. After an overnight recovery period, either 160 or 480 mg/kg body wt. PCE was administered as an aqueous Alkamuls® emulsion by bolus gavage in a total volume of 3 ml/kg body wt. Ten (160 mg/kg body wt.) or 20% (480 mg/kg body wt.) Alkamuls EL-620 was used to prepare stable emulsions of PCE in 0.9% saline. The actual concentration of PCE in each dosage formulation was determined from a standard curve made by diluting various amounts of PCE in isooctane for analysis by headspace gas chromatography (GC). Following dosing, blood samples were withdrawn from the arterial cannulas of the unrestrained and unanesthetized animals via a three-way stopcock by a 1 ml syringe. Serial blood samples (2 to 75 μl, depending upon the anticipated blood concentration) were taken at time intervals of 1 min to 24 hr for up to 4 days after dosing. Blood samples were quickly transferred to 8 ml headspace vials, capped immediately with teflon-lined latex rubber septa in aluminum seals,

and crimped tightly. Some blood samples required dilution with ice-cold saline in order that they could be analyzed within the linear range of the electron capture detector of the GC. As necessary, blood withdrawal was followed by a heparin flush to maintain cannula patency. Food was withheld during surgical recovery (approximately 18 hr) but was available ad libitum during blood sampling.

For the determination of PCE concentrations in tissues, rats were transferred to individual polypropylene cages (48 x 25 x 20 cm), food restricted (10 ± 0.25 g/day) for 72 hr, and subsequently fasted for 18 hr prior to being dosed as described for blood sampling. Groups of six rats were sacrificed by cervical dislocation followed by decapitation at 1, 6, 15, 30, 40, 50, 60, and 90 min after dosing. Approximately 0.5 to 1.0 g samples of brain, liver, perirenal fat, and skeletal muscle were excised with 2.5 to 3 min from each animal and immediately placed into chilled scintillation vials containing 2 ml of 0.9% saline and 8 ml of isooctane. Tissues were homogenized as quickly as possible (5 to 15 seconds) with an Ultra-Turrax® homogenizer to minimize volatilization of PCE prior to being vortex-mixed for 30 seconds. The homgenates were then centrifuged at 2500 x g for 10 min at 4°C in the capped scintillation vials. An aliquot of the isooctane layer (5 to 20 μl) was either transferred directly to a 20 ml headspace vial. The vials were capped immediately with teflon-lined latex rubber septa in aluminum seals and crimped tightly.

A Sigma Model 300 GC equipped with a HS6 headspace sampler and an electron capture detector (ECD) was used for the analysis of PCE in blood. Analyses were carried out using a stainless-steel column (182 x 0.317 cm) packed with 3% OV-17 (100-120 mesh). The GC operating conditions were: headspace sampler temperature, 80°C; injection port temperature, 200°C; column temperature, 90°C; ECD temperature, 360°C; flow rate for argon:methane (95:5) carrier gas, 60 ml/min. For PCE analysis of isooctane tissue extracts, a Perkin-Elmer Model 8500 GC with a HS-101 headspace autosampler and ECD was employed under the same conditions as those previously listed. All sample vials were heated thermostatically in one of the two headspace sampler units, pressurized with the carrier gas, and a pre-set volume of volatilized isooctane and PCE was injected into the GC column. PCE concentrations were calculated from daily standard curves made by diluting various amounts of PCE in isooctane for GC analysis and corrected for the percent recovery characteristic of blood and tissue samples. Percent recovery of PCE was determined by injecting solutions of PCE in isooctane into samples of blood and each of the four tissue types with a Hamilton gas-tight syringe. The blood and tissues were then homogenized in saline/isooctane as previously described and aliquots of the isooctane analyzed for PCE content. Percent recoveries ranged from 86% for fat to 92% for muscle. The limit of detection for PCE was approximately 1 ng.

Fourteen rats were transferred to individual polypropylene cages (48 x 25 x 20 cm) with corn cobb bedding and stainless steel wire lids. Rats were food-restricted during a period in which they were trained to lever-press for undiluted evaporated milk presentation (0.05 ml for 7 seconds) on a fixed ratio-40 schedule of reinforcement. Rats were initially hand shaped in daily 30 min sessions. Once rats learned to respond independently, the ratio of responses to reinforcers was gradually increased to 40 and the session length extended to 90 min. Rats were allowed to respond in daily 90 min sessions spaced 24 hr apart

until their response rates stabilized, a process requiring 10 to 15 days. The criterion for stable behavior was three successive sessions in which the number of responses per second varied by less than 10%.

Upon the completion of each operant session, rats were returned to their home cage and given 10 ± 0.25 g of food which was promptly eaten. Since 24 hr separated each operant session, this feeding regimen resulted in a fasting period of approximately the same duration as that employed in the study of PCE's pharmacokinetics. Once rats exhibited stable behavior, they were gavaged with either a 10 or 20% aqueous Alkamuls emulsion vehicle (3 ml/kg body wt.), immediately placed in a modular test cage, and monitored for operant behavior for 30 min. Twenty-four hr later, rats were dosed with either 160 or 480 mg/kg body wt. PCE in the appropriate vehicle (3 ml/kg body wt.), and their behavior monitored again. The behavior of six and eight rats was monitored after administration of the low and high doses, respectively.

Operant sessions took place in a modular test cage for rodents equipped with a response lever, a liquid delivery through and dipper, and a stimulus light above the delivery trough that remained lit during the availability of the milk reinforcer. The modular test cage was placed inside a 1.0 M^3 Rochester-type dynamic flow inhalation chamber that served to isolate animals from extraneous external stimuli. The test cage was interfaced via LabLinc with an IBM compatible 386 computer running COSMOS software that applied the operant performance schedules and recorded the number of responses and reinforcers in each 5-min interval of the operant sessions.

RSTRIP (Version 3.1) was used to fit PCE blood concentration versus time profiles to polyexponential equations and for calculating AUC, t½, and Cmax. Tests for differences in the concentrations of PCE in blood and tissues were made with two sample t-tests. The baseline response of each rat was calculated as the mean number of responses in each 5 min interval of the three operant sessions used to meet the stability criterion. Trends in respondse(s) were examined by fitting a straight line to the baseline response of each rat and calculating a t-statistic based on the mean ± SD or regression coefficients (slopes) for comparison to critical t values. Response ratios (vehicle/baseline, (PCE + vehicle)/baseline, and (PCE + vehicle)/vehicle) for each of the eighteen 5 min intervals in the operant sessions were calculated so that each rat served as his own control. These ratios were subjected to a mixed model repeated measures analysis of variance (RMANOV) with fixed factors of time and treatment, and a random factor of rat nested within treatment. In the event that time effects failed to satisfy multisample sphericity, test statistics of time effect and time x treatment interaction were modified using the Geisser-Greenhouse adjustment factor. Despite non-significant time x treatment interactions, two sample t-tests were used to determine the 5 min intervals during which response ratios differed between dose groups. Paired t-tests within each dose group were used to determine the intervals during which response ratios significantly differed from one another. Due to equipment failure, response data from portions of two operant sessions used to meet the stability criterion were lost. These data were presumed to be equal to that in the last 5 min interval successfully recorded. Following gavage with 480 mg/kg body wt. PCE< two rats failed to respond in all but two intervals. Exclusion of these non-responders did not change the conclusions drawn from variance analysis, but did slightly modify P values.

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Unless otherwise noted, data analysis was exclusive of the non-responders. The minimum level of significance was set at $p \leq 0.05$ for all tests.

The blood concentration versus time profiles for rats following gavage at both doses (160 and 480 mg/kg) are presented in Figs. 1 and 2. The long elimination half-life ($t\frac{1}{2}$) of PCE relative to that of most volatile halocarbons was demonstrated by its presence in blood for several days after dosing. The pharmacokinetic parameter estimates for each treatment group are summarized in Table 1. The differences in AUC and Cmax between the two doses were nearly proportional to the difference in PCE dose. A 3-fold increase in dose resulted in AUC and Cmax values that were 2.7 and 3.6-fold higher, respectively. Blood $t\frac{1}{2}$ did not differ with dose.

The blood and brain concentration versus time profile during the 90 min immediately following dosing are presented in Figs. 3 and 4, respectively. This 90-min post dosing period corresponds to the time course over which SCOB was monitored. PCE was rapidly absorbed from the gastrointestinal tract as evidenced by its presence in blood as early as 1 min after dosing. As might be anticipated for a highly perfused tissue, PCE was also present in the brain at this time. Following a 10 to 15 min phase of very rapid PCE uptake, blood and brain concentrations slowly increased as they approached a steady-state equilibrium. Absolute maximum blood concentrations were not reached until 30 and 90 min after administration of the low and high doses, respectively. Following low dose gavage, the maximum brain concentration occurred at 60 min, but still increasing 90 min after administration of 480 mg/kg PCE.

In both the blood and brain, the ratio of PCE concentrations resulting from the two doses was greatest at 1 min (9.5 in blood and 3.0 in brain). Blood PCE concentrations significantly differed with dose at all sampling times during the 90 min immediately following dosing ($p \leq 0.0018$). Despite a 3-fold difference in mean brain PCE concentrations at 1 min, the difference was not statistically significant ($p = 0.072$) due to variability within the high dose group. Brain concentrations at 6, 15, and 20 min also were not significantly different across doses ($p = 0.1080$, $p = 0.2139$, and $p = 0.4488$, respectively), but were so at all subsequent sampling times ($p \leq 0.0068$).

Tissue dose time courses for PCE were also determined in fat, liver, and muscle (Figs. 5 and 6). The rate of blood perfusion and lipid content of these tissues had a significant impact on organ deposition in the rat. Based upon relative tissue concentrations at 1 and 6 min, the well perfused liver accumulated PCE at the highest rate, followed by the fat and muscle. Based on concentrations at 1 and 6 min, PCE accumulation by the brain was most similar to that of the fat, not the liver as might be predicted. Prior to 30 min post dosing, tissue concentrations rarely differed with dose. At 90 min post dosing, at which time behavioral monitoring was discontinued, PCE concentrations were greatest in the fat, followed by the liver, brain, and muscle.

The baseline response rates of rats in the low and high dose treatment groups ranged from 1.04 to 3.04 (mean ± SD, 1.88 ± 0.75) and from 0.99 to 2.62 (1.63 ± 0.58) responses per second, respectively (Figs. 7 and 8). Neither treatment group exhibited a significant trend in baseline responding, i.e., baseline response rates did not have a tendency to significantly increase or

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decrease over the course of the operant sessions. However, straight lines fit to the baseline responses of eleven of the fourteen rats had negative slope values indicating slight decreases in response rates did occur over time.

The repeated measures analysis of variance on vehicle/baseline (V/B) ratios indicated no significant time x treatment interaction (p = 0.3349), but significant treatment (p = 0.0159) and time (p = 0.0254) effects. Despite the high dose group having a higher mean V/B ratio during sixteen of the eighteen operant session intervals, group differences were significant only between 5-10 (p = 0.0341) and 10-15 (p = 0.0184) min. Response rates of the treatment groups averaged 91.6 ± 5.2 and 111.0 ± 18.2% of baseline after administration of the 10 and 20% aqueous emulsion vehicles, respectively (Figs. 9 and 10). Mean V/B ratios of the low dose group were less than one during fourteen session intervals. In contrast, mean V/B ratios of the high dose group were greater than one in fifteen intervals. These deviations from unity by the low dose group were significant only between 0-5, 5-10, 10-15, 30-35, and 40-45 min (p = 0.0099, 0.0107, 0.0197, 0.0252, and 0.0297, respectively), while none of the mean V/B ratios of the high dose group significantly differed from one.

Variance analysis of (PCE + vehicle)/baseline (PV/B) ratios indicated no significant time x treatment interaction (p = 0.2794) or treatment effect (p = 0.1870), but a significant time effect (p = 0.005) (Figs. 11 and 12). Despite the low dose group having a higher PV/B ratio during fourteen of the eighteen session intervals, group differences were not significant during any interval. Response rates of the treatment groups averaged 99.4 ± 9.8 and 83.6 ± 16.0% of baseline after administration of the low and high PCE doses, respectively. the inclusion of data on the two-non-responders reduced the mean response rate of the high dose group to 62.9 ± 40.6% of baseline. Mean PV/B ratios of the low dose group were less than one in eight intervals, including five of the first seven (Fig. 13). Mean PV/B ratios of the high dose group were less than one in thirteen intervals including eleven of the first twelve (Fig. 14). These deviations from unity by the low dose group were significant only between 0-5, 50-55, and 80-85 min (p = 0.0329, 0.0221, nd 0.0396, respectively), while V/B ratios of the high dose group significantly differed from one at 0-5 (p = 0.0002) and 10-15 (p = 0.0220) min.

When (PCE + vehicle)/vehicle (PV/V) ratios were submitted to variance analysis, there was no significant time x treatment interaction (p = 0.4153), but a significant treatment (p = 0.0012) and time (p = 0.0230) effect. Even though the low dose group had a higher mean PV/V ratio during all of the session intervals, group differences were only significant between 0-5 (p = 0.0357), 10-15 (p = 0.0051), and 15-20 (p = 0.0163) min. Response rates of the treatment groups averaged 107.2 ± 13.3 and 75.8 ± 17.7% of vehicle after administration of the low and high PCE doses, respectively. The inclusion of data on the two non-responders reduced the mean response rate of the high dose group to 57.0 ± 37.7% of vehicle. Mean PV/V ratios of the low dose group were greater than one in seventeen intervals. On the otherhand, mean PV/V ratios for the high dose group were less than one in seventeen intervals. None of the deviations form unity were significant in the low dose group, while response ratios during the first four intervals (p = 0.0018, 0.0512, 0.0135, and 0.0376, respectively) were significantly different form one in the high dose group. Inclusion of data from

the two non-responders in the high dose group resulted in significant deviations from one in ten intervals, including none of the first ten.

Table 1. Pharmacokinetic Parameters Following Oral Administration of Perchloroethylene in Rats[1]

Dose (mg/kg)	AUC $(\mu g \cdot min/mL)$ [2]	$t_{\frac{1}{2}}$ (min) [3]	$C_{max}(\mu g/mL)$ [4]
160	9508 ± 730	504 ± 46	24.7 ± 2
480	25256 ± 1162	555 ± 78	89.2 ± 7

1. Values are the Mean ± SE of 6 rats per dose.
2. Area under the curve that describes the concentration of PCE in blood as a function of time.
3. Elimination half-life or the amount of time required for the blood concentration of PCE to be reduced by 50%.
4. Maximum concentration of PCE in blood.
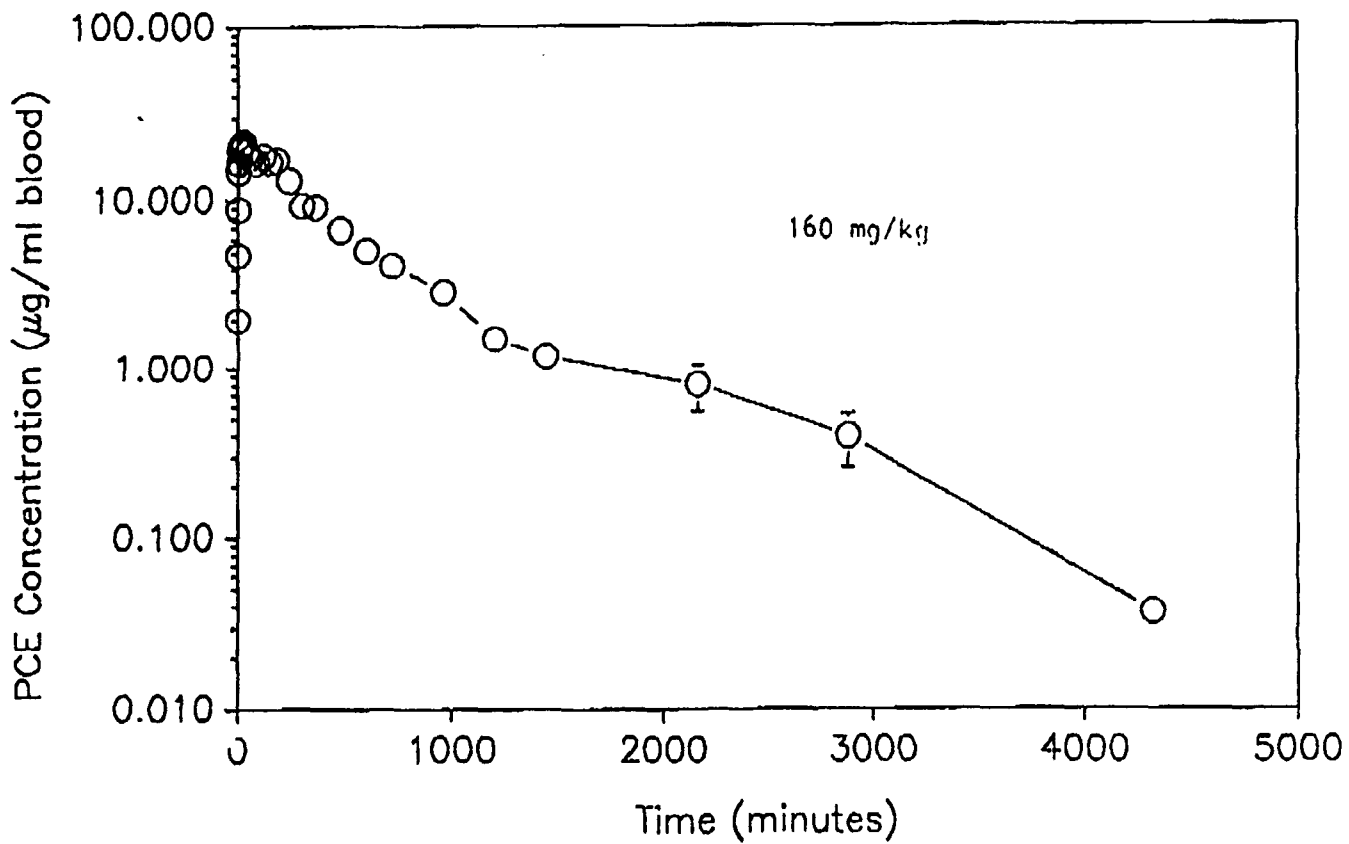
Figure 1. The uptake and clearance of PCE from the blood of rats following oral gavage with 160 mg/kg PCE in a 10% aqueous Emulphor emulsion. Each point represents the mean ± SE of six animals.
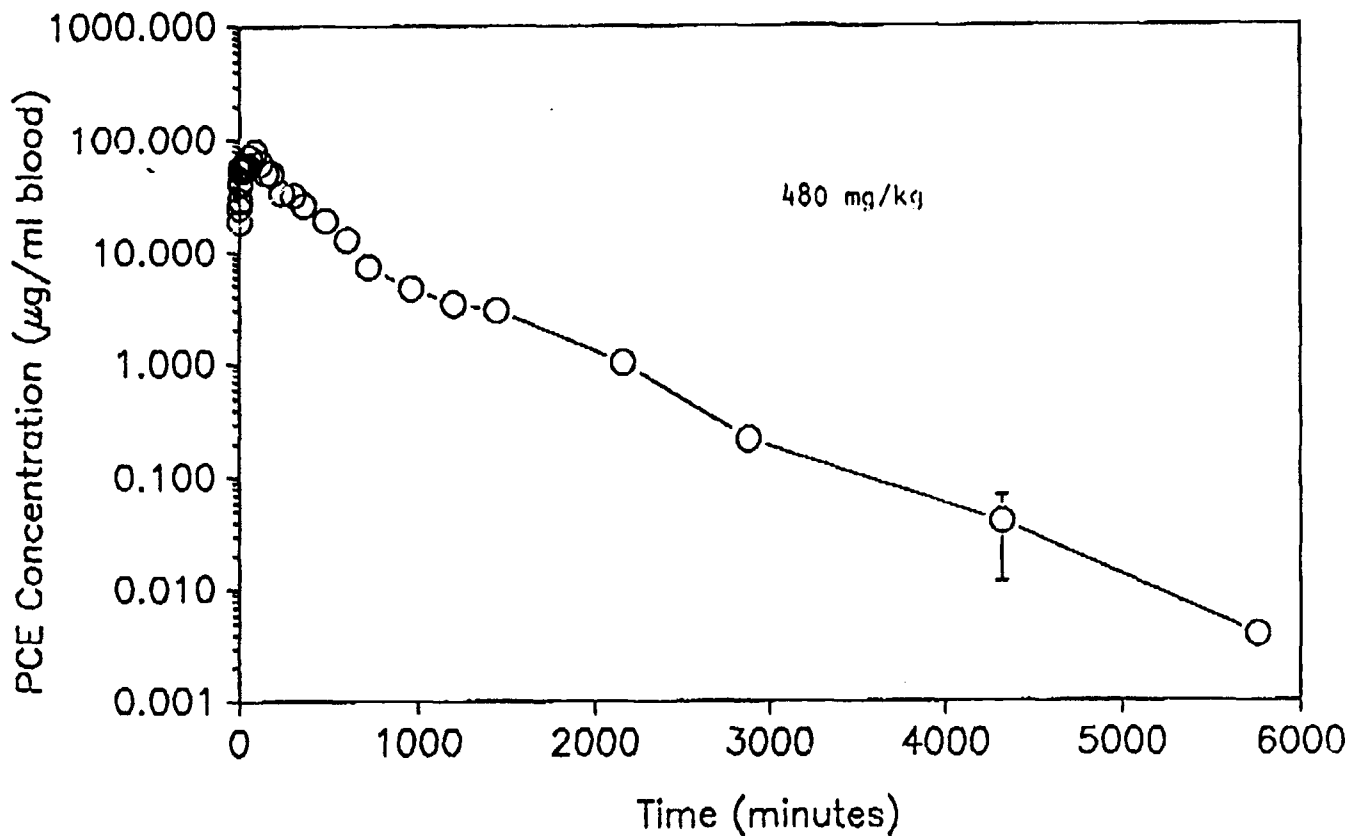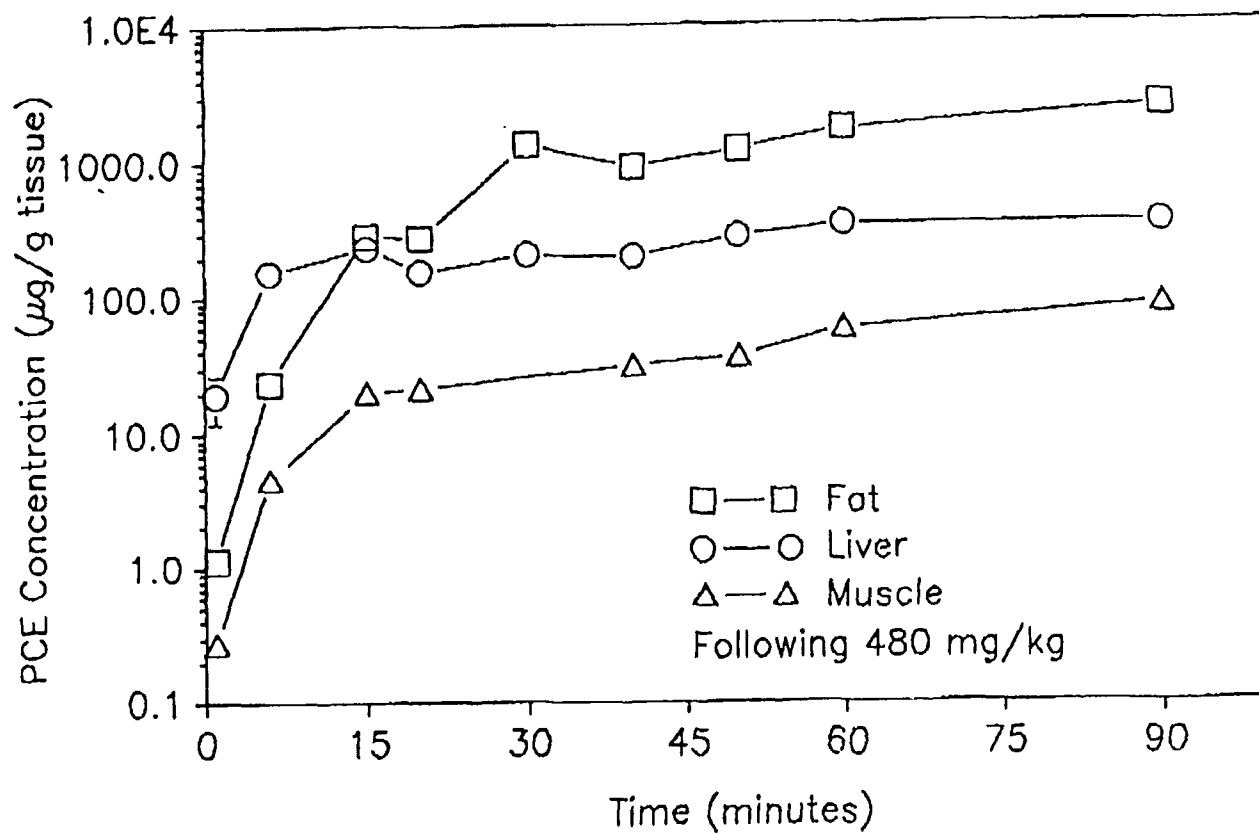
Figure 2. The uptake and clearance of PCE from the blood of rats following oral gavage with 480 mg/kg PCE in a 20% aqueous Emulphor emulsion. Each point represents the mean ± SE of six animals.
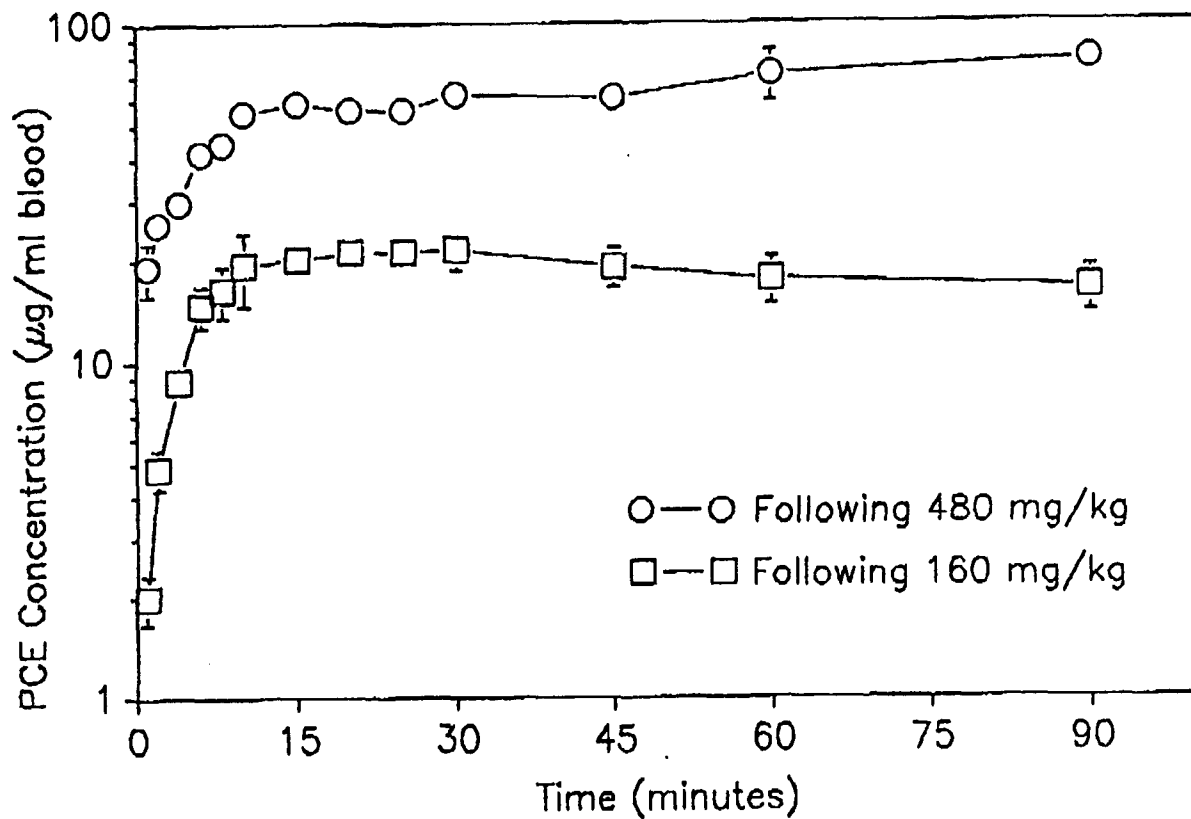
Figure 3. The uptake of PCE in blood during the 90 minutes immediately following oral gavage with 160 mg/kg and 480 mg/kg PCE in 10% and 20% aqueous Emulphor emulsions, respectively. Each point represents the mean ± SE of six animals.
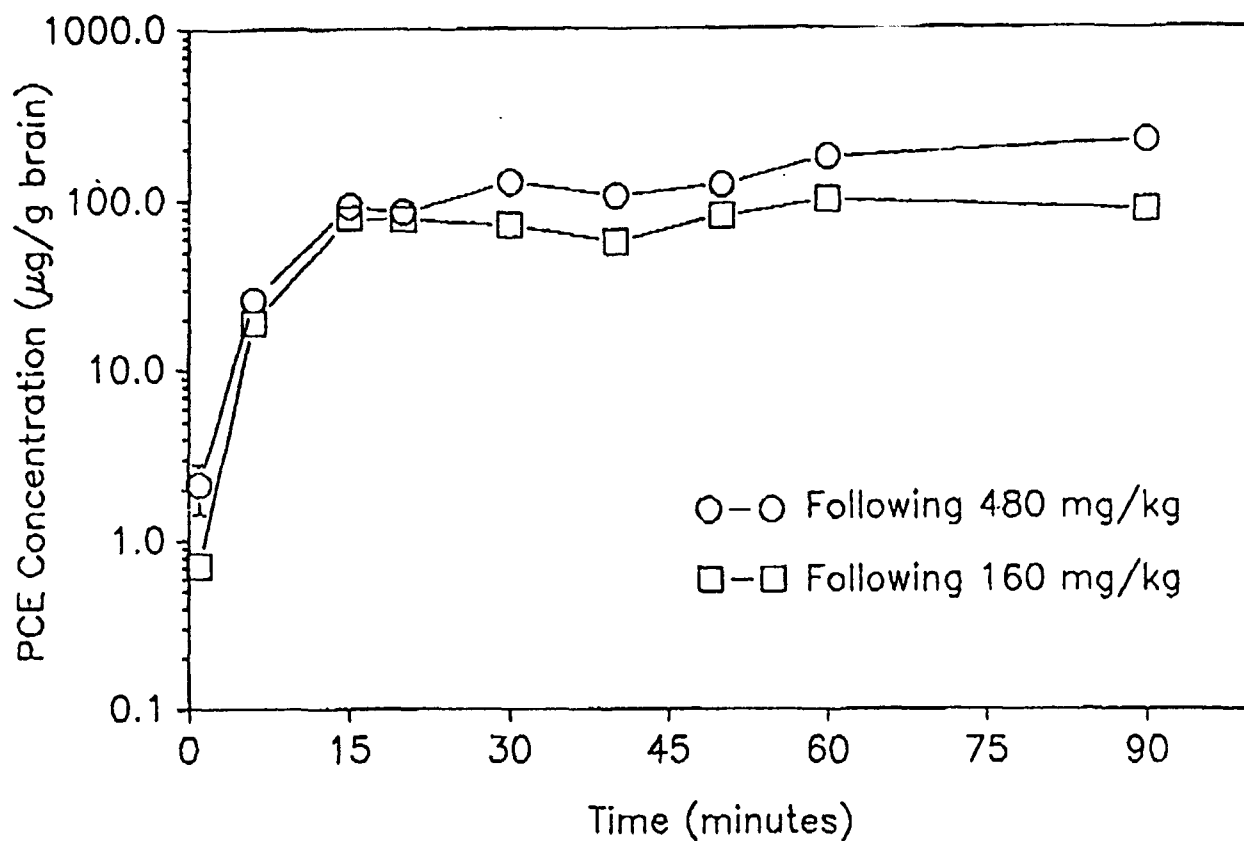
Figure 4. The uptake of PCE in brain during the 90 minutes immediately following oral gavage with 160 mg/kg and 480 mg/kg PCE in 10% and 20% aqueous Emulphor emulsions, respectively. Each point represents the mean ± SE of six animals.
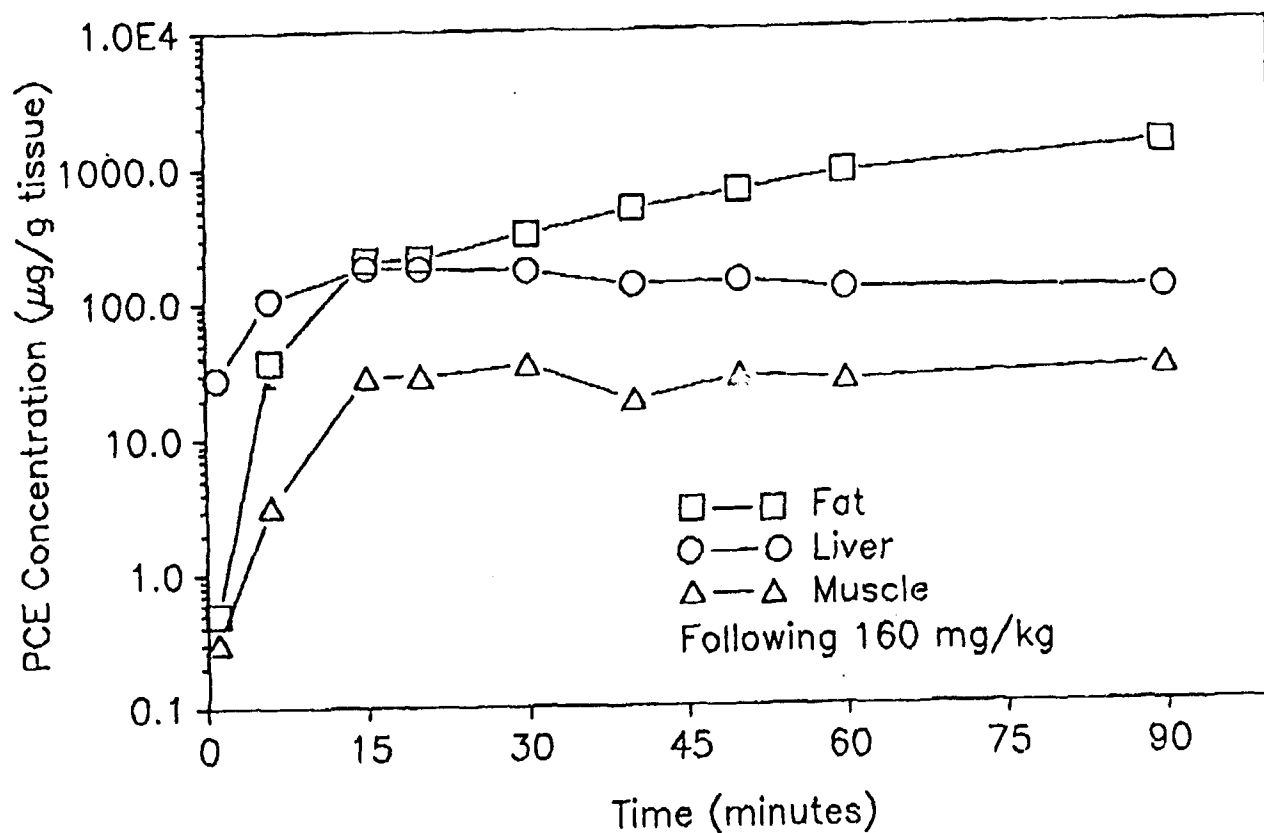
Figure 5. The uptake of PCE in fat, liver, and muscle during the 90 minutes immediately following oral gavage with 160 mg/kg PCE in a 10% aqueous Emulphor emulsion. Each point represents the mean ± SE of six animals.

Figure 6. The uptake of PCE in fat, liver, and muscle during the 90 minutes immediately following oral gavage with 480 mg/kg PCE in a 20% aqueous Emulphor emulsion. Each point represents the mean ± SE of six animals.
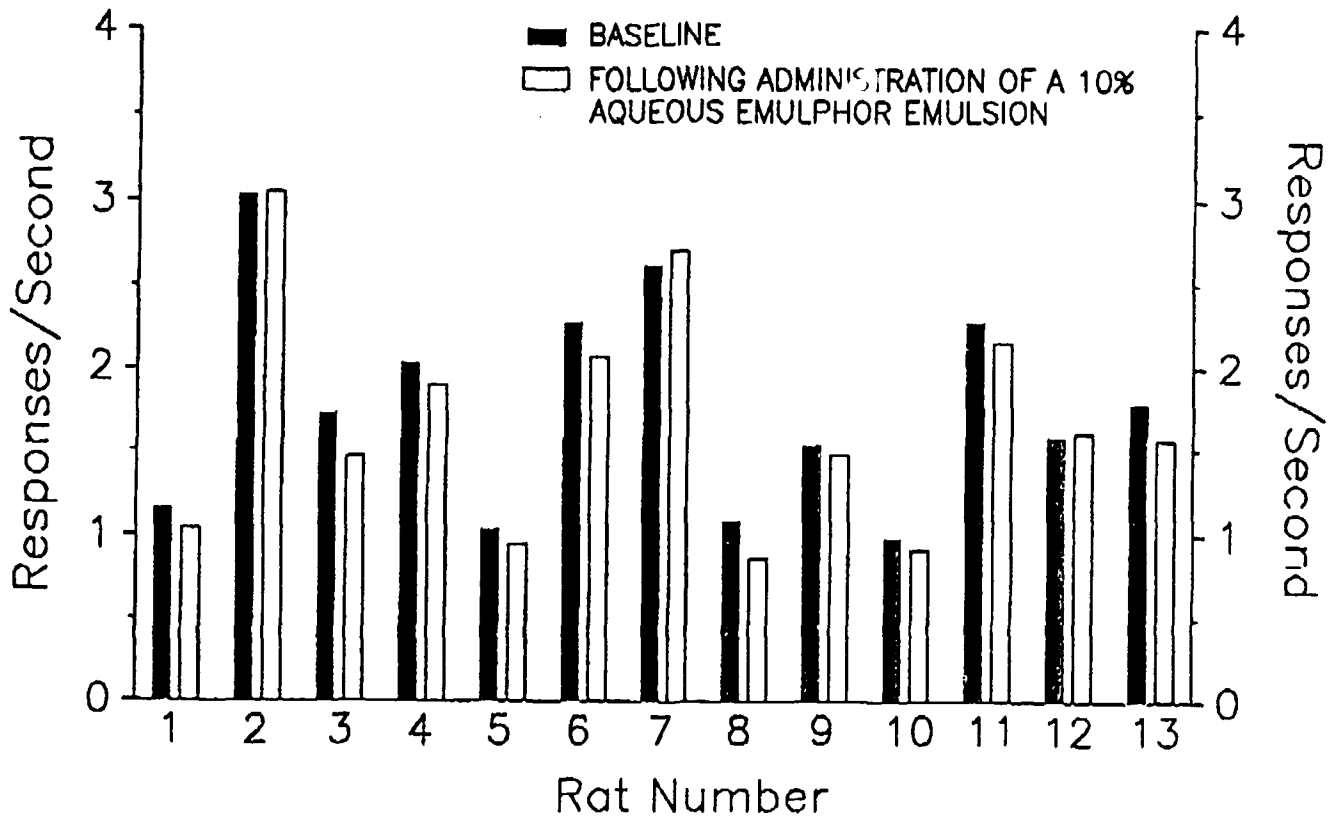
Figure 7. Operant response rates of thirteen rats during the 90 minutes immediately following either no treatment (baseline) or oral gavage with 1 ml of 10% aqueous Emulphor emulsion vehicle.
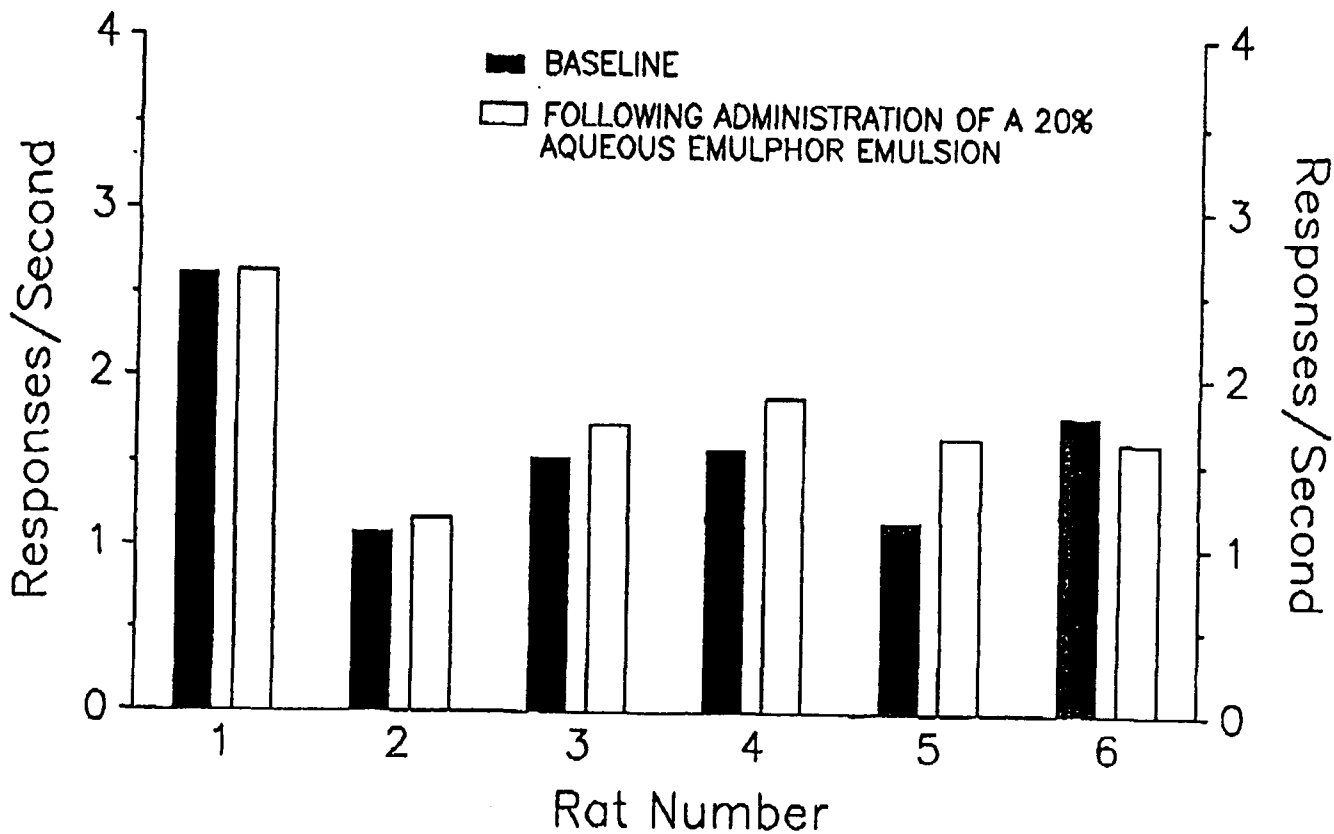
Figure 8.   Operant response rates of -- six -- rats during the 90 minutes immediately following either no treatment (baseline) or oral gavage with 1 ml of 20% aqueous Emulphor emulsion vehicle.
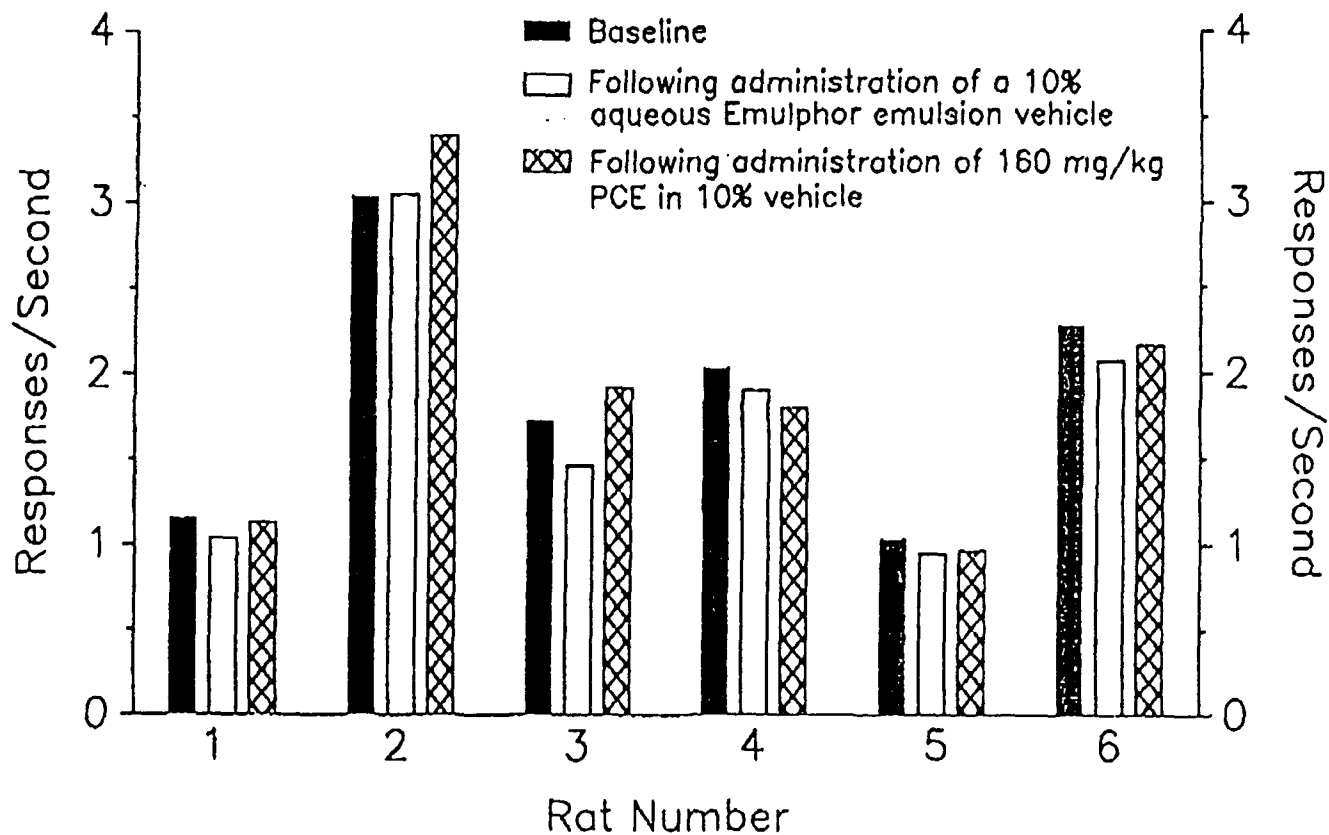
Figure 9. Ratio of operant responses following oral gavage with 160 mg/kg PCE in a 10% aqueous Emulphor emulsion to operant responses following oral gavage with emulsion vehicle alone. Each point represents the mean ± SE of six animals as calculated for each 5 minute interval of the 90 minute operant session.
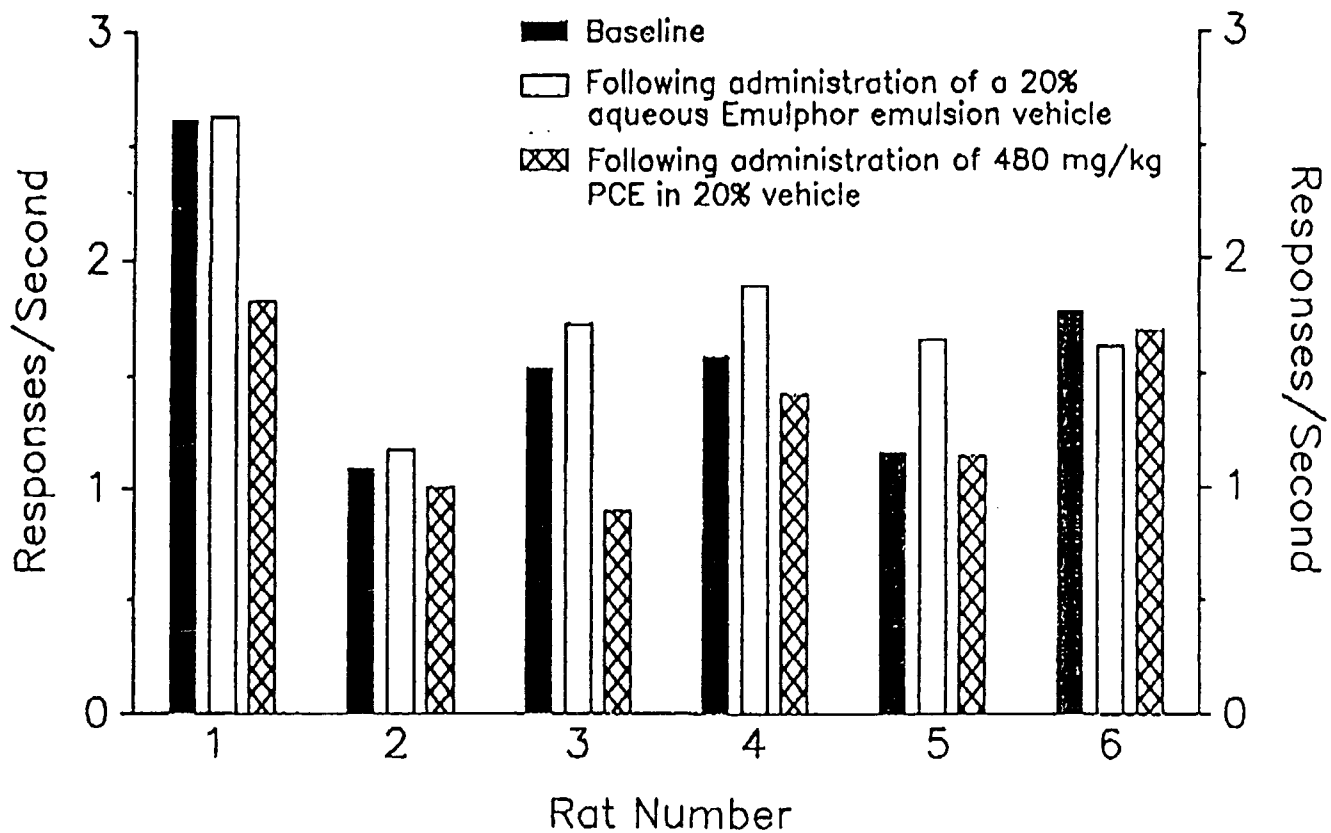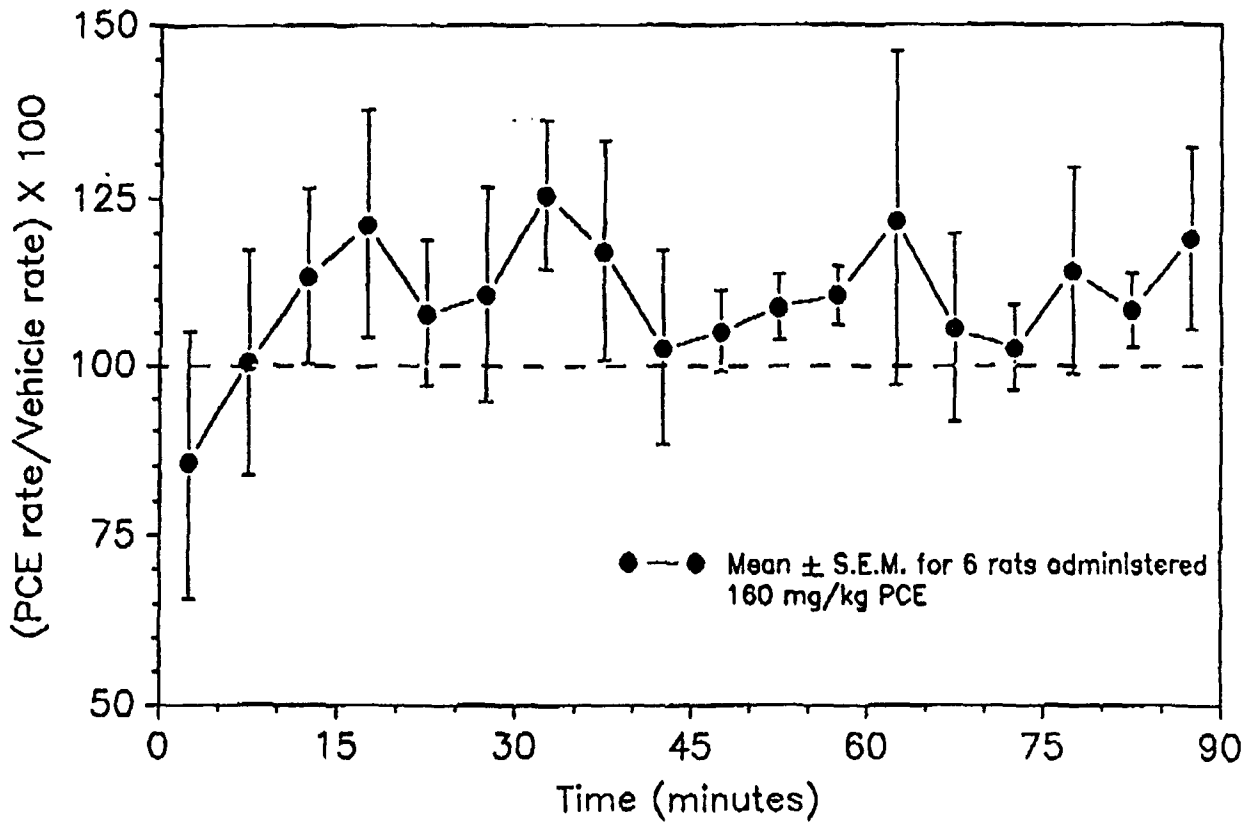
Figure 10. Ratio of operant responses following oral gavage with 480 mg/kg PCE in a 20% aqueous Emulphor emulsion to operant responses following oral gavage with emulsion vehicle alone. Each point represent the mean ± SE of six animals as calculated for each 5 minute interval of the 90 minute operant session.
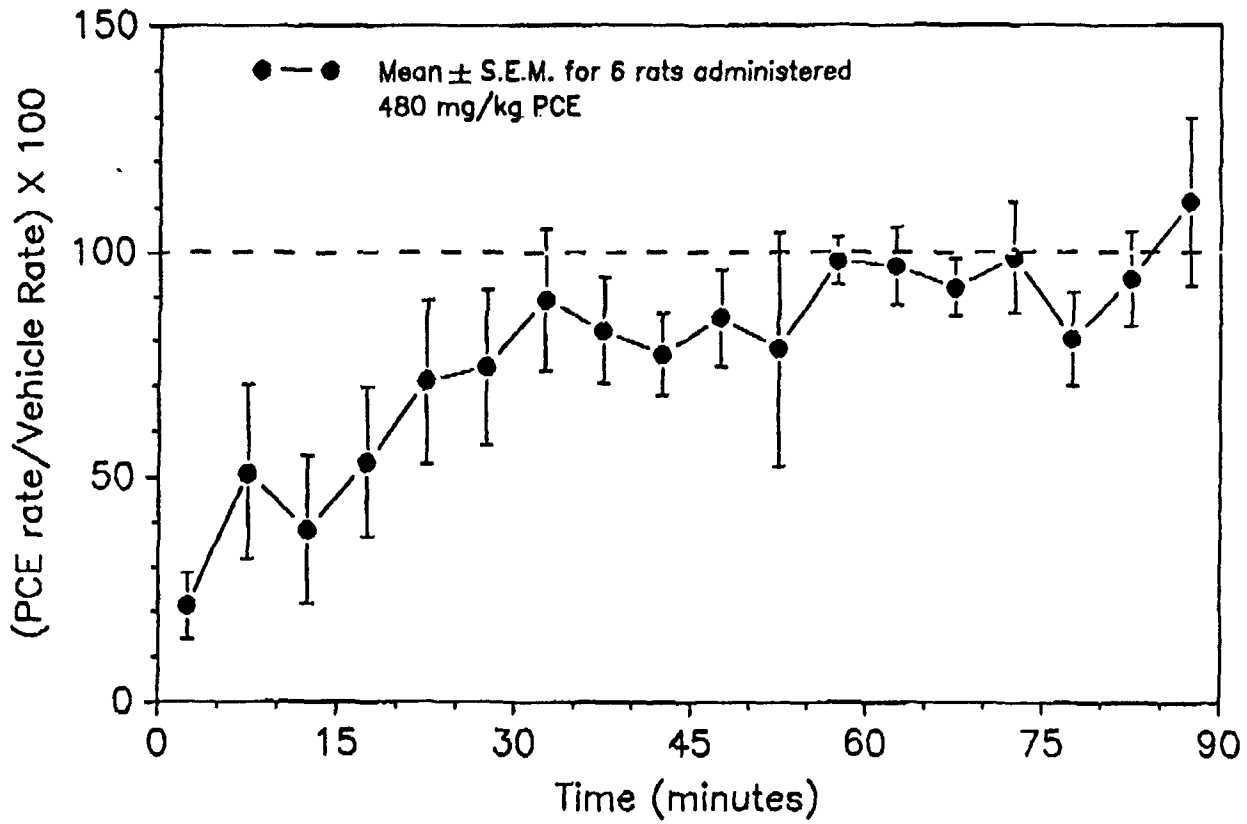
Figure 11. Relationship of PCE blood concentrations to mean response ratios. Each point represents the average of the mean response ratios during the 5 minute intervals immediately preceding and following times of blood collection. Solid lines are second order regressions.
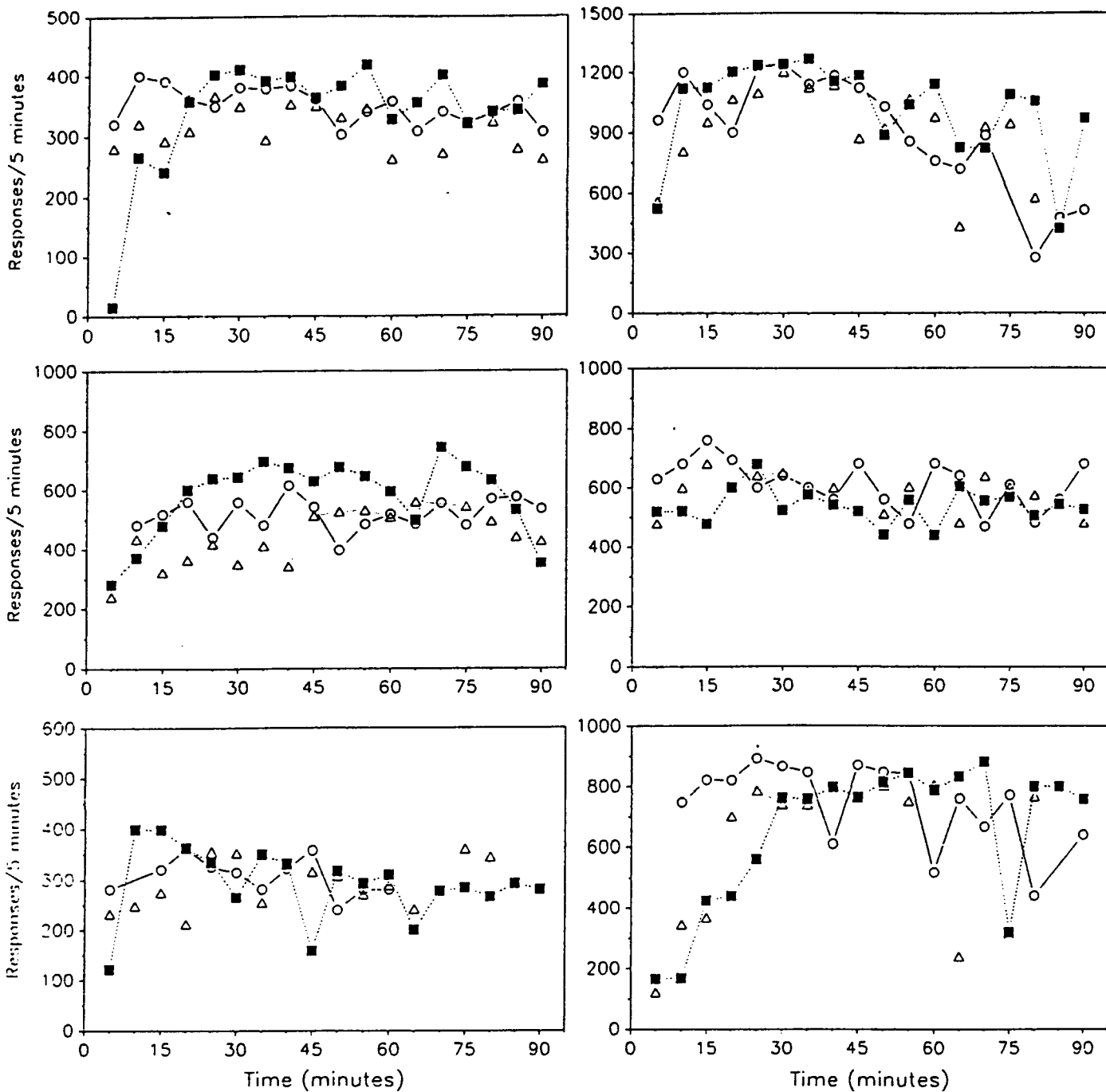
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Figure 12. Relationship of PCE brain concentrations to mean response ratios. Each point represents the average of the mean response ratios during the 5 minute intervals immediately preceding and following times of brain collection. Solid lines are second order regressions.

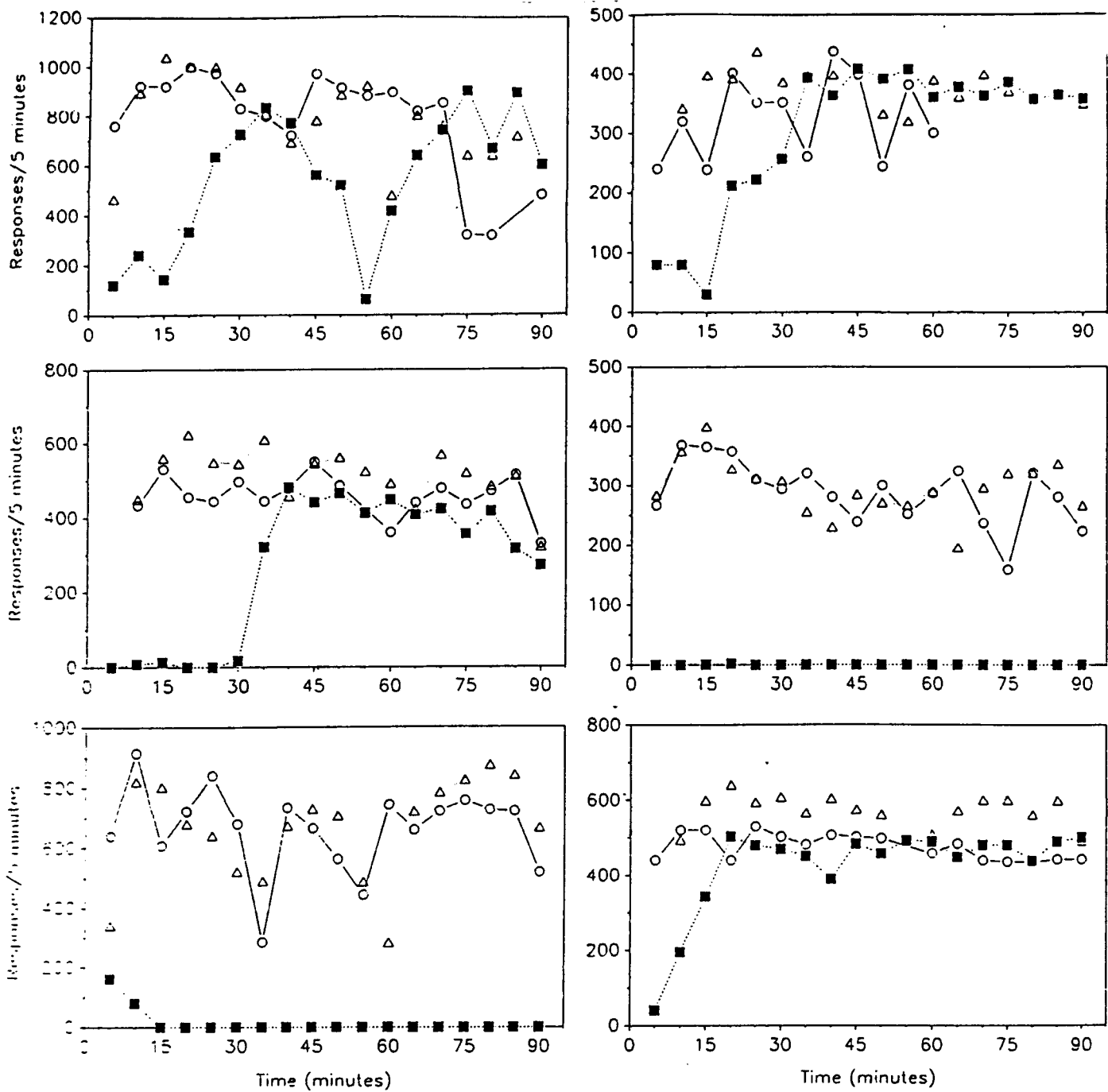Figure 13. Individual animal SCOB plots for rats receiving 160 mg/kg PCE as a single oral bolus.

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Figure 14. Individual animal SCOB plots for rats receiving 480 mg/kg PCE as a single oral bolus.

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IX. COMPARISON OF NEUROBEHAVIORAL TOXICITY   BETWEEN SINGLE AND MULTIPLE INHALATION EXPOSURES TO TRI IN RATS

In response to the heightened interest in the CNS as a target organ, we have examined the scheduled-controlled operant behavior (SCOB) of rats during solvent exposures, and measured blood and brain solvent concentrations in comparable animals under identical exposure conditions. The use of SCOB as a behavioral measure is well documented. In typical studies of operant behavior, a specific response (e.g., a lever press) is followed by reinforcement (e.g., a drink of milk) delivered on an intermittent schedule. Eventually, the lever pressing of the subject is reliably maintained by the reinforcement resulting in remarkably stable rates of lever pressing over long periods of time. Solvent-induced changes in such performance may result from very specific changes in the CNS, toxicity in other organ systems, or the sensory-aversive properties of the solvent itself. Schedule-controlled operant behavior is used by all of the federal laboratories concerned with behavioral effects of chemicals in drugs, its use has been endorsed by the National Academy of Sciences, and it is the only test of complex neurobehavioral function required under EPA's 1993 "Solvent Rule". Most importantly, the use of SCOB yields data that are sufficiently quantitative and objective to be suitable for correlations with measures of blood and brain concentrations.

The recreational abuse of solvents has became a significant public health problem. Researchers at Virginia Commonwealth University (VCU) with whom we are collaborating believe solvents that produce and profile of behavioral effects that are both excitatory and depressant are especially prone to abuse. These researchers believe that locomotor activity is a straightforward means of comparing the behavioral profiles of solvents, as well as their efficacious and potencies. Although seemingly simple, locomotor activity is a complex behavior comprised of a variety of motor acts, such as horizontal and vertical movement, sniffing, and grooming. A change in the frequency of this behavior could reflect solvent-induced changes in any one or more sensory or motor functions, alterations in excitability or motivational states, or perturbations of regulatory states (i.e., diurenal cycles, energy balance of the animal). The measurement of locomotor activity is rapid and simple, and its use as a behavioral measure is well documented. Like SCOB, locomotor activity counts are relayed directly to a computer for analysis, ensuring objectivity and accurate quantitation. Through a collaborative effort with researchers at VCU, locomotor activity in mice will also be examined in relation to blood and brain concentrations. The determination of blood and brain concentration time courses for the same solvents and exposure scenarios employed in the measurement of locomotor activity should enhance the understanding of the biphasic nature of the behavioral response to many solvents. It will also allow the relative potency of the solvents to be based upon dose-response curves, where dose is blood or brain dose, rather than administered dose.

The major disadvantages of SCOB are that it is extremely time consuming and labor intensive, due in large part to the necessity for animal training. It was therefore of interest to determine whether accurate dose-response curves for solvent-induced changes in SCOB could be generated by exposing individual rats to multiple solvent concentrations, rather than groups of rats to a single solvent concentration. The ability to do so is contingent upon whether solvent

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Specially-tooled Coulbourn Instruments Operant Test Cage
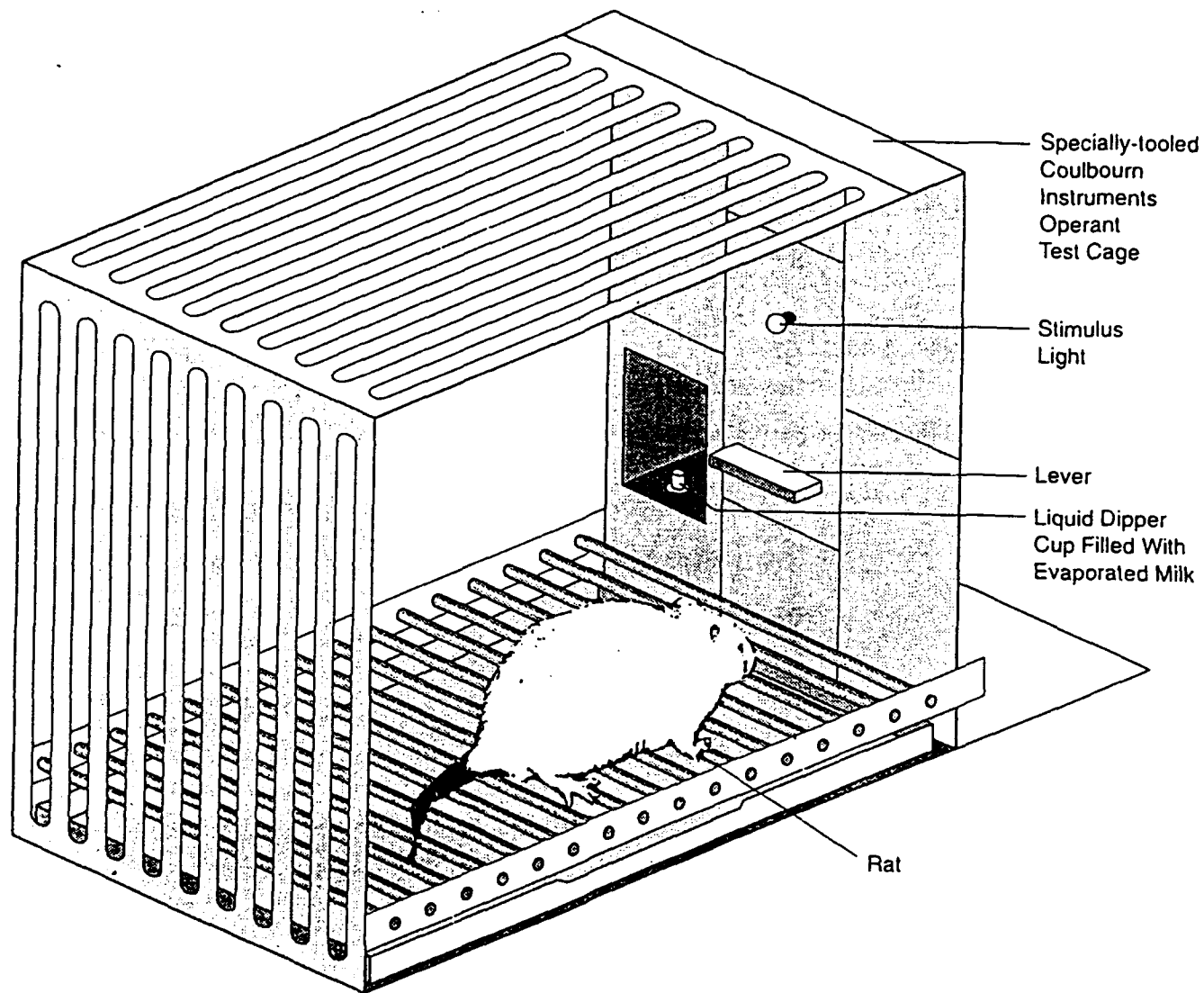
Stimulus Light

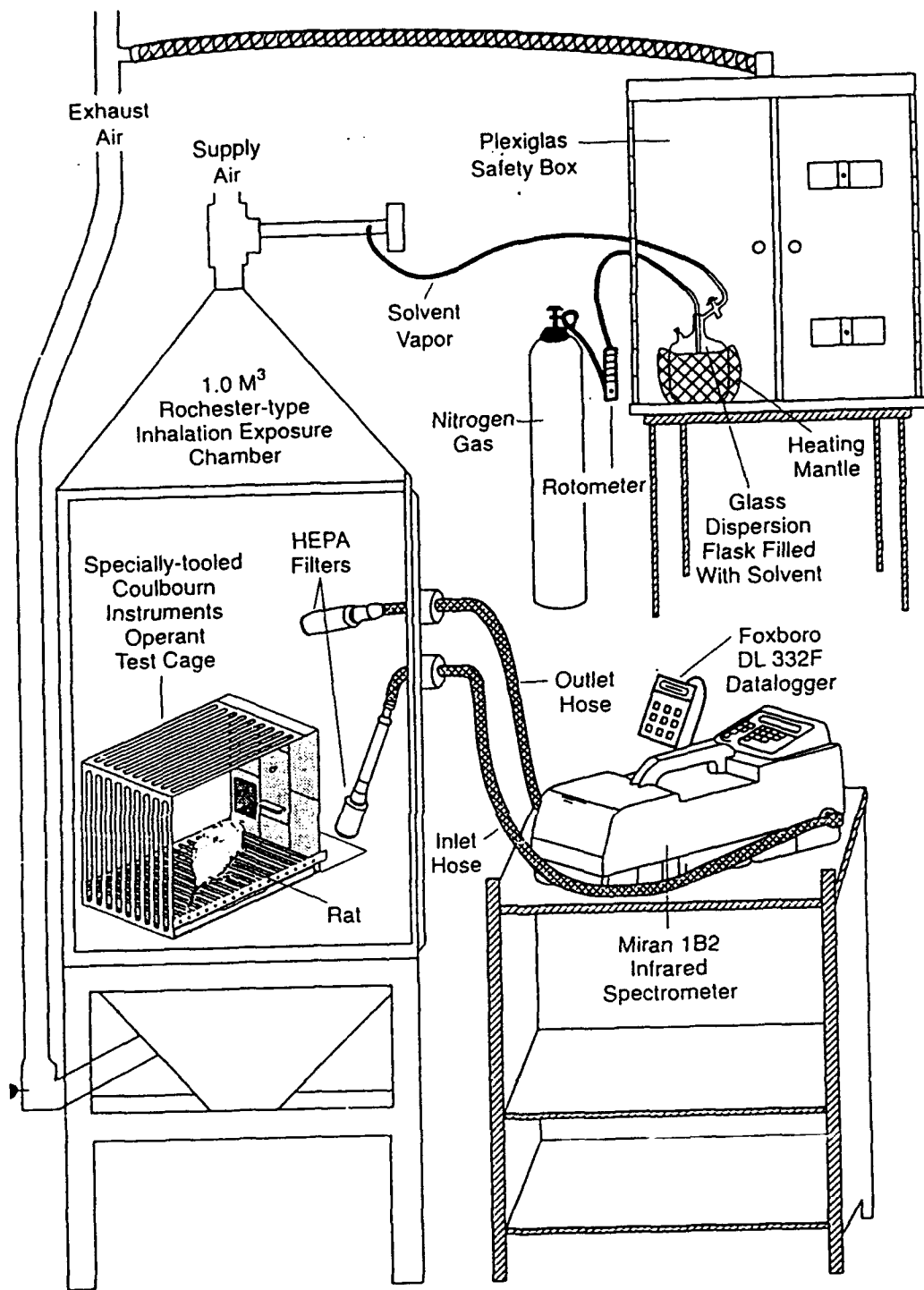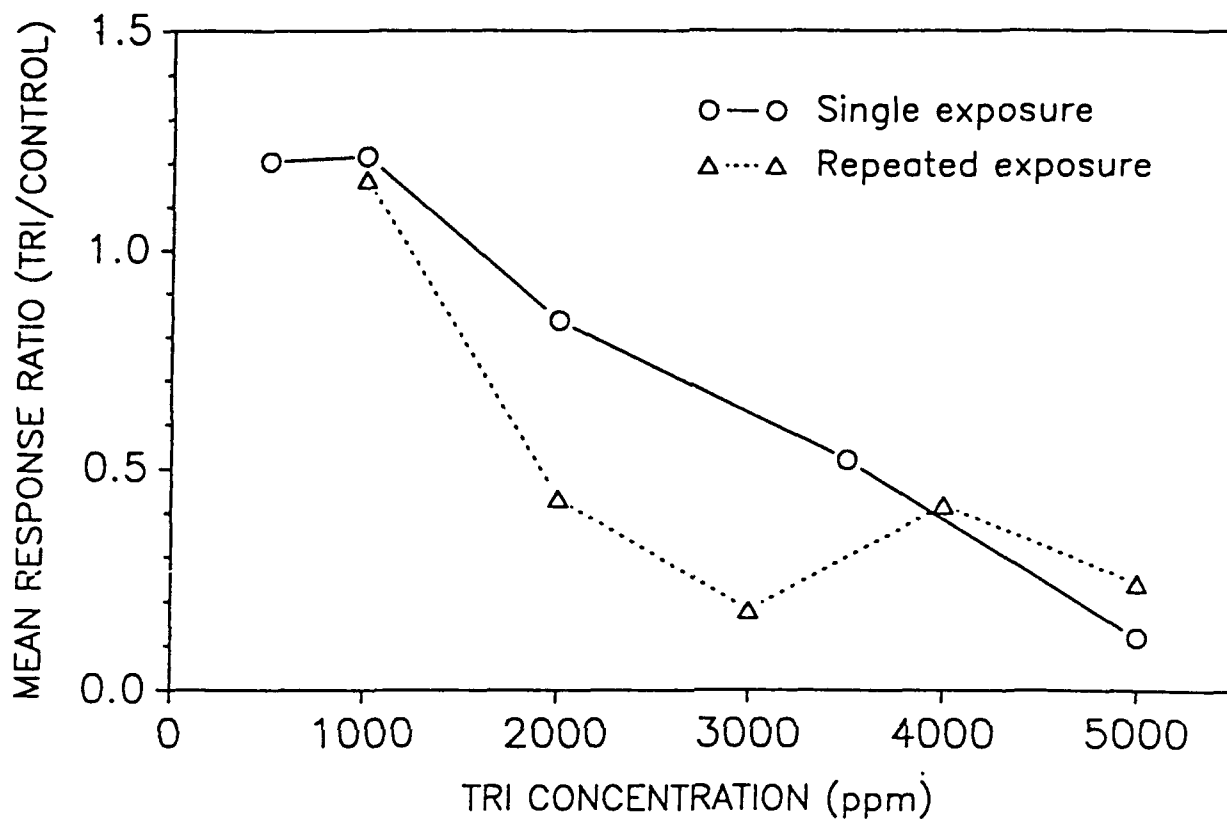Lever

Liquid Dipper Cup Filled With Evaporated Milk

Rat

Figure IX-1
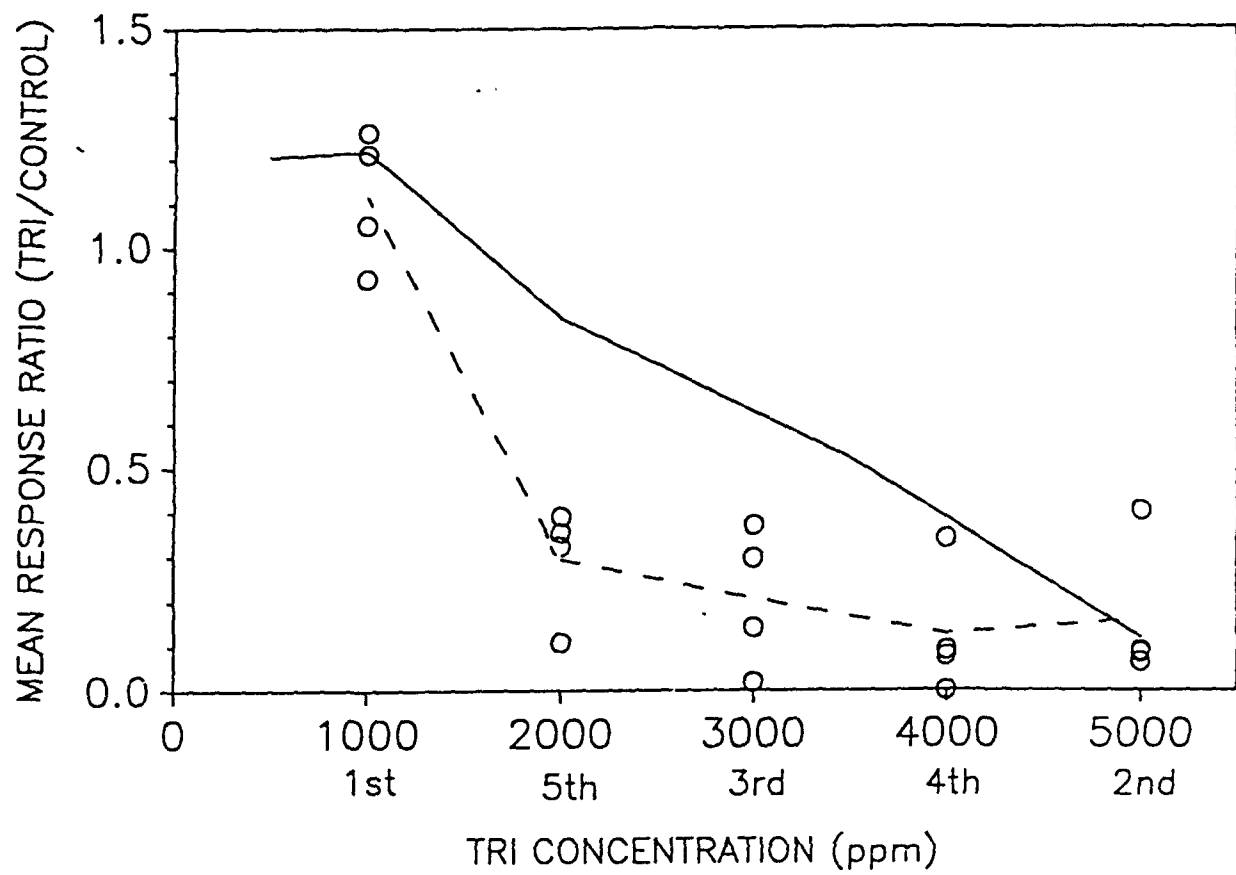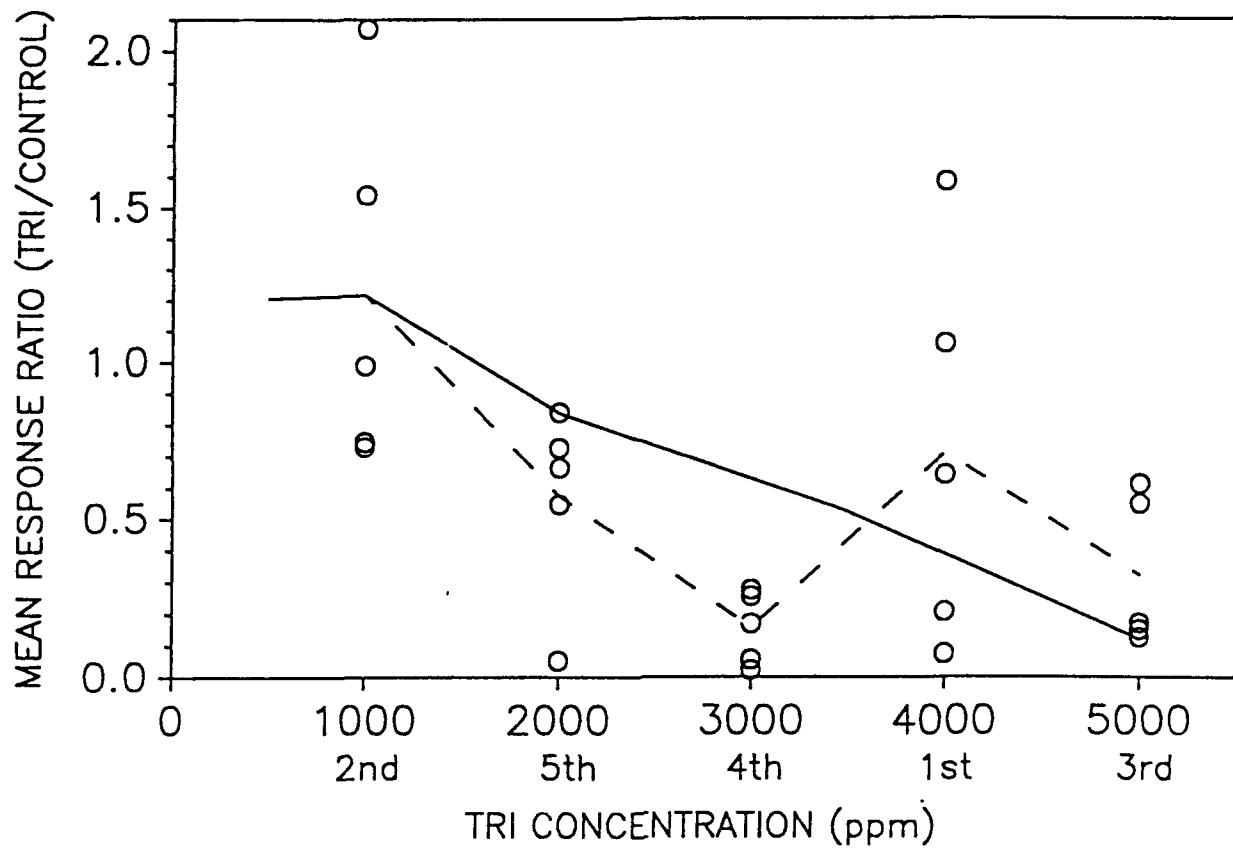
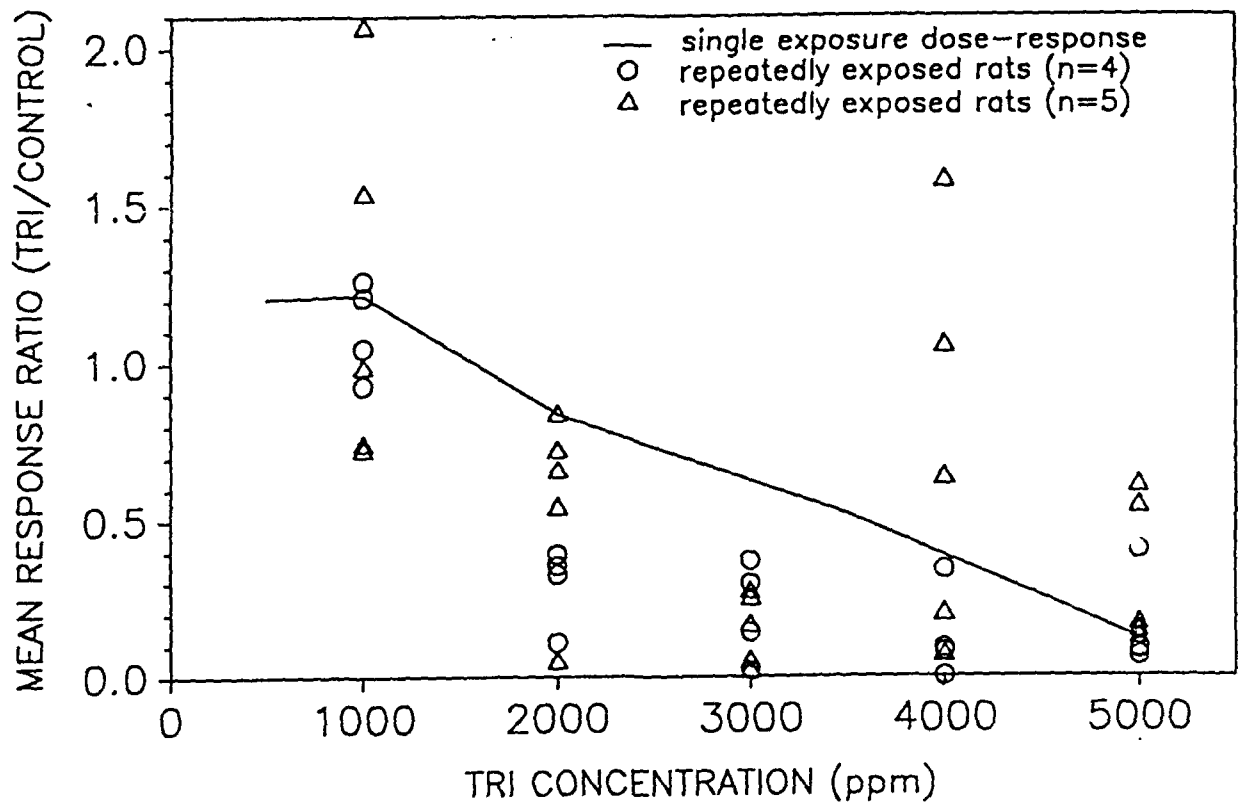Figure IX-2

Figure IX-3

Figure IX-4

Figure IX-4

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Figure IX-5

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X. DETERMINATION OF THE BLOOD AND TARGET TISSUE PHARMACOKINETICS OF INHALED 1,1,2-TRICHLOROETHYLENE (TCE) AND ITS MAJOR METABOLITES IN MICE

Studies with B6C3F1 mice have linked two metabolites of TCE, trichloroacetate (TCA) and dichloroacetate (DCA), with liver cancer. TCE is metabolized to a transient epoxide which rapidly undergoes intramolecular rearrangement to form trichloroacetylaldehyde, which is either oxidized to TCA or reduced to trichloroethanol. DCA is formed via a dechlorination reaction. The human TCE metabolic profile is qualitatively, although not quantitatively, the same as that of test animals. For the purpose of risk assessment, the liver and lung of the B6C3F1 mouse represent the most sensitive organs in TCE cancer bioassays. Therefore, a comprehensive PBPK model for TCE for use in cancer risk assessment should not only predict the concentration time course of TCE in these organs, but of its active metabolites as well. Although no such model currently exists, a massive research effort is underway with this goal in mind. Experimental data for validation purposes will thus be needed on TCE and its metabolites in blood, liver, and lung. Quantification of the parent compound in fat is also important since this tissue is the primary repository for TCE, and thus a critical determinant of TCE's pharmacokinetics. As previously mentioned, it is advantageous to have quantitative toxicity data with which to correlate blood and tissue concentrations. The blood and tissue concentration time course determination thus employed B6C3F1 mice subjected to an exposure scenario for which positive cancer bioassay data exist.

Physiologically-based pharmacokinetic models have recently become an important tool in cancer risk assessments for several chemicals, including TCE. It is absolutely necessary to assess the precision of PBPK models by comparing model predictions to experimentally-determined blood and tissue concentration time courses distinct from those on which the models are based. Unfortunately, there exist very few comprehensive experimental data sets for TCE model validation. Further progress in applying PBPK models to TCE risk assessment is therefore dependent upon obtaining blood and tissue concentration time course data through direct measurement studies. The opportunity to perform such a study in the Toxic Hazards Division at Wright-Patterson Air Force Base recently presented itself. Collaboration with this DOD laboratory will provide the ability to extend the modelling efforts to the metabolites as well as the parent compound.

Experimentally-naive, male B6C3F1 mice (28-32 g) were acclimated on a 12-hr light-dark cycle in a temperature- and humidity-controlled room for a minimum of 7 days prior to use. Tap water and commercial rodent chow were provided ad libitum. This project was conducted in the Toxic Hazards Division, Armstrong Laboratory, Wright-Patterson Air Force Base, Dayton, OH.

Sixteen mice were exposed to 600 ppm TCE to determine blood and tissue concentrations of the parent compound and its metabolites during and following exposure. A relatively new nose-only exposure system was adapted for this purpose (Fig. 1). The nose-only system can support fifty-two animal holding tubes (350 ml each), only seventeen of which were utilized. Sixteen tubes held mice, one tube was connected to a GC to monitor the TCE concentration, and the remaining tubes were closed off with rubber stoppers. Trichloroethylene vapor was generated using a 250 ml gas washing bubbler filled with TCE that was

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maintained at 0°C with a slush ice bath. The TCE concentration was monitored using a Varian 3400 GC with a loop injection system and a flame ionization detector. The target TCE concentration of 600 ppm was maintained by very slight adjustments of the exhaust flow.

Just prior to exposure, sixteen mice were loaded into their respective tubes. When a sampling point along the time course arrived, a tube (with mouse inside) was removed from the exposure system and the hole stoppered without affecting the TCE concentration in the other tubes. One tube was removed at the following times during exposure: 15 min, 30 min, 1 hr, 3 hr, 5 hr, and 7 hr. The mice in these tubes were immediately sacrificed for blood and tissue collection. Immediately following removal of the tube at 7 hr, all remaining tubes were removed (i.e., the maximum duration of exposure was 7 hr). The mice from these tubes were placed in individual holding cages with access to food and water ad libitum. A mouse was removed from its holding cage and sacrificed for blood and tissue collection at each of the following times post exposure: 5 min, 15 min, 30 min, 45 min, 1.5 hr, 3 hr, 5 hr, 7 hr, 9 hr, and 18 hr. All mice were sacrificed by CO_2 asphyxiation, blood was withdrawn from the inferior vena cava, and liver, lungs, and perirenal fat were excised. The blood and liver samples were divided into two parts, one for parent compound analysis and the other for metabolite analysis. The lung and fat tissue volumes were insufficient to divide and were only used to determine TCE concentrations. Blood and tissue levels of TCE were determined by the method of Chen *et al.* (1993). For metabolite analysis, TCE and DCA were derivitized to their methyl esters and 2,2-dichloropropionic acid was used as an internal standard. The methyl esters and 2,2,2-trichloroethanol were analyzed by ECD-GC following liquid injection. The above scenario was repeated five times in order that the blood and tissue concentration time course would accurately reflect the variation between animals.

Thus far, only TCE data have been tabulated. The TCE concentration time courses for blood, liver, lungs and fat are shown in Fig. 2-5, respectively. In addition to the experimentally-measured blood and tissue concentrations, the figures also contain PBPK model predictions (solid lines). In all cases, the model predicts a more rapid rise in TCE concentrations than is reflected by the measured values. With the exception of the liver, the model overpredicts the maximum TCE concentration, although the rate of TCE elimination is predicted fairly accurately. The model also predicts that blood and tissue concentrations will reach a steady-state equilibrium which is not reflected in the experimental data. The reason for the discrepancies between model predictions and measured values are unknown. A previous attempt to model TCE pharmacokinetics in this concentration range in the B6C3F1 mouse was also unsuccessful. The validity of the experimental data will be tested through direct and systematic replication at WPAFB in the near future.
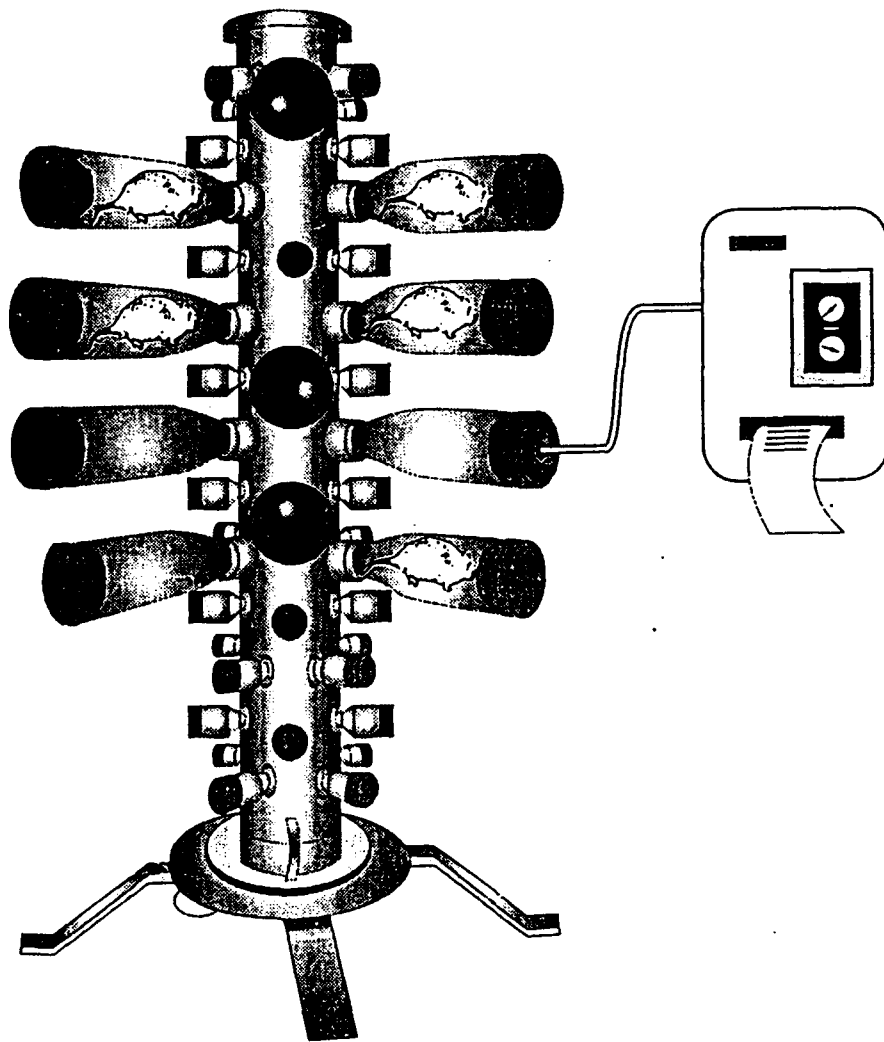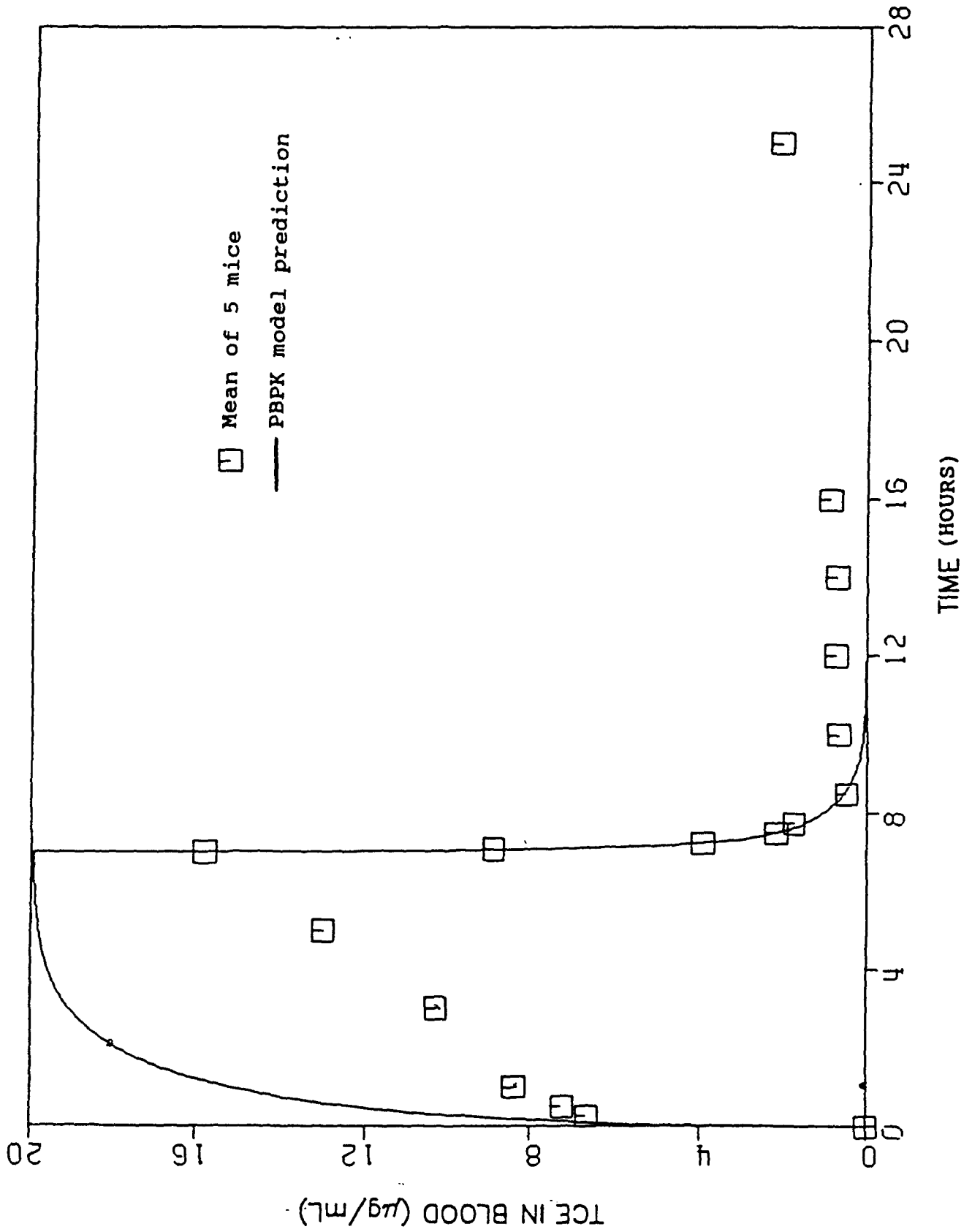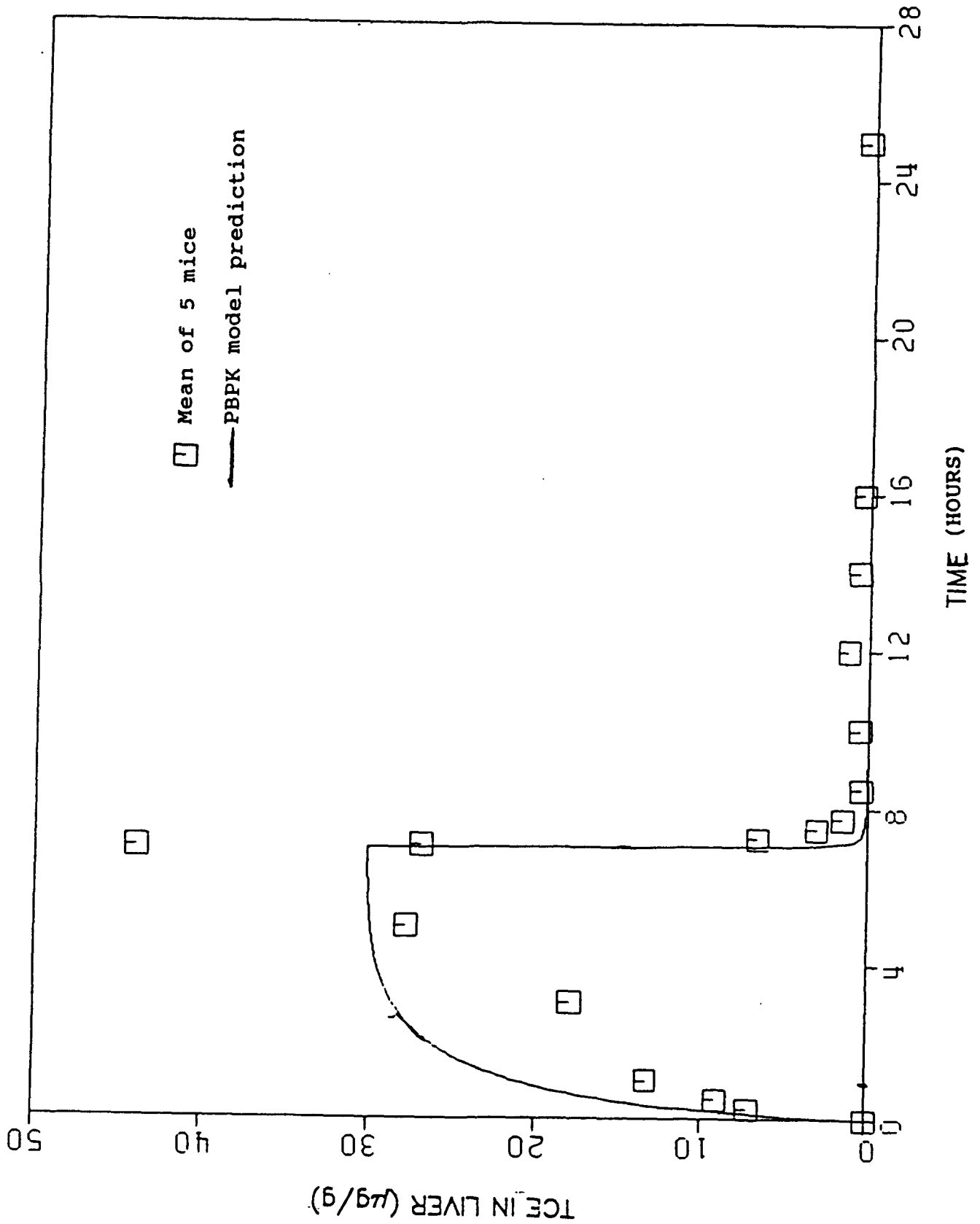
Figure 1

FIGURE 2

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FIGURE 3

TIME (HOURS)

TCE IN LIVER (μg/g)

☐ Mean of 5 mice

── PBPK model prediction

FIGURE 4

FIGURE 4

FIGURE   5

TIME (HOURS)

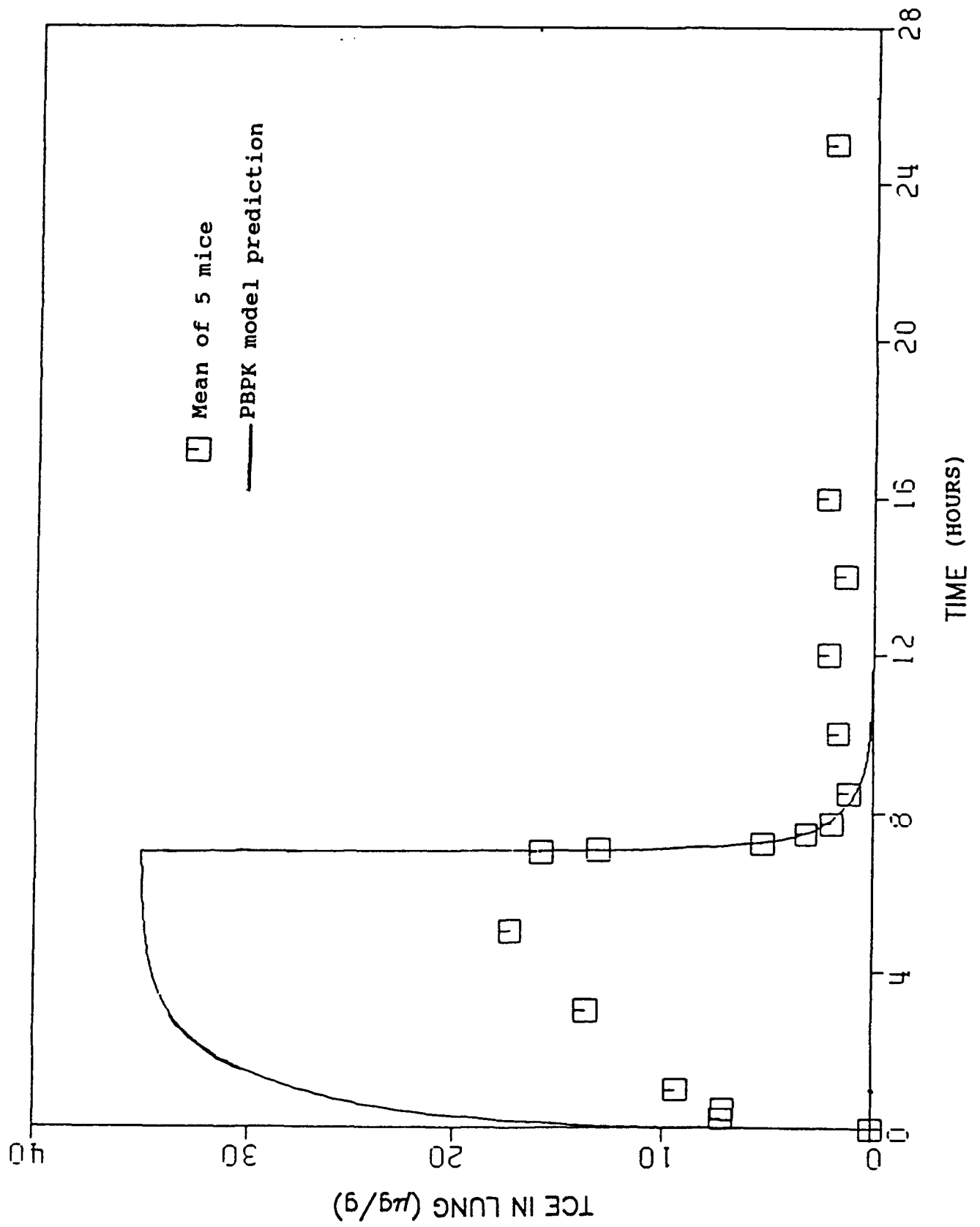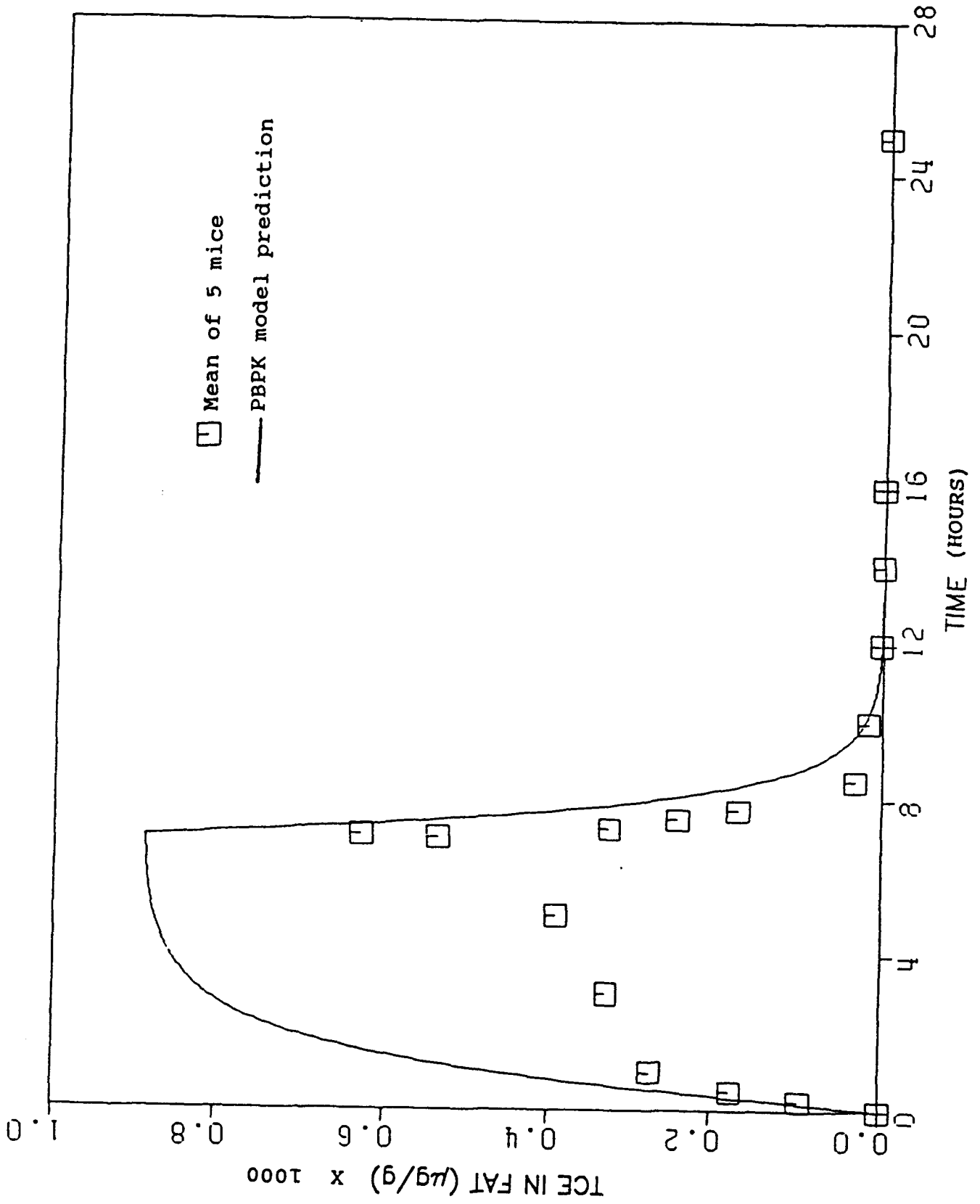TCE IN FAT (μg/g) x 1000

☐ Mean of 5 mice

—— PBPK model prediction

XI. COLLABORATIVE ARRANGEMENTS

The proposed project has been conducted at the Department of Pharmacology and Toxicology (P & Tx) and the Department of Pharmaceutics in the University of Georgia (UGA) College of Pharmacy. the Principal Investigator has been Dr. Cham E. Dallas (CED), who has been responsible for overall coordination of the project. He has provided a 20% commitment to this project. In addition to coordinating the project, CED personally conducted all of the respiratory elimination studies that were done. Under CED's direction, the assay was developed for measuring halocarbon levels in the tissues of animals following exposure, which has been of significant utility in the present investigation. A primary focus of CED's studies has been to provide data sets for the development and validation of physiologically-based pharmacokinetic (PBPK) models. Dr. James V. Bruckner (JVB) has provided a 10% commitment to the project as a Co-Principal Investigator. He has directed a number of research projects on the oral toxicity and pharmacokinetics of volatile organic compounds over the past 10 years. JVB is also experienced in the application of pharmacokinetic data to risk assessments, having served on a number of committees and advisory groups for federal agencies concerned with health effects of VOCs. Dr. James M. Gallo (JMG) has served as a Co-Investigator (5% commitment), and provided expertise in the field of pharmacokinetics (PK). The major focus of his work has been the physiologically-based pharmacokinetic (PBPK) modeling, including the derivation of methods for estimation of mass transfer coefficients and partition coefficients for PBPK models. JMG had primary responsibility for design of PK studies and analysis of data, development and refinement of PBPK models, and assessment of the model's ability to predict halocarbon disposition in humans. Dr. Randall Tackett (RT) served as a Co-Investigator on the project (10% commitment), and was responsible for the kinetic experiments in the dog in this project. RT heads an active laboratory staffed by postdoctoral associates and graduate students, in which a number of toxicodynamic studies in the dog and the rat have been conducted. Dr. Tom Reigle (TR) also served as a Co-Investigator on the project (20% commitment). TR provided valuable assistance in the selection and purchase of the appropriate testing equipment that can be used for both rats and dogs, and was involved in the design and conduct of all the neurobehavioral studies.

Mr. Alan Warren (AW) is a doctoral student who has been conducting his dissertation research involving the research objectives of this grant. Alan is the recipient of a three-year award from the Department of Defense, managed by the Southeastern Center for Electrical Engineering Education (SCEEE). This award provides for his graduate assistantship stipend and approximately $2000 annually for travel and minor expenses. As the SCEEE award coincides almost exactly with the period of this Air Force grant, this is an important (and much appreciated) collaborative effort. AW has personally been involved in the development of the neurobehavioral testing protocols, and has provided a very perseverant effort toward the success of this critical part of the project. Mr. Li You (LY), a doctoral student, worked on the analytical measurements of halocarbon concentrations in animal tissues, and the neurobehavioral studies with mice. LY has set up the operant testing system for this second species, and has completed two papers which have been accepted for publication on the correlation of neurobehavioral depression and halocarbon pharmacokinetics in mice.

Dr. Xiao Mei Chen (XMC) has served as a full-time postdoctoral associate on the project. XMC has a medical degree from the People's Republic of China, and has worked in the current Air Force project since its inception until her return to China in September, 1993. She was successful in her work in the development of the assay for the measurement of halocarbons in the tissues of exposed animal and conducted these tissue measurements for ia and po exposures for PER and TET in rats and dogs. Dr. Peter Varkonyi (PV) is a postdoctoral associate from Hungary who has worked "hands-on" with the development of the PBPK models for halocarbon pharmacokinetics. Dr Varkonyi was replaced this year by Dr. Tharin Limsakun, who has had experience with the Simusolv program we have been using to run the PBPK model simulations. Dr. Limsakun has started on the project, and spent the first weeks on the job learning the procedures from Dr. Varkonyi. Dr. Limsakun is now working on several data sets generated for VOC pharmacokinetics in a collaborative training effort with the U.S. EPA. Mr. Hitesh Mehta worked on this project during the summer quarter of 1994 as part of the NIH Minority Research Apprenticeship Program, in which Dr. Dallas is an annual participant as a host laboratory. Mr. Mehta worked full-time for 3 months and assisted Mr. You and Mr. Warren in their pharmacokinetic studies. Mr. Srinvasa Muralidhara has been employed part-time (25%) on the project. He participated in the analysis of halocarbons in biological samples, computer programming, the conduct of inhalation studies of dynamic exposure chambers, and the compiling of laboratory records.

Dr. Dallas and Mr. Warren now have an established collaborative relationship with Dr. Robert Balster (RB) and his laboratory at the Medical College of Virginia in Richmond, Virginia. RB is an acknowledged leader in the study of the neurobehavioral effects of halocarbon solvents. Comparison of the theoretical and technical approaches are underway in ongoing investigations between the two laboratories.

XII. INTERACTION WITH DOD LABORATORIES

Mr. Warren spent approximately 10 weeks (Oct. 1, 1993 - Dec. 15, 1993) at Wright-Patterson Air Force Base (WPAFB), as part of an opportunity afforded him as a recipient of a Department of Defense Science and Engineering Fellowship. While at WPAFB, Mr. Warren worked with his Air Force Laboratory mentor, Dr. Jeff Fisher, as well as other Air Force and contract scientists with expertise in pharmacokinetic modeling and toxicodynamics. The studies conducted during this time are discussed in Section X.

APPENDIX A

Development of a Physiologically Based Pharmacokinetic Model for Perchloroethylene Using Tissue Concentration-Time Data. In Press, *Toxicology and Applied Pharmacology*, 1994.

Development of a Physiologically Based Pharmacokinetic Model for Perchloroethylene Using Tissue Concentration–Time Data[1,2]

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Development of a Physiologically Based Pharmacokinetic Model for Perchloroethylene Using Tissue Concentration–Time Data. DALLAS, C. E., CHEN, X. M., O'BARR, K., MURALIDHARA, S., VARKONYI, P., AND BRUCKNER, J. V. (1993). *Toxicol. Appl. Pharmacol.* 127, 000–000.

The tissue disposition of perchloroethylene (PCE) was characterized experimentally in rats in order to (1) obtain input parameters from *in vivo* data for the development of a physiologically based pharmacokinetic (PBPK) model, and (2) use the PBPK model to predict the deposition of PCE in a variety of tissues following inhalation exposure. For the derivation of model input parameters, male Sprague–Dawley rats received a single bolus of 10 mg PCE/kg body wt in polyethylene glycol 400 by ia injection through an indwelling carotid arterial cannula. Other male Sprague–Dawley rats inhaled 500 ppm PCE for 2 hr in dynamic exposure inhalation chambers. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, perirenal fat, and blood were taken for up to 72 hr following ia injection, during the 2-hr inhalation exposure, and for up to 72 hr postexposure. Blood and tissue PCE concentrations were analyzed using a gas chromatography headspace technique. Following ia administration, the tissues exhibited similar terminal elimination half-lives ($t_{1/2}$). As comparable tissue $t_{1/2}$s are consistent with a blood-flow-limited model, tissue:blood partition coefficients were calculated for noneliminating compartments by division of the area under the tissue concentration–time curve (AUC) by the blood AUC. Liver PCE concentration versus time data were employed in the calculation of *in vivo* metabolic rate constants. A PBPK model was developed using these parameters derived from the ia data set and used to predict tissue PCE concentrations during and following PCE inhalation. Predicted tissue levels were in close agreement with the levels measured over time in the seven tissues and in blood. Tissue concentration–time data can thus provide valuable input for parameter estimation and for validation of PBPK model simulations, as long as independent *in vivo* data sets are used for each step. © 1994 Academic Press, Inc.

Tetrachloroethylene, or perchloroethylene (PCE), is a volatile halogenated hydrocarbon that is widely used as a synthetic intermediate and a general solvent in operations such as metal degreasing and dry cleaning. Because of its widespread use, PCE is commonly found as a contaminant of air and groundwater (ATSDR, 1993). PCE has been identified in samples from 714 of 1300 hazardous waste sites on the EPA's National Priorities List (HAZDAT, 1992). It has been estimated that 688,110 workers employed at 49,025 industrial facilities may be exposed to PCE (NOES, 1990). Occupational exposure limits have been based on PCE's ability to reversibly inhibit central nervous system functions. Inhalation of high concentrations of the halocarbon can also sensitize the myocardium to catecholamines, resulting in cardiac arrhythmias. Hepatotoxicity is seen rarely in workers, even those exposed to very high vapor levels (ATSDR, 1993). Of greater concern, particularly at environmental exposure levels, is the potential for PCE to cause cancer. Tumors have been reported in mice and/or rats in oral (NCI, 1977) and inhalation (NTP, 1986) carcinogenicity bioassays. Thus, there is considerable interest at present in risks PCE may pose to human health in both occupational and environmental settings.

Several groups of investigators have developed physiologically based pharmacokinetic (PBPK) models to simulate

PCE disposition in laboratory animals and humans (Guberan and Fernandez, 1974; Chen and Blancato, 1987; Ward *et al.*, 1988; Bois *et al.*, 1990). In PBPK models, the body is divided into anatomical compartments representing individual organs or groups of organs which share a common characteristic. A mass balance differential equation is written for each compartment in the model, based upon anatomical and physiological parameters (i.e., tissue volumes and blood flow rates) for the test species, and upon physicochemical (i.e., partition coefficients) and biochemical parameters (i.e., metabolic rate constants) for each chemical (Gerlowski and Jain, 1983). Solution of the set of mass balance differential equations generates chemical concentrations as a function of time in each compartment/tissue.

Assessment of the accuracy of predicted chemical concentrations over time in tissue compartments is important to determining PBPK model reliability. Efforts to establish the accuracy of PBPK model simulations of systemic uptake and elimination of volatile organic compounds (VOCs) have largely involved VOC blood and exhaled-breath data (Andersen *et al.*, 1987; Ward *et al.*, 1988; Reitz *et al.*, 1988; Fisher *et al.*, 1989). Blood levels over time, as a measure of bioavailability, have classically been used as an index of the level of chemical in the body and are therefore representative of tissue levels and target organ effects. This assumption can be misleading in that blood concentrations may not be reflective of the concentration of chemical or active metabolite at the site of action in a target tissue. A more logical measure of target organ exposure might be the area under the tissue concentration versus time curve for the active chemical or the peak blood concentration, depending upon the chemical and its mechanism of action.

There has been very little published to date on the ability of PBPK models to accurately predict time integrals of tissue exposure to halocarbons and other VOCs, apparently due to a paucity of tissue concentration versus time data sets. This lack of data is largely due to the considerable time and effort required in such studies and to technical problems with quantitation of the volatile chemicals in solid tissues. The only tissue data Reitz *et al.* (1988) had to use in testing their 1,1,1-trichloroethane (TRI) 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-hr inhalation exposures to [^{14}C]TRI. Measurement of radioactivity, of course, does not distinguish between parent compound, metabolites, and ^{14}C which has entered the carbon pool. It should also be recognized that VOCs are very rapidly absorbed from the lungs (Dallas *et al.*, 1983, 1989) and gastrointestinal tract (D'Souza *et al.*, 1985; Putcha *et al.*, 1986) into the bloodstream and transported to tissues. Manifestations of central nervous system effects (Bruckner and Peterson, 1981) and hepatocytotoxicity (Rao and Recknagel, 1968; Lowrey *et al.*, 1981; Luthra *et al.*, 1984)

can occur within a few minutes of the onset of VOC exposure. Thus, it is important to fully characterize entire time courses of tissue uptake and elimination of VOCs, particularly during the "critical early minutes" of exposure, when many events important in cytotoxicity and cellular dysfunction occur.

The present study was conducted to generate comprehensive tissue concentration versus time data sets to use for determination of *in vivo* input parameters and for validation of a PBPK model for PCE exposure of rats. The halocarbon was administered by ia injection and the resulting time-course data used to derive *in vivo* parameters including tissue: blood partition coefficients and metabolic rate constants. A PBPK model developed on this basis was then tested for its accuracy by comparison of simulations with observed tissue concentration–time data during and following inhalation exposure of rats to PCE.

METHODS

Adult male Sprague–Dawley rats (325–375 g), obtained from Charles River Laboratories (Raleigh, NC) were utilized in these studies. The animals were maintained on a constant light–dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. Rats 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 7-day acclimation period, at which time they were approximately 12 weeks old. PCE exposures were initiated between 1000 and 1200 hr each day.

1,1,2,2-Perchloroethylene (tetrachloroethylene) of 99%+ purity was obtained from Aldrich Chemical Company (Milwaukee, WI). The purity of the chemical was verified by gas chromatographic analysis. A cannula was surgically implanted into the right common carotid artery of one group of rats. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg body wt 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 cannulas were protected from manipulation by exteriorizing and surgically taping them at the back of the neck. The cannulated animals were allowed complete freedom of movement during a 24-hr recovery period. All animals employed in the ia studies were freely moving and unanesthetized. PCE was given through the arterial cannula as a single bolus in a dose of 10 mg/kg body wt using polyethylene glycol 400 (1 mg/kg body wt) as a vehicle.

Additional groups of rats were exposed to 500 ppm PCE for 2 hr in 1.0-m^3 Rochester-type constant/dynamic flow chambers. Air or nitrogen was passed through a glass dispersion flask with PCE at a constant rate and directed into the chamber influent air stream. A heating mantle was placed around the dispersion flask and narrow temperature limits maintained for continuity of volatilization. The entire halocarbon-generating system was enclosed in a specially fabricated safety box. The box was maintained under constant negative pressure during exposure sessions, as were the inhalation chambers. Exhaust air from the chambers and the generation box was vented through HEPA and activated charcoal filters so that the chemical was removed before release of effluent air to the environment.

The chambers were operated at flow rates of 7 to 15 ft^3/min ($\frac{1}{4}$ to $\frac{1}{2}$ change of the chamber volume per min). A negative pressure of 20 to 50 mm Hg was maintained at all times during operation of the chambers. PCE concentrations in the chamber air were monitored by withdrawing 1-ml samples of air from the chamber and injecting them into a Tracor MT560 gas chromatograph (GC) equipped with a flame ionization detector (FID) (Tracor Instruments, Austin, TX). Characterization of the

chamber test atmospheres verified that the PCE concentrations in the individual wire-mesh cages, which comprised the breathing zone of the animal, were within 10% of the samples withdrawn for the chamber monitoring during the exposure. Standards were prepared in each of four 9-liter bottles, equipped with Teflon stoppers with needles from which air samples could be taken. Air samples were procured from the bottles and chambers with a gas-tight, 1-ml syringe and injected directly onto an 8-ft × $\frac{1}{8}$-in stainless steel column packed with 0.1% AT 1000 on GraphPak. Operating temperatures were: 150°C, injection port; 200°C, FID detector; 350°C, isothermal column operation.

In a typical exposure sequence, groups of five rats were placed into sets of wire-mesh exposure cages and positioned in the exposure chamber. Each animal was individually housed, so the animals could not limit their inhalation of the halocarbon vapors by burying their nose in one another's fur.

Groups of rats (n = 4) were anesthetized with ether and euthanized at the following times following ia dosing: 1, 5, 10, 15, 30, and 60 min; and 2, 4, 6, 12, 36, 48, and 72 hr. For inhalation exposures, sampling times for the groups of five rats were 15, 30, 60, 90, and 120 min during exposure; and 0.25, 0.5, 1, 2, 4, 6, 12, 24, 36, 48, and 72 hr postexposure. Blood samples were obtained by cardiac puncture of the left ventricle. Approximately 1-g samples of brain, liver, kidney, lung, heart, perirenal fat, and skeletal muscle were then quickly excised and placed into previously chilled 20-ml glass vials containing 2 ml of ice-cold saline and 8 ml of isooctane. Each tissue was then homogenized for an established time interval with a Ultra-Turrax SDT homogenizer (Tekmar Co., Cincinnati, OH). These times were kept as brief as possible in order to minimize volatilization of the halocarbon. Brain, liver, and fat were the most easily homogenized, requiring only 3–4 sec. Kidney, lung, and heart required 5–8 sec. Skeletal muscle was the most difficult to homogenize; it required 20 sec. The homogenates were then centrifuged at 1800g for 10 min at 4°C in capped vials. An aliquot of the isooctane layer was transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal. This tissue extraction procedure has been recently described in detail by Chen et al. (1993).

A Sigma model 300 GC equipped with a HS-6 headspace sampler and an electron capture detector (ECD) (Perkin–Elmer Co., Norwalk, CT) was used for the analysis of PCE in biological samples. Analyses were carried out using a stainless steel column (182 × 0.317 cm) packed with 10% FFAP (Alltech Associates, Deerfield, IL). The GC operating conditions were: headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; ECD temperature, 400°C; flow rate for argon/methane carrier gas, 60 ml/min. Each sample vial was heated thermostatically to 90°C for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas and 20 μl of volatilized isooctane, and PCE injected automatically into the GC column. The limit of detection for PCE was approximately 1 ng, or 6.7 parts of PCE per billion parts of air in the sample vial. The percentage recovery of PCE from spiked blood and tissue samples ranged from 81 to 99% (Chen et al., 1993). Reproducibility, as reflected by SE values in this recovery experiment, was quite good. Thus the analytical technique proved to be a sensitive, reliable means of quantifying PCE in the large number of biological samples utilized in the current investigation.

The disposition of PCE during and following inhalation exposures in the rat was predicted using a PBPK model (Fig. 1), developed using tissue concentration–time data following ia administration. This model is similar to the model previously developed by Ramsey and Andersen (1984) for other VOCs in that it provides for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood-flow-limited organ representations. The current model differs primarily in that it includes a greater number of tissue compartments. Another difference is the use of a mass transfer coefficient in the lung compartment, which is based on the alveolar-permeability-area product for methylene chloride
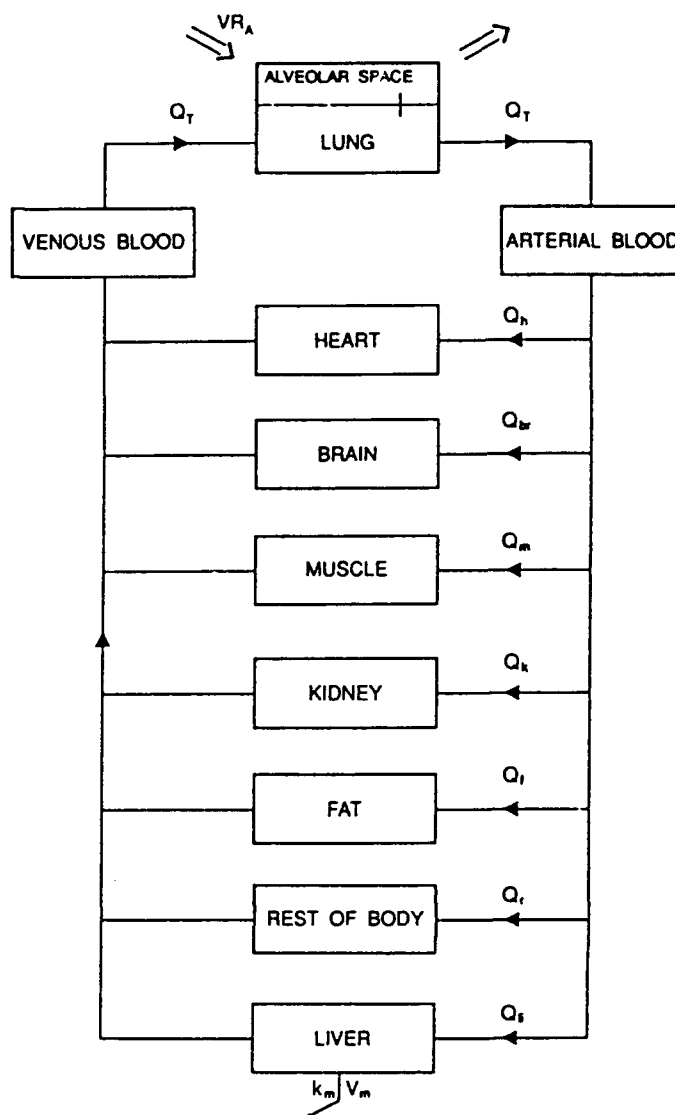

FIG. 1. Diagram of the physiological pharmacokinetic model used to simulate the disposition of PCE in blood and tissues during and following inhalation exposures in rats. The parameters used to describe the model are included in Table 1.

(Angelo and Pritchard, 1984). Values measured in our laboratory for cardiac output, tissue blood flows (Delp et al., 1991), tissue volumes, and lipid content (Manning et al., 1991) in male Sprague–Dawley rats were employed (Table 1). Alveolar ventilation values for Sprague–Dawley rats inhaling 500 ppm PCE were determined in a companion study (Dallas et al., 1994), where the airflow created by the animal's inspiration was recorded both during and following PCE exposure. For the current study using unrestrained animals, 70% of the minute volume was utilized as the alveolar ventilation rate for the model. In vivo tissue:blood partition coefficients were calculated by the area method of Gallo et al. (1987) from ia data obtained in the present study. The metabolic parameters, K_m and V'_{max}, and blood:air coefficients were estimated from the observed data by nonlinear regression analysis (Table 1). Thus, the present model is a detailed and accurate description of the anatomy and physiology of the test subject, the male Sprague–Dawley rat. The inclusion of more individual tissues, as opposed to "lumped" tissue groups used in previous models, makes it

TABLE 1
Parameters Used in the Physiological Based Pharmacokinetic Model for PCE in the Rat

Parameters	
Alveolar ventilation (ml/min)	132[a]
Inhaled PCE concentration (mg/ml)	3.42 (509 ppm)
Body weight (g)	340
Alveolar mass transfer coefficient	500 ml/min

Tissue volumes	Percentages of body weight
Liver	3.39
Kidney	0.77
Fat	5.0
Heart	0.33
Lung	0.37
Muscle	35.36
Brain	0.6
Blood	7.4
Rest of body	46.78

Cardiac output	1.57 (ml/min·g) body wt $(g)^{0.75}$

Blood flows	Percentages of cardiac output
Liver	15.73
Kidney	13.13
Fat	6.56
Heart	4.73
Lung	100
Muscle	26.11
Brain	2.21
Blood	$100\% = 1.57$ (ml/min·g) body wt $(g)^{0.75}$
Rest of body	31.53

Partition coefficients	
Blood:air	19.8
Fat:blood	152.5
Lung:blood	2.47
Liver:blood	5.25
Muscle:blood	2.98
Brain:blood	4.37
Heart:blood	2.68
Kidney:blood	4.45
Rest of body:blood	2.98

Metabolism constants	
V_{max} (µg/min)	0.15
k_m (µg/ml)	0.019

[a] Alveolar ventilation for 50 ppm PCE exposure was determined to be slightly higher, at 151 ml/min, as experimentally determined by Dallas *et al.* (1994).

possible to predict chemical concentrations in a larger number of specific target organs.

Areas under PCE concentration–time curves (AUCs) for blood and tissues were determined from the time of initiation of exposure to infinity. Total body clearance was determined by dividing the administered dose by the blood AUC. The maximum PCE concentration reached in blood and tissues (C_{max}) and the time after dosing that it occurred (T_{max}) were determined by observation of the available data points. The terminal elimination half-life ($t_{1/2}$) was determined according to the formula $0.693/\beta$, where β is the terminal elimination rate constant.

Differential mass balance equations, incorporating the parameters listed in Table 1, were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution of the equations provided predicted PCE concentrations over time.

RESULTS

Measurement of PCE in tissues following its ia injection (Table 2, Figs. 2 and 3) revealed that the chemical was eliminated somewhat more rapidly from the liver than from other tissues. Extrahepatic tissues, other than fat and lung, exhibited similar $t_{1/2}$ values, ranging from 412 to 443 min (Table 2). Elimination from the fat was relatively slow. The blood $t_{1/2}$ was also quite long.

Tissue deposition, following ia injection of PCE, appeared to be governed by rate of blood perfusion and lipid content of the tissues. Highly perfused organs such as liver, kidney, and brain had relatively high C_{max} and AUC values. Nonlipoidal tissues, such as skeletal muscle, heart, and lung, had lower C_{max} and AUC values. The highest PCE levels were found in most tissues at the initial sampling time (i.e., 1 min post ia injection). The T_{max} for adipose tissue, however, was substantially longer. As would be anticipated for a chemical as highly lipophilic as PCE, the fat exhibited markedly higher C_{max} and AUC values than those of the other tissues.

Tissue:blood partition coefficients calculated from the ia tissue time-course data are included in Table 1. Due to the high degree of lipophilicity of PCE, the fat:blood value is almost two orders of magnitude greater than that for the nonfat tissues. Highly perfused tissues such as liver, kidney, and brain had similar values, which were approximately twofold larger than values for the other nonfat tissues.

Pharmacokinetic parameter estimates following the 2-hr inhalation exposure to 500 ppm PCE are presented in Table 3. The C_{max} achieved in adipose tissue was 9–18 times

TABLE 2
Pharmacokinetic Parameter Estimates Following ia Injection of Rats with 10 mg PCE/kg body wt[a]

Tissue	Area under curve $\int_0^\infty c$ (µg·min/ml)	Half-life (min)	C_{max} (µg/g)	T_{max} (min)
Liver	1737	389	41.0	1
Kidney	1759	412	36.0	1
Fat	60335	466	64.4	30
Heart	1059	439	19.0	1
Lung	909	479	11.3	1
Muscl	1178	443	5.7	10
Brain	1730	443	21.7	1
Blood	396	496	4.6	1

[a] Each value represents the value for tissues of four rats pooled at each of 14 time-points, ranging from 1 min to 72 hr.
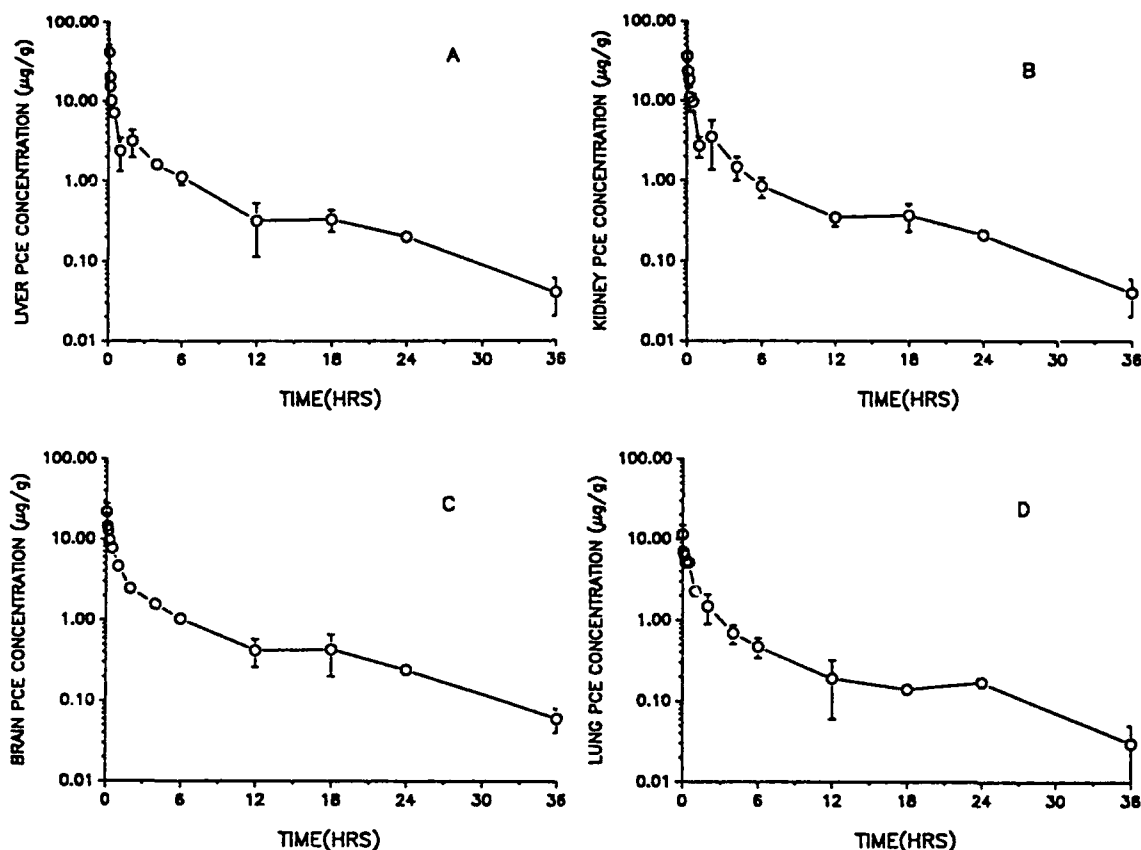
FIG. 2.   PCE concentrations measured in the (A) liver, (B) kidney, (C) brain, and (D) lung of rats following ia injection of a single bolus of 10 mg/kg of PCE through an indwelling carotid arterial cannula. Each point represents the mean ± SE for four rats.

higher than in nonfat tissues. The AUC $\int_0^\infty$ for fat was 45–80 times greater than AUC values for the nonfat tissues. Among nonfat tissues, the brain had the highest AUC $\int_0^\infty$ and C_{max} values. Slightly lower values were observed for the liver. As anticipated for a moderately perfused, nonlipoidal tissue, skeletal muscle had the lowest C_{max} and shortest $t_{1/2}$. The muscle AUC was similar to that of tissue having a low lipid content (i.e., heart, kidney, and lung). Over the time course of inhalation exposures, the actual vapor concentration achieved in the chambers was 509 ± 23.7 ppm ($\bar{x}$ ± SD) (n = 12).

A PBPK model was developed, using the parameter estimates from the ia tissue disposition data and physiological values measured in male Sprague–Dawley rats, to predict the pharmacokinetics of PCE in the body during and following inhalation exposure. Model predictions of PCE tissue concentrations were compared to the experimentally determined (i.e., observed) PCE concentration versus time profiles in Figs. 4 and 5. Overall, concentrations of PCE both during and following inhalation exposure were well predicted by the model. Simulations of PCE levels in the liver and kidney were in close agreement with observed levels during and immediately following exposure, with very

small overpredictions during the terminal elimination phase (Figs. 4A and 4C). Concentrations of PCE in the fat were accurately forecast during the 2-hr inhalation period, while levels in heart, lung, brain, and blood were modestly but consistently underpredicted during this time. PCE elimination from extrahepatic tissues was quite accurately forecast during the 48- to 72-hr postexposure monitoring period. Fat concentrations were slightly underpredicted, and levels in each other tissue slightly overpredicted during the terminal elimination phase.

DISCUSSION

Findings in the present study demonstrate that PCE is very rapidly absorbed from the lungs into the systemic circulation and available for uptake by tissues throughout the body. Experiments in humans reveal that inhaled PCE resides in the alveolar air for only seconds before being absorbed (Opdam and Smolders, 1986). This is reflected in the current investigation by the observation of high blood PCE levels at the first sampling time (i.e., 1 min) after the beginning of exposures. Plots of PCE blood levels versus time of inhalation are atypical for halocarbons in that PCE
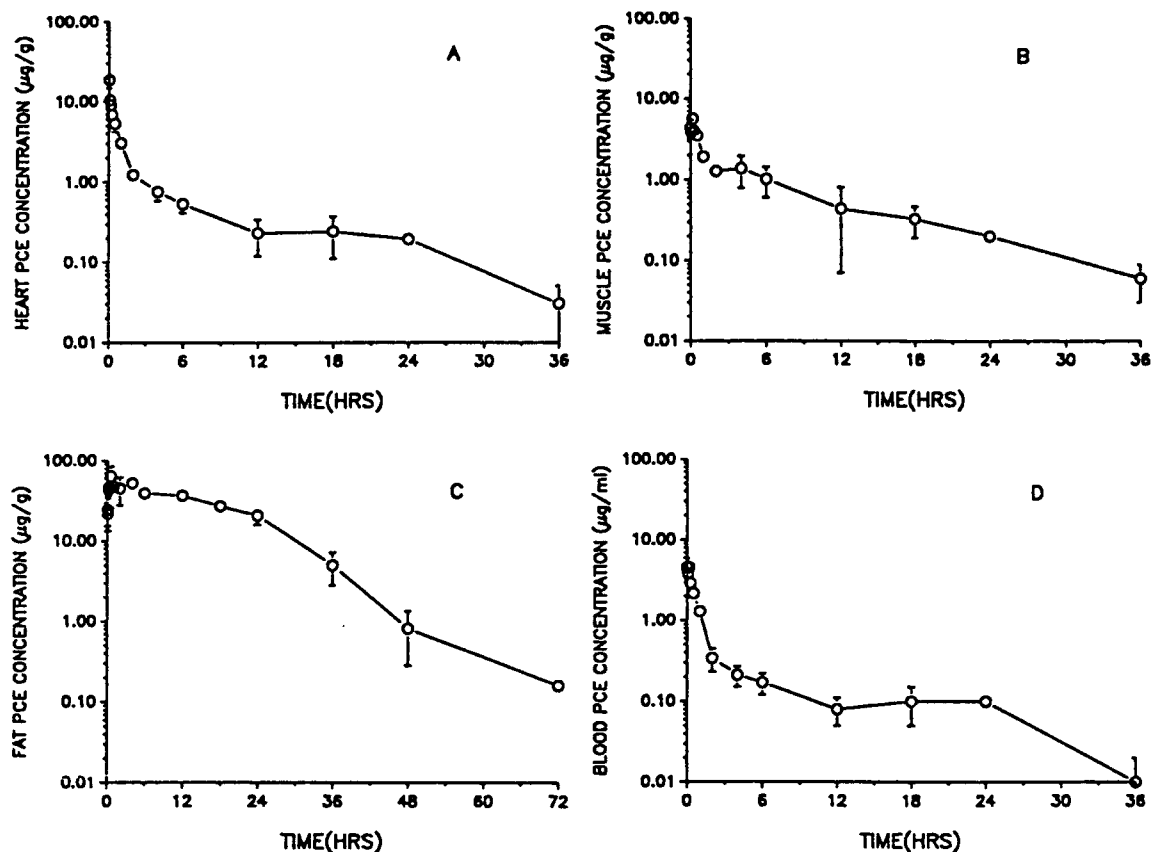
FIG. 3. PCE concentrations measured in the (A) heart, (B) skeletal muscle, (C) fat, and (D) blood of rats following ia injection of a single bolus of 10 mg/kg of PCE through an indwelling carotid arterial cannula. Each point represents the mean ± SE for four rats.

levels continue to rise sharply throughout exposures. Fernandez et al. (1976) also reported a progressive increase in PCE concentrations in the exhaled breath of persons during 8-hr exposures to 100 ppm PCE. Rapid approach to near steady-state concentrations is typically seen for other halo-

TABLE 3
Pharmacokinetic Parameters Estimates for a 2-hr Inhalation Exposure of Rats to 500 ppm PCE[a]

Tissue	Area under curve $\int_0^\infty$ ($\mu g \cdot min/ml$)	Half-life (min)	C_{max} ($\mu g/g$)
Liver	31247	423	152.4
Kidney	25868	425	107.5
Fat	1493190	578	1536.3
Heart	23179	328	106.6
Lung	18596	406	94.6
Muscle	24458	335	87.3
Brain	32975	455	173.9
Blood	8464	322	44.9

[a] Each value represents the value for tissues of five rats pooled at each of 16 time points, ranging from 15 min after the initiation of exposure to 72 hr postexposure.

carbons, such as 1,1-dichloroethylene (Dallas et al., 1983), TRI (Dallas et al., 1989), and trichloroethylene (TCE) (Dallas et al., 1991). These three halocarbons have short $t_{1/2}$s, relative to PCE. As near steady state is generally reached in 4 to 5 $t_{1/2}$s during ongoing exposures, the aforementioned profile for PCE would be anticipated, since the $t_{1/2}$ for PCE was determined here in ia experiments to be approximately 8 hr.

Results of the current study revealed that PCE is quickly taken up by most tissues upon inhalation or ia injection. PCE is a small, uncharged, lipid-soluble molecule which should rapidly diffuse through membranes of capillaries and other cells. This appears to be the case, since the T_{max} for most tissues following ia injection of PCE was 1 min (i.e., the initial sampling time). The T_{max} for skeletal muscle was somewhat longer (i.e., 10 min), likely the result of the slower perfusion rate and higher tissue mass of muscle relative to ʻhe other tissues. Adipose tissue, with the slowest perfusion rate, exhibited the longest T_{max}.

There is a paucity of data on the tissue distribution of PCE. Pegg et al. (1979) did publish blood PCE concentration-time profiles for postinhalation and for an oral exposure of rats. Pegg et al. (1979) and Frantz and Watanabe
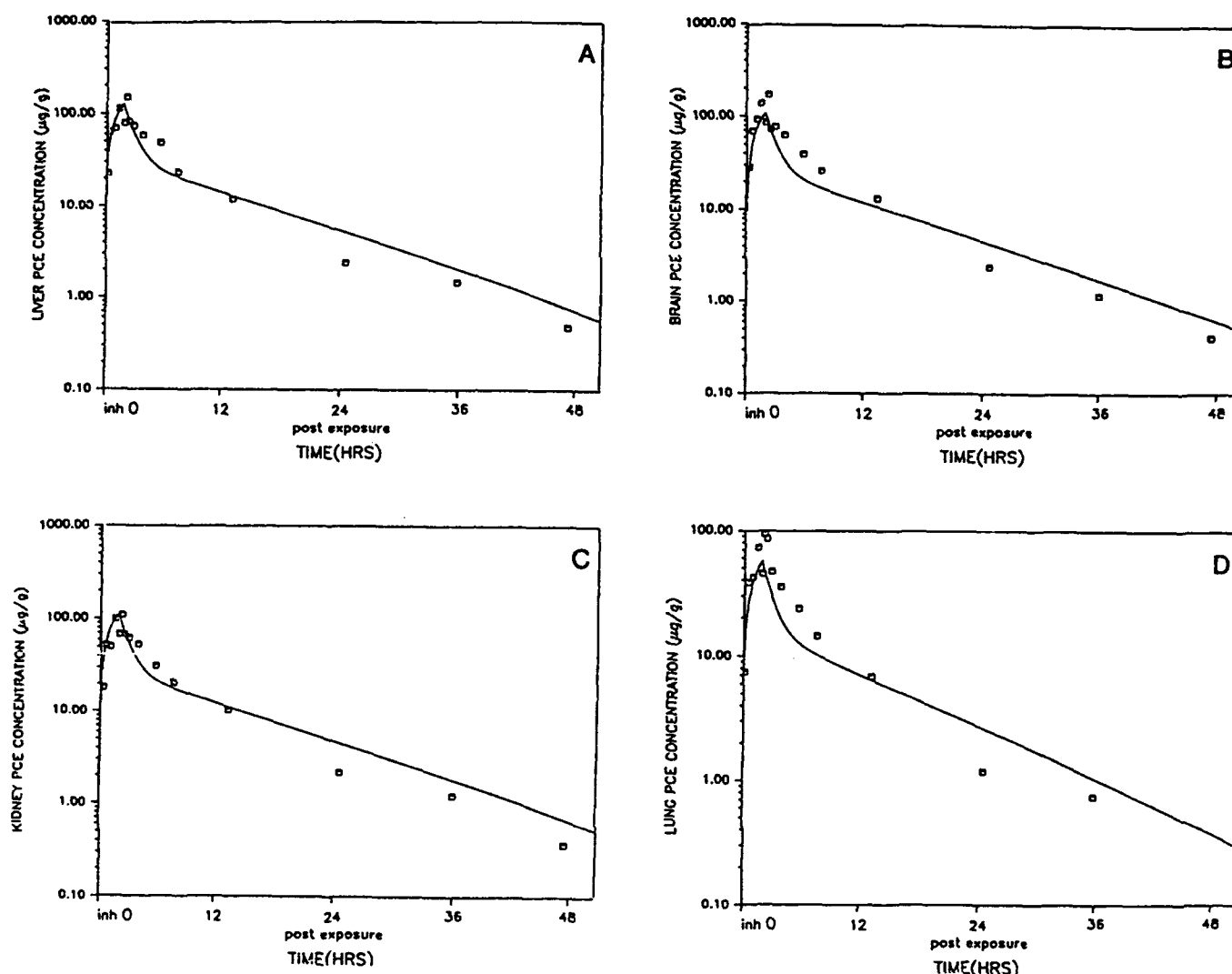
FIG. 4. Observed (□) and model-predicted (—) PCE concentrations in the (A) liver, (B) brain, (C) kidney, and (D) lung of rats during and following 2-hr inhalation exposures to 500 ppm PCE in rats. Each point represents the mean value for five rats.

(1983) measured levels of radioactivity in several organs of rats at a single time point (i.e., 72 hr) post [^{14}C]PCE exposure. Pegg et al. (1979) did differentiate between total and irreversibly (i.e., covalently) bound radiolabel in the liver. Other investigators have examined autoradiograms of animals exposed to [^{14}C]PCE vapors, in order to establish the qualitative distribution pattern of the chemical in the body. Ghatous et al. (1986) saw marked uptake of radioactivity in highly perfused tissues such as brain, liver, kidney, and lung, as well as in nasal mucosa, blood, and fat of mice after 10 min of inhalation of PCE. Savolainen et al. (1977) published the most extensive quantitative tissue data set to date. These researchers exposed male Sprague–Dawley rats 6 hr daily for 4 days to 200 ppm PCE, then euthanized two rats at each of the following times during the 5th day of exposure: 0, 2, 3, 4, and 6 hr. PCE levels were lowest in the blood and lungs, similar in the cerebrum and liver, and

highest in perirenal fat. Marth (1987) found comparable PCE concentrations in the liver, brain, and kidney of mice which consumed 0.05 or 0.1 mg/kg daily in their drinking water for 8 weeks. The spleen contained very high levels of PCE, which the investigator attributed to ongoing hemolysis and accumulation of PCE from the hemolyzed erythrocytes in the spleen. No comprehensive, scientifically sound tissue PCE concentration time-course profiles, however, were located for any route of exposure.

As mentioned under Results, the tissue distribution of PCE is apparently governed largely by the perfusion rate and the lipid content of organs. The liver, brain, and kidneys exhibited high PCE concentrations soon (i.e., 1 min) after ia injection of the chemical. All of the organs are highly perfused and each has a relatively high lipid content, except the kidneys. Two other lean, highly perfused organs, the heart and lungs, showed rapid PCE uptake, but low
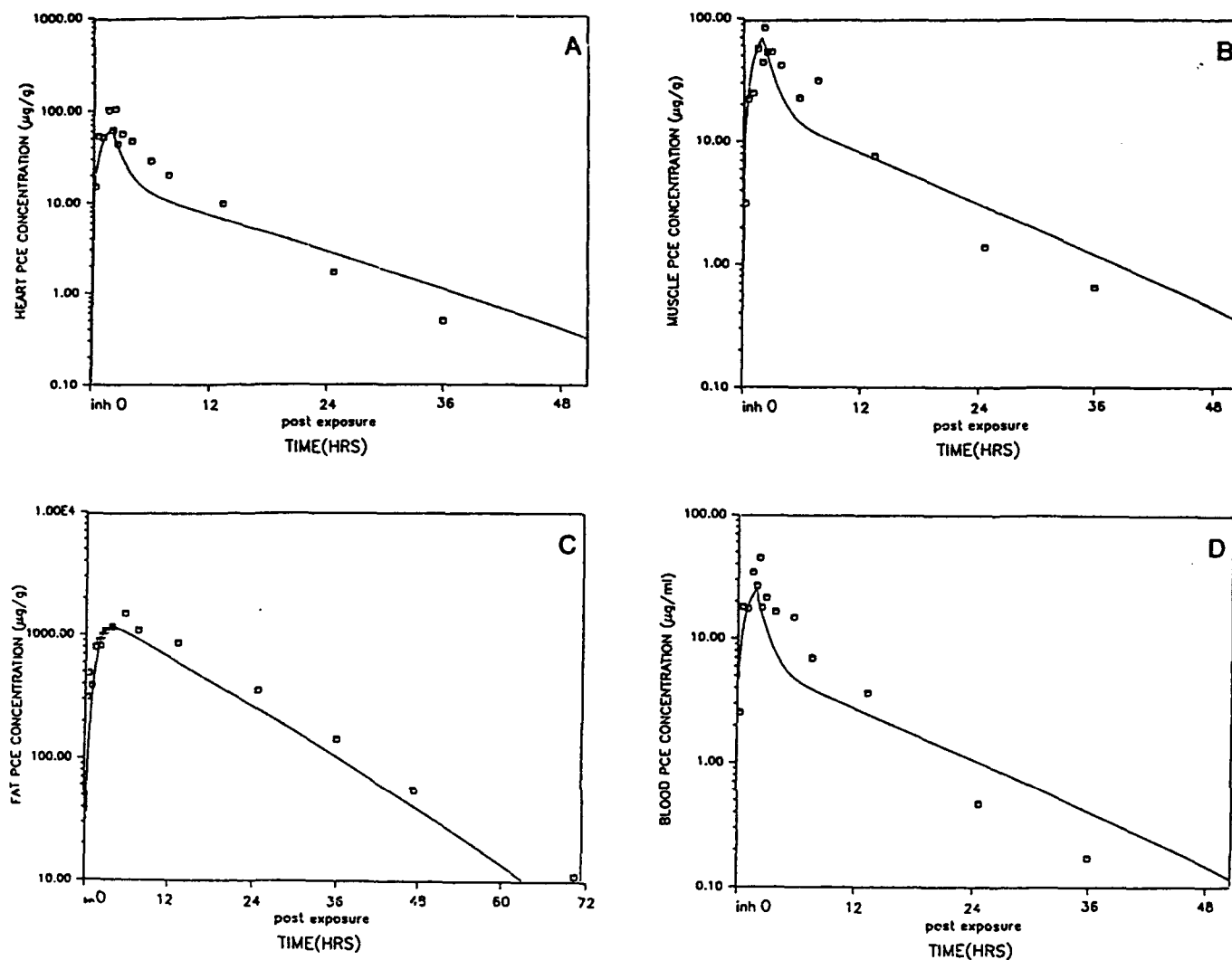
FIG. 5. Observed (□) and model-predicted (—) PCE concentrations in the (A) heart, (B) skeletal muscle, (C) fat, and (D) blood of rats during and following 2-hr inhalation exposures to 500 ppm PCE in rats. Each point represents the mean value for five rats.

PCE concentrations. Skeletal muscle, a tissue with a moderate perfusion rate and low lipid content, exhibited relatively slow, limited uptake of PCE. Bergman (1983) monitored the tissue distribution of a series of organic solvents in mice by whole-body autoradiography. He observed very rapid uptake of inhaled solvents such as TCE and carbon tetrachloride by the brain and other well-perfused organs. By 30 min postexposure, there had been redistribution of much of each halocarbon to adipose tissue. An analogous pattern was seen in the current experiments. PCE accumulated slowly but attained very high levels in fat. This phenomenon would be expected for deposition of a lipophilic chemical in a poorly perfused, lipoidal tissue. PCE levels progressively increased in each tissue, as they did in blood, during the 2-hr inhalation sessions. Savolainen et al. (1977) reported similar increases in rats throughout a 6-hr, 200 ppm PCE exposure.

PCE is eliminated from tissues of the body of the rat at slow but comparable rates. The $t_{1/2}$s following ia injection of a 10-mg/kg dose range from 389 min for liver to 496 min for blood (Table 2). The relatively rapid decline in PCE levels in the liver may reflect hepatic metabolism, although the capacity of rats to metabolize higher doses of PCE is limited (Pegg et al., 1979; Schumann et al., 1980). The blood $t_{1/2}$ of 8.27 hr in the current ia experiment is comparable to the values of 6.94–7.43 hr calculated by Pegg et al. (1979) and Frantz and Watanabe (1983) from inhalation and oral data. PCE which reenters the bloodstream from adipose tissue is, of course, available for reuptake by other tissues of the body. Thus, $t_{1/2}$ values for most organs monitored in the present study were quite similar. The slow systemic elimination of PCE may be attributed to a combination of factors including prolonged release from fat, limited metabolism, and a relatively high blood:air partition coeffi-

cient compared to those of other VOCs, resulting in prolonged exhalation.

In the present investigation, PBPK model-predicted PCE concentrations in tissues of rats were in close agreement with direct measurements of the chemical over time. Previous validations of PBPK models for PCE and other VOCs have had to primarily rely on observed blood and exhaled breath data (Chen and Blancato, 1987; Travis, 1987; Ward et al., 1988). For example, the model of Reitz et al. (1988) reliably forecast blood and exhaled breath levels of TRI in mice, rats, and humans during and following inhalation exposures. Their PBPK model was versatile enough to forecast the kinetics of TRI in rats given the chemical iv and orally, as well as to predict target organ (i.e., liver) concentrations in humans who consume TRI in their drinking water. However, no actual tissue data were available for humans or other species for verification of the model predictions of target organ concentrations, other than levels of total radioactivity at a single time point (i.e., 6 hr) postexposure.

There have been relatively few attempts to model the uptake and elimination of halocarbons and other VOCs in tissues. In papers published to date (Ramsey and Andersen, 1984; Reitz et al., 1988; Paustenbach et al., 1988), the modelers had to utilize limited tissue concentration data of other investigators. Modeling was largely limited to simulations of VOC levels in the blood and fat. In the current investigation, tissue concentration versus time data from in experiments were used to develop a PBPK model to describe the kinetics of PCE in blood and a variety of tissues and employed in simulating inhalation exposure. As described under Methods, the physiological and physicochemical input parameters were carefully measured in rats of the same sex and strain as those used in the PCE exposures. There have been considerable differences in the estimates of the metabolic parameters K_m and V_{max} for PBPK models of PCE in the rat (Hattis et al., 1990), with differences between 60- and 15-fold, respectively, reported in the literature. Our V_{max} value is even lower than this range (2.9 nM/ml kg. in the units reported in that review article). However, these previous higher estimates of V_{max} were not based on liver concentration–time data, which were available for estimation of this parameter in the current study.

There were some dissimilarities between in vivo partition coefficients utilized in the current modeling effort and in vitro values used in many previous PBPK models. The in vivo blood:air partition coefficient that was determined for PCE was similar to an in vitro value (18.9) published by Gargas et al. (1989). The in vivo tissue:blood partition coefficients used in the present study, however, were 1.4, 1.8, and 2.8 times higher for liver, fat, and muscle, respectively, than in vitro values determined by Gargas et al. (1989). These differences in partition coefficients may be due to the use of Fischer-344 rats by Gargas et al. (1989) and Sprague-Daw-

ley rats in the current investigation. The differences may also be attributable to the utilization of in vitro versus in vivo techniques. The in vitro procedure of Gargas et al. (1989) involved the use of tissue homogenates, in which normal tissue architecture and cellular structure were disrupted. Artifactual changes may introduce changes in partitioning of PCE in some tissues. It seems preferable to utilize partition coefficients measured in animals during actual exposures, as these should be the most accurate parameters and therefore help reduce uncertainty in modeling. It was necessary for Ward et al. (1988), for example, to adjust the fat and muscle partition coefficients to obtain better fits to their experimental data. In the current modeling effort, predicted tissue concentration versus time data agreed quite well with PCE levels measured in tissues without having to alter any input parameter to optimize simulations. Thus the present model, used in conjunction with accurately determined in vivo input parameters, is a more detailed and representative description of physiological structure, which reliably predicts blood and tissue time courses of PCE in rats.

The use of experimental tissue concentration–time data and accurate measurement of physiological values can be of significant value in the development and validation of PBPK models. As such models can more reliably predict time integrals of target organ exposures to chemicals, they should improve the accuracy of risk assessments of PCE and other VOCs.

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APPENDIX B

Use of a Physiologically Based Model to Predict Systemic Uptake and Respiratory Elimination of Perchloroethylene. In Press, *Toxicology and Applied Pharmacology*, 1994.

Use of a Physiologically Based Model to Predict Systemic Uptake and Respiratory Elimination of Perchloroethylene[1,2]

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Use of a Physiologically Based Model to Predict Systemic Uptake and Respiratory Elimination of Perchloroethylene. DALLAS, C. E., MURALIDHARA, S., CHEN, X. M., RAMANATHAN, R., VARKONYI, P., GALLO, J. M., AND BRUCKNER, J. V. (1994). *Toxicol. Appl. Pharmacol.* 127, 000–000.

The pharmacokinetics of inhaled perchloroethylene (PCE) were studied in male Sprague–Dawley rats to characterize the pulmonary absorption and elimination of the volatile organic chemical (VOC). The direct measurements of the time course of PCE in the blood and breath were used to evaluate the ability of a physiologically based pharmacokinetic (PBPK) model to predict systemic uptake and elimination of PCE. Fifty or 500 ppm PCE was inhaled for 2 hr through a miniaturized one-way breathing valve by unanesthetized male Sprague–Dawley rats of 325–375 g. Serial samples of the inhaled and exhaled breath streams, as well as arterial blood, were collected during and following PCE inhalation and analyzed by headspace gas chromatography. PCE-exhaled breath concentrations increased rapidly to near steady state (i.e., within 20 min) and were directly proportional to the inhaled concentration. Uptake of PCE into the blood was also rapid, but blood levels continued to increase progressively over the course of the 2-hr exposure at both exposure levels. Cumulative uptake, or total absorbed dose, was not proportional to the exposure level. A PBPK model was developed from *in vivo* parameters determined from tissue concentration-time data in a companion ia study (Dallas *et al.*, 1994, *Toxicol. Appl. Pharmacol.* 127, 000–000). PCE concentrations in the blood and exhaled breath during and following PCE inhalation were well predicted by the PBPK model. Despite species differences in blood:air and lung:air partition coefficients, the model was used to account for similar levels of PCE and other VOCs in the expired air of rats and humans. The model also accurately simulated percentage uptake and cumulative uptake of PCE over time. The model's ability to predict systemically absorbed doses of PCE under a variety of exposure scenarios should be useful in assessment of risks in occupational and environmental settings. © 1994 Academic Press, Inc.

Perchloroethylene (1,1,2,2-tetrachloroethylene) (PCE) is a volatile organic chemical (VOC) which is used in large quantities in industry for metal degreasing, dry cleaning of fabrics and textiles, and as an intermediate in the production of other chemicals. Approximately 500,000 workers in the United States are estimated to be occupationally exposed to PCE (NIOSH, 1978). Measurements of workplace air concentrations of PCE in the dry cleaning industry have yielded mean 8-hr time-weighted averages of 28.2–88.2 ppm (Materna, 1985) and 4–149 ppm (Ludwig *et al.*, 1983). PCE has been found in indoor air in residences using PER-contaminated water supplies (Highland *et al.*, 1985; Andelman, 1985). Central nervous system (CNS) effects including dizziness, headache, sleepiness, incoordination, and impairment of performance on psychophysiological tests have been reported in humans acutely exposed to levels of 100–200 ppm PCE and higher (Stewart *et al.*, 1961, 1970; Hake and Stewart, 1977). High-level inhalation exposures to PCE have also been reported to result in mild hepatorenal toxicity in some humans (Hake and Stewart, 1977), as well as mild hepatotoxicity (Kylin *et al.*, 1963), biochemical changes in the brain including reduced RNA content (Sa-

volainen *et al.*, 1977), and CNS depressant effects (Rowe *et al.*, 1952; Goldberg *et al.*, 1964) in laboratory animals.

Although PCE produces CNS depression as well as mild hepatorenal toxicity in sufficiently high doses, its potential as a carcinogen is of primary concern at low occupational and environmental levels. High, chronic doses of PCE have been shown to cause an increased incidence in hepatocellular carcinomas in B6C3F1 mice but not in rats (NCI, 1977; NTP, 1986). There was also an increase in mononuclear cell leukemia in male and female Fischer 344 rats, as well as a low incidence of renal tubular cell tumors in the male rats (NTP, 1986). Some epidemiological studies of dry cleaning workers have not found an excess incidence of cancer (Brown and Kaplan, 1987), while others have (Duh and Asal, 1984; Blair *et al.*, 1990). Such investigations have stimulated interest in conducting valid cancer risk assessments of PCE (ATSDR, 1993).

Physiologically based pharmacokinetic (PBPK) models have been used increasingly in risk assessments of PCE and other VOCs, particularly in the species-to-species and high-to-low dose extrapolations often necessary to apply animal bioassay data to low-level human exposures. It is important to determine the quantity of chemical absorbed systemically (i.e., the absorbed dose), as it is often quite different from the inhalation or oral exposure level (i.e., the administered dose). Systemic absorption of inhaled PCE has been demonstrated to be dependent upon a number of factors, including lean body mass, respiratory rate, duration of exposure, and inhaled concentration in limited human experiments (Hake and Stewart, 1977; Monster, 1979). Valid PBPK models offer one the ability to predict systemic uptake and elimination of chemicals in laboratory animals and humans under a variety of exposure conditions. A PBPK model for PCE was recently developed and validated in a companion paper (Dallas *et al.*, 1994). The model was based on ia time-course data sets from laboratory experiments in rats, and the accuracy of its predictions verified by comparison with the actual time course of PCE in tissues of rats inhaling the chemical. The current work was undertaken to determine whether this model can accurately forecast systemic uptake of PCE over time as well as respiratory elimination of the volatile halocarbon.

Objectives of this study were to (1) directly determine the systemically absorbed dose of PCE during inhalation exposures, by simultaneously measuring PCE in the inhaled and exhaled breath; and (2) evaluate the ability of a recently developed PBPK model (Dallas *et al.*, 1994) to accurately predict the systemic uptake and elimination of PCE, by comparison of simulated and observed levels of the chemical over time in the blood and breath of rats.

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 dark 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 325 to 375 g. Solvent exposures were initiated at approximately the same time each day (1000–1200 hr).

Test material. Perchloroethylene (tetrachloroethylene), of 99%+ purity, was obtained from Aldrich Chemical Company (Milwaukee, WI). The purity of the chemical was verified by gas chromatographic analysis.

Animal preparation. An indwelling carotid artery 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), and xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). The cannulated animals were freely moving during a 24-hr recovery period.

Inhalation exposures. A face mask specifically designed to fit the Sprague–Dawley rat of this size 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 animal. This established separate and distinct airways for the inhaled- and exhaled-breath streams. The use of such a device for pharmacokinetic studies of inhaled halocarbons in small animals has been described in detail (Dallas *et al.*, 1986). A known concentration of PCE was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of the VOC 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, the one-way breathing valve, and an empty 70-liter gas collection bag. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposures (Battelle-Geneve, Switzerland). PCE inhalation exposures of 2-hr duration were initiated only after stable breathing patterns (i.e., respiratory rate and minute volume) were established. During this exposure period and for up to 8 hr afterward, serial-inhaled and exhaled-breath samples were taken at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for PCE content by headspace gas chromatography.

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

Calculations of PCE uptake and elimination were conducted utilizing equations presented in a previous VOC inhalation study in rats (Dallas *et al.*, 1989). Since the V_E and the exhaled breath PCE concentration at each sampling point were measured, subtraction of the quantity of PCE exhaled from the amount inhaled yielded the quantity of PCE taken up during sequential sampling periods. By summing these values, the cumulative uptake, or systemically absorbed dose, was determined. The percentage uptake during each exposure period was determined by dividing the cumulative uptake by the total inhaled dose for that time period.

A PBPK model was used to describe the uptake and elimination of PCE in the rat. It was assumed that a blood-flow-limited model was appropriate

to characterize the tissue distribution of PCE. Most previous PBPK models for VOCs have utilized blood-flow-limited organ representations (Ramsey and Andersen, 1984; Angelo and Pritchard, 1984; Ward *et al.*, 1988; Dallas *et al.*, 1989, 1991). Compartmental volumes and organ blood flows were determined in this laboratory for the male Sprague–Dawley rat (Delp *et al.*, 1991; Manning *et al.*, 1991). *In vivo* tissue:blood partition coefficients were calculated from tissue concentration–time data for PCE following its ia injection in a companion study (Dallas *et al.*, 1993) using the area method of Gallo *et al.* (1987). The metabolic parameters K_m and V_{max} and blood:air partition coefficients were estimated from the observed ia time-course data for liver and blood by nonlinear regression analysis. Alveolar ventilation was determined to be 50% of the minute volume, accounting for dead space in the animal and in the miniaturized breathing valve. Alveolar ventilation values were measured in the present study. The alveolar mass transfer coefficient was based on the alveolar permeability–area product for methylene chloride (Angelo and Pritchard, 1984). The lung:air partition coefficient was derived using the area method by Gallo *et al.* (1987). Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of PCE in the rat were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution of the equations provided predicted PCE blood, expired air, and tissue concentrations over time. The model-predicted cumulative uptake values were the sum of the simulated amounts of PCE in each tissue compartment in the model.

Analysis of PCE in air and blood. The concentrations of PCE in the inhaled- and exhaled-breath samples collected during inhalation exposures were measured with a Tracor MT560 gas chromatograph (GC) (Tracor Instruments, Austin, TX). Standards were prepared in each of four 9-liter bottles equipped with Teflon stoppers with needles from which air samples could be taken with a syringe. 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 × $\frac{1}{8}$-in stainless steel column packed with 0.1% AT 1000 on GraphPak. 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 make-up gas flow rate to the detector of 20 ml/min.

Concentrations of PCE in the blood were measured by a GC headspace technique (Chen *et al.*, 1993). Blood samples were withdrawn from the arterial cannula via a stopcock into a 1-ml syringe. Depending on the anticipated blood PCE concentration, from 25 to 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 metal washers and tightly crimped. Each sample vial was then placed into the HS-6 autosampler unit of a Sigma 300 GC (Perkin–Elmer) where it was heated to 90°C by a thermostat. A predetermined volume of the vapor was then injected automatically into the column for analysis. The column used was an 8-ft × $\frac{1}{8}$-in stainless steel column packed with 10% FFAP chromasorb W-AW (80–100 mesh). Operating temperatures were 200°C, injection port; 400°C, ECD detector; and 110°C column oven. The carrier gas was 5% argon–methane at a flow rate of 40 ml/min with a make-up gas flow rate of 20 ml/min to the detector. The limit of detection was about 1 ng. or 6.7 ppb. in air. The percentage recovery from spiked blood samples was approximately 95% (Chen *et al.*, 1993).

RESULTS

PCE concentrations inhaled by the animals were determined by analysis of air samples taken from a sampling port immediately adjacent to the breathing valve. Actual inhaled PCE concentrations for the six rats in each group

TABLE 1
Parameters Used in the Physiologically Based Pharmacokinetic Model for PCE in the Rat

Parameters	
Alveolar ventilation ml/min Inhaled PCE concentration (mg/ml)	108 (50 ppm); 94.5 (500 ppm)
Body weight (g)	0.35 (50 ppm); 3.55 (500 ppm)
Alveolar mass transfer coefficient	340 500 ml/min
Tissue volumes (ml)	**Percentages of body weight**
Liver	3.39
Kidney	0.77
Fat	5.0
Heart	0.33
Lung	0.37
Muscle	35.36
Brain	0.6
Blood	7.40
Rest of body	46.78
Cardiac output	1.57 (ml/min · g) body wt (g)$^{0.75}$
Blood flows (ml/min)	**Percentages of cardiac output**
Liver	15.73
Kidney	13.13
Fat	6.56
Heart	4.73
Lung	100
Muscle	26.11
Brain	2.21
Blood	100% = 1.57 (ml/min · g) body wt (g)$^{0.75}$
Rest of body	31.53
Partition coefficients	
Blood:air	18.9
Fat:blood	152.5
Lung:blood	2.48
Liver:blood	5.25
Muscle:blood	2.98
Brain:blood	4.37
Heart:blood	2.68
Kidney:blood	4.45
Rest of body:blood	2.98
Metabolism constants	
V_{max} (μg/min)	0.15
K_m (μg/ml)	0.019

were 528.2 ± 21.9 ppm ($\bar{x}$ ± SD) for the 500-ppm exposures and 53.1 ± 5.1 ppm ($\bar{x}$ ± SD) for the 50-ppm exposures.

Substantial respiratory elimination of unchanged PCE was evident during the 2-hr inhalation exposure period. Near steady-state PCE levels were achieved in the exhaled breath within 20 min and maintained for the duration of the exposures. The near steady-state concentrations were 2.1–2.4 μg/ml in the exhaled air of the 500 ppm rats (Fig. 1B) and 0.20–0.22 μg/ml in the 50 ppm animals (Fig. 2B). Thus, the exhaled breath levels during the 2-hr exposures
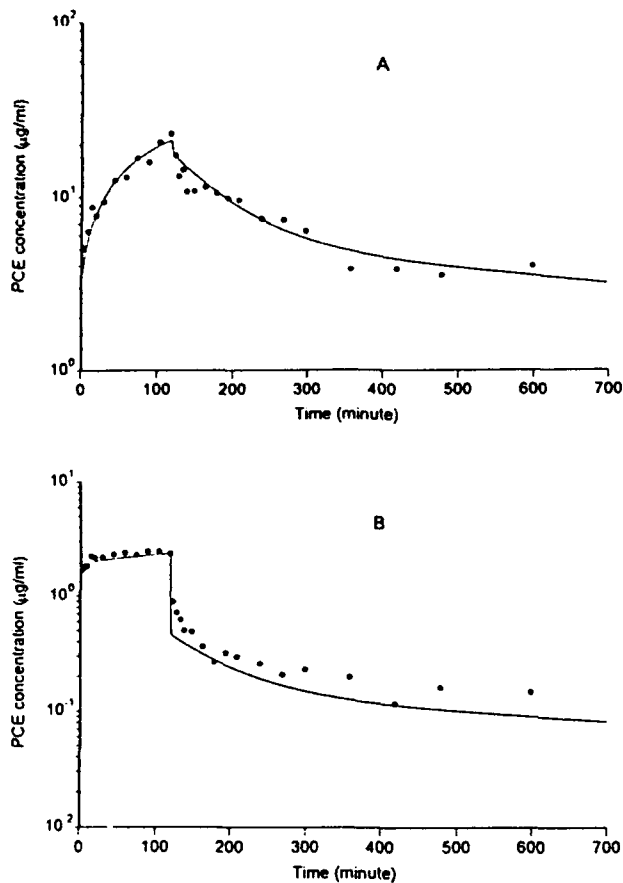
FIG. 1. Observed (●) and predicted (—) PCE concentrations in the (A) arterial blood and (B) exhaled air of rats during and following a 2-hr, 500 ppm inhalation exposure. Each point represents the mean value for six rats.

were directly proportional to the inhaled PCE concentrations.

PCE was rapidly absorbed from the lungs, as relatively high arterial blood concentrations of PCE were measured at the first sampling time (i.e., 2 min). Unlike the pattern of PCE levels in the exhaled breath, the concentration of PCE in the blood progressively increased over the course of the 2-hr session at both exposure levels. The rates of increase of the PCE concentrations in the blood were greater in the 500 ppm group (Fig. 1A) than those in the 50 ppm group (Fig. 2A). Arterial blood PCE concentrations were not proportional to the inhaled concentrations. After the initial rapid uptake phase during the first 30 to 60 min of exposure, blood levels in the 500 ppm rats were 12 to 17 times higher than those in the 50 ppm rats. Upon cessation of PCE inhalation, the chemical was eliminated very quickly during the first minutes, particularly from the exhaled breath. Following this initial phase, PCE levels in the blood and breath declined slowly.

Plots of the cumulative uptake of PCE over time are shown in Fig. 3. Visual observation of the measured data

points reveals a more pronounced deviation from linearity in the 500 ppm group than that in the 50 ppm group. Total cumulative uptake of PCE during the 2-hr exposure to 500 ppm was determined to be 28.1 ± 4.3 mg ($\bar{x}$ ± SD), or 79.9 mg/kg body wt. The 2-hr exposure to 50 ppm PCE resulted in a cumulative uptake of 3.9 ± 0.9 mg ($\bar{x}$ ± SD), or 11.2 mg/kg body wt. Thus, cumulative uptake was not proportional to inhaled concentration. The percentage systemic uptake of PCE is shown in Fig. 4. Percentage uptake was relatively constant after the first 20 min of inhalation, approximately 40% in the 500 ppm group and approximately 50% in the 50 ppm group.

The PBPK model predictions of blood and exhaled-breath concentrations of PCE are shown in Figs. 1 and 2. Both observed and predicted exhaled-breath concentrations of PCE rapidly achieved near steady state following the initiation of exposures. Predicted concentrations of PCE in the exhaled air both during and following 500 ppm (Fig. 1B) and 50 ppm (Fig. 2B) inhalation exposures agreed very well with the observed concentrations. The steep increase in PCE blood concentrations throughout the 500
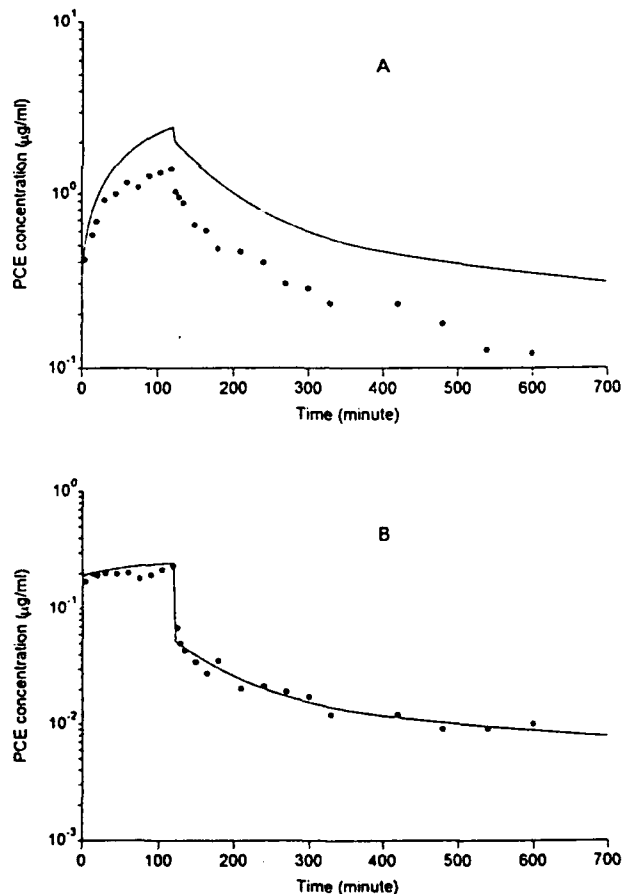

FIG. 2. Observed (●) and predicted (—) PCE concentrations in the (A) arterial blood and (B) exhaled air during and following a 2-hr, 50 ppm inhalation exposure. Each point represents the mean value for six rats.

ppm exposure (Fig. 1A) was accurately simulated by the model. PCE blood concentrations postexposure were also well simulated. The uptake of PCE in the blood during the second hour of the 50 ppm exposure (Fig. 2A) was overpredicted, as were postexposure blood levels. A saturable, high-affinity metabolic pathway with a somewhat greater capacity could have accounted for this disparity in predicted and measured blood levels. We adopted the practice, however, of using a training data set from a separate experiment (i.e., V_{max}' and K_m based on the ia data in the companion study (Dallas et al., 1993)) to derive input parameters for the PBPK model to obtain predictions in the current study.

The PBPK model was also utilized to generate predictions of cumulative uptake and percentage uptake of PCE during the 2-hr inhalation sessions. Cumulative uptake of PCE was well predicted over the course of the 500 ppm inhalation exposure (Fig. 3A). During the 50-ppm exposure, a slight underprediction during the initial hour of PCE inhalation became more pronounced during the last hour, resulting in a forecast of cumulative uptake which was 20% less than the observed value at 120 min (Fig. 3B). In the second hour of the 500 ppm exposure, model simulations

FIG. 4. The percentage systemic uptake of PCE over time during inhalation exposures to 500 (A) or 50 (B) ppm PCE for 2 hr. Each point represents the mean for six rats. The percentage uptake of the inhaled dose was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter, as described under Methods. Model predictions of the percentage uptake are delineated by the solid lines.
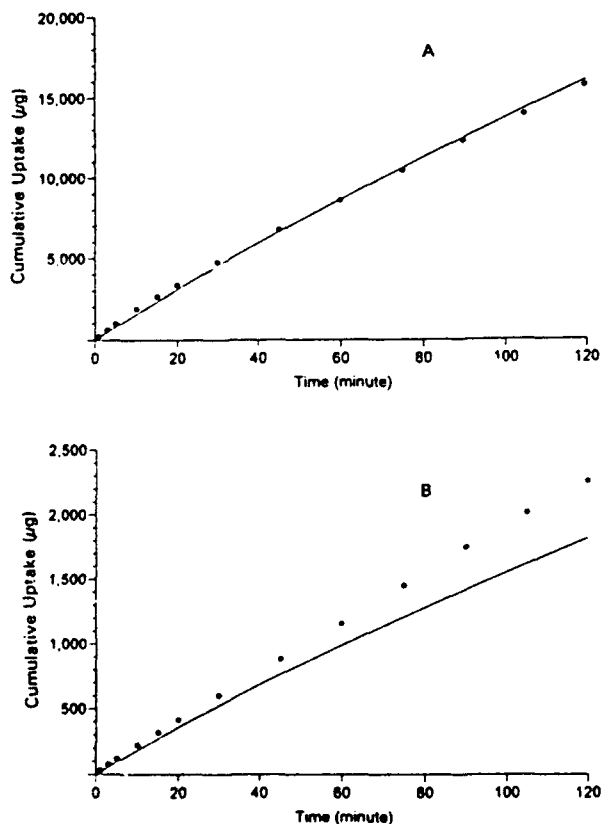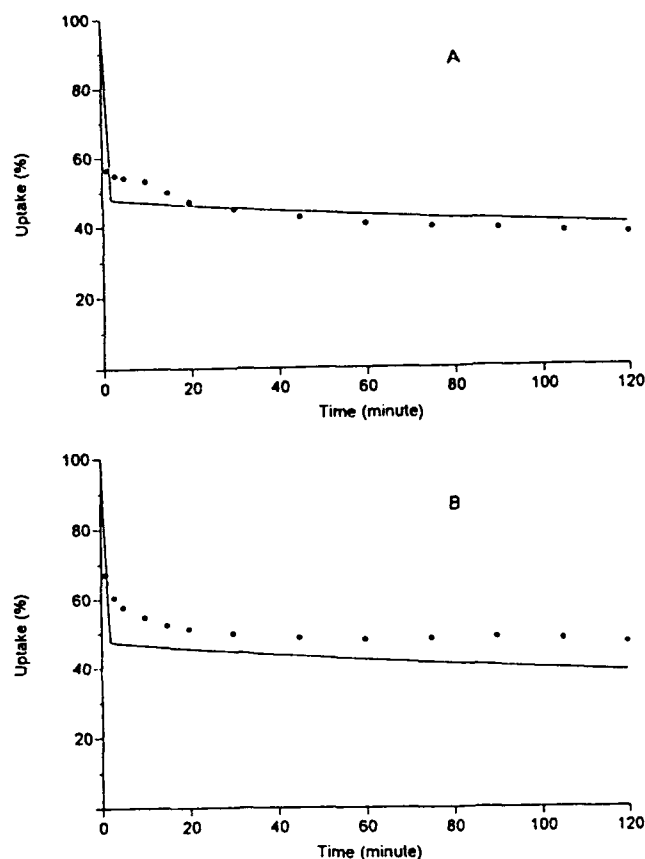

FIG. 3. Cumulative uptake of PCE during inhalation of 500 (A) or 50 (B) ppm PCE for 2 hr. The quantity of PCE retained during successive 10-min intervals was calculated as described under Methods. Each point represents the mean for six rats. Model predictions of cumulative uptake are delineated by the solid lines.

of percentage uptake were within 2% of the observed values (Fig. 4A). For the 50 ppm exposure, the predicted percentage uptake was consistently about 5% below the observed percentage uptake (Fig. 4B). As discussed earlier, a somewhat greater metabolic rate in the rats used in the current investigation than in those used in the companion ia study by Dallas et al. (1993) could be responsible for the underprediction of percentage systemic uptake.

Model predictions of exhaled breath concentrations were also conducted using different lung:air partition coefficients in order to determine the influence of this parameter on exhalation of PCE in dissimilar species. Employing the assumption of equivalent tissue:blood coefficients in species, including lung:blood partition coefficients, a lung:air partition coefficient was calculated by use of the equation

$$r_a = r_1 r_{b:a},$$

where r_a is lung:air partition coefficient, r_1 is lung:blood partition coefficient, and $r_{b:a}$ is blood:air partition coefficient.

TABLE 2

Predicted PCE Exhaled Breath Concentrations [μg/ml]
Using Different r_a Values[*]

r_a	50 ppm		500 ppm	
	1 hr	2 hr	1 hr	2 hr
46.87 (rat)	0.25	0.26	2.36	2.48
25.54 (human)	0.26	0.28	2.40	2.70

[*] The influence of different r_a (lung:air partition coefficient) values on exhaled breath concentrations predicted by a PBPK model is evaluated for a 1- or 2-hr inhalation exposure to 50 or 500 ppm PCE in rats and humans.

In the current rat model, $r_a = 46.87$, $r_l = 2.48$, and $r_{b:a} = 18.9$. Use of the human $r_{b:a}$ value of 10.3 of Bois $et\ al.$ (1990) and the r_l of 2.48 resulted in a human r_a of 25.54. Model predictions of PCE exhalation over time were made using these two r_a values of 46.87 for rats and 25.54 for humans. The predictions of exhaled PCE concentrations in rats and humans after 1 and 2 hr of exposure are included in Table 2. The entire time courses for the 500 and 50 ppm exposures are shown in Figs. 5A and 5B, respectively. It can be seen that even a doubling of the lung:air partition coefficient resulted in only a small difference in exhaled concentrations in rats and humans at each exposure level. The larger r_a value resulted in a slightly lower predicted PCE concentration in exhaled breath.

DISCUSSION

PCE is readily absorbed from the lungs into the blood. This is manifest in rats by a substantial percentage uptake and relatively high arterial blood levels at the initial sampling times (i.e., 1 and 2 min, respectively) after inhalation of PCE begins (Figs. 1A and 2A). Although percentage uptake is initially quite high, it decreases as a function of time for the first 20 min. Systemic uptake remains relatively constant thereafter for the duration of the 2-hr exposure (Fig. 4), with percentage uptake slightly higher (i.e., 50%) at the lower (i.e., 50 ppm) exposure level. This finding is evidence of a limited capacity of the male Sprague–Dawley rat to metabolize PCE. The plots of cumulative uptake, as well as total uptake provide evidence of saturable PCE metabolism in Sprague–Dawley rats. Deviation from linearity in plots of cumulative uptake versus time is more evident at the higher (i.e., 500 ppm) exposure level. The 10-fold increase in administered dose results in just a 7.2-fold increase in the total absorbed dose over the 2-hr exposure. This phenomenon of saturable metabolism of PCE in Sprague–Dawley rats is also reported by Schumann $et\ al.$ (1980) and Pegg $et\ al.$ (1979). The latter group report that B6C3F1 mice have a significantly greater capacity to metabolize PCE than do the rats. Humans appear to have a quite limited capacity to

metabolize PCE. The data of Ohtsuki $et\ al.$ (1983) and Seiji $et\ al.$ (1989) demonstrate that PCE metabolism is saturated at inhaled concentrations >100 ppm. Uptake of inhaled concentrations of 72–144 ppm PCE diminishes as a function of time in humans, decreasing to approximately 62% (Monster $et\ al.$, 1979), a value somewhat higher than those measured in rats in the current study.

The systemic uptake and elimination patterns of PCE are characteristic of a lipid-soluble, poorly metabolized chemical. Uptake of an inhaled halocarbon should be determined largely by its solubility in the blood and tissues and its rate of metabolism (Monster, 1979). Blood levels progressively increased during the 2-hr exposures in the current study, though they seemed to be approaching steady state by 2 hr in the 50 ppm group. Stewart $et\ al.$ (1961) saw a similar pattern in blood levels in humans inhaling 194 ppm PCE, with apparent steady state reached after 3 hr of exposure. PCE concentrations in tissues, as in blood, steadily increased in rats inhaling 500 ppm of the halocarbon (Dallas

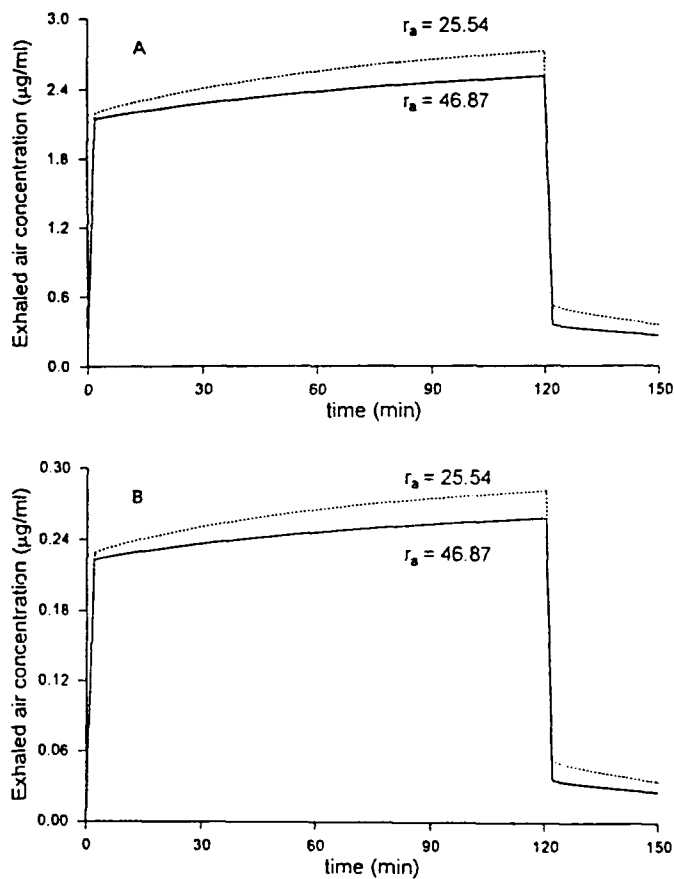FIG. 5. Model predictions of exhaled breath PCE concentrations using different lung:air partition coefficients (r_a). Lung:air partition coefficients of 46.87 for rats and 25.54 for humans were calculated according to the equation described under Results. Predicted levels of PCE in the exhaled breath of humans (dashed lines) and rats (solid lines) are compared during and following 500 (A) and 50 (B) ppm exposures to PCE.

et al., 1993). The pattern of uptake into and elimination from fat was quite different. PCE uptake by fat was slower but much greater in magnitude, due to a low perfusion rate and a very high adipose tissue:blood partition coefficient. Although blood samples were not taken long enough postexposure in this companion study to define the terminal elimination phase, blood levels appeared to diminish more rapidly in the low-dose (i.e., 50 ppm) animals. This is indicative that metabolism, albeit of limited capacity, contributes to the systemic clearance of lower doses of PCE.

As for many other VOCs, the major route of elimination of PCE in laboratory animals and man is exhalation of the parent compound. There have been several studies involving direct measurements of the respiratory elimination of PCE in humans. This allows interspecies comparisons of the quantity of the halocarbon which is eliminated in the breath. Following inhalation of 100 ppm by humans for 2 hr, PCE concentrations in the exhaled breath at 1 and 2 hr postexposure were 0.06 and 0.047 μg/ml. Assuming a linear scale-up from the 50 ppm data in the current investigation to 100 ppm, the PCE concentration in the expired air of rats at these two time points would be 0.07 and 0.04 μg/ml, respectively. Similarly, postexposure exhaled breath PCE levels in other human studies (Stewart et al., 1961, 1970) were comparable to levels of PCE measured in the breath of rats in the current study. Similar concentrations of halocarbons in the expired air of rats and humans were also noted in studies of 1,1,1-trichloroethane (TRI) (Dallas et al., 1989) and 1,1,2-trichloroethylene (TCE) (Dallas et al., 1991). Since the blood:air partition coefficients for PCE, TRI, and TCE are markedly higher in rats than in humans (Gargas et al., 1989), it might be anticipated that this physicochemical difference would result in greater respiratory elimination of the halocarbons by humans when there are equivalent blood levels.

Consideration of species differences in blood:air and tissue:air partition coefficients is necessary in order to understand why human and rat exhaled-breath concentrations can be so similar. In the PBPK model of Bois et al. (1990) for PCE, rat blood:air and tissue:air partition coefficients are significantly greater than corresponding human partition coefficients. When one uses these values to calculate tissue:blood coefficients, the tissue:blood coefficients for rats and humans are quite similar.

In the current PBPK model, chemical input occurs at the lung:air interface and is characterized by a mass transfer coefficient and a lung:air partition coefficient. Since the value of the mass transfer coefficient would not be rate-limiting for chemical uptake, the influence of changes in the lung:air partition coefficient (r_a) on exhaled breath concentration was assessed. Model simulations were conducted with two different r_a values, calculated as described under Results, for rats and for humans. The only model parameter that varied in the comparison was r_a. The differences

between the exhaled PCE concentrations predicted using the two different r_a values were very small, for both the 50 and the 500 ppm exposures. It can be anticipated, then, that the concentration of PCE exhaled by humans and rats can be similar, despite dissimilar blood:air, or lung:air partition coefficients. This disparity in blood:air partition coefficients may be offset by higher tissue:pulmonary perfusion and respiratory rates in rats.

Overall, concentrations of PCE in the blood and breath were accurately predicted during and following inhalation exposures by the current PBPK model. Only in the 50 ppm rats were arterial blood levels overpredicted. The reason for this discrepancy is unclear. There have been few other attempts to model the time course of PCE in blood or exhaled breath. The percentage of PCE exhaled over time following inhalation of the halocarbon was well predicted in rats by a PBPK model of Ward et al. (1988). These investigators compared their simulated values to experimental data previously published by Pegg et al. (1979). It was necessary for Ward and co-workers, however, to increase their fat:air partition coefficient from 1638 to 2300 in order to more closely simulate PCE concentrations in expired air. The in vivo fat:blood partition coefficient of 152.5, derived in our companion study (Dallas et al., 1993) from adipose tissue time-course data, corresponds to a fat:air partition coefficient of 2881. Guberan and Fernandez (1974) developed a PBPK model with four compartments, which they used to make preliminary forecasts of alveolar PCE concentrations in humans who inhaled the chemical. In subsequent work, Ward et al. (1988) utilized their own PBPK model and the experimental human data of Stewart et al. (1961) and Fernandez et al. (1976). Predictions by the model of Ward and his colleagues of PCE levels in expired air during and post inhalation exposures were in close agreement with the empirical data. Noone to our knowledge, however, has previously modeled the time course of PCE in the bloodstream during or following inhalation exposures, until the current series of experiments described herein and in the companion paper (Dallas et al., 1994).

The ability of the present PBPK model to predict the disposition of inhaled PCE should make it useful in health risk assessments in occupational and environmental settings. One of the model's most important assets is its ability to accurately forecast systemic uptake of the chemical over time over a range of exposure concentrations. Although risk assessments often have been based upon the administered dose, it is now accepted that the (systemically) absorbed dose should be determined and utilized instead. The absorbed dose may be time-, concentration-, and species-dependent. Although Fernandez et al. (1976) measured alveolar PCE levels in humans and noted that uptake is the product of the minute volume and the difference between inspired and alveolar concentrations, no reports of systemic absorption of PCE by their or other groups of investigators

were found in the literature. Time integrals of target organ exposure to PCE, as described in the companion study (Dallas *et al.*, 1993), can be quite useful in conducting risk assessments. Andersen (1987) points out that the most appropriate tissue dose surrogate is a time integral of the concentration of active form of a chemical. The CNS depressant and cardiac arrhythmigenic actions of PCE are caused by the parent compound. It is generally accepted that one or more reactive metabolites are responsible for PCE's cytotoxic (Buben and O'Flaherty (1985) and carcinogenic actions (ATSDR, 1993), but it is not clear which metabolite(s) are responsible. Thus, a number of researchers have used PBPK models to predict total PCE metabolism and associated cancer risks (Chen and Blancato, 1987; Bogen and McKone, 1988; Bois *et al.*, 1990). Hattis *et al.* (1990) examined the appreciable differences in predictions of risk among different PCE models and found they were largely due to the choice of disparate metabolic parameters. There were also disagreements about the relative importance of competing linear and saturable metabolic pathways in different species. Thus, it is clear that more research should be conducted on the mechanisms of PCE cytotoxicity and carcinogenicity, so the appropriate active metabolite(s) can be identified and monitored in future experiments and PBPK modeling efforts.

ACKNOWLEDGMENT

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APPENDIX C

A Physiologically-Based Pharmacokinetic Model Useful in Prediction of the Influence of Species, Dose and Exposure Route on Perchloroethylene Pharmacokinetics. In Press, *Environmental Research*, 1994

A Physiologically-Based Pharmacokinetic Model Useful in Prediction

of the Influence of Species, Dose and Exposure Route

on Perchloroethylene Pharmacokinetics

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ABSTRACT

The ability of a physiologically-based pharmacokinetic (PBPK) model to predict the uptake and elimination of perchloroethylene (PCE) in venous blood was evaluated by comparison of model simulations with experimental data for two species, two routes of exposure and three dosage levels. Unanesthetized male Sprague-Dawley rats and beagle dogs were administered 1, 3, or 10 mg PCE/kg bw in polyethylene glycol 400 as a single bolus, either by gavage or by intraarterial (ia) injection. Serial blood samples were obtained from a jugular vein cannula for up to 96 h following dosing. PCE concentrations were analyzed by headspace gas chromatography. For each dose and route of administration, terminal elimination half-lives in rats were shorter than in dogs, and area under the blood concentration-time curves were smaller in rats than in dogs. Over a 10-fold range of doses, PCE blood levels in the rat were well predicted by the PBPK model following ia administration, and slightly underpredicted following oral administration. PCE concentrations in dog blood were generally overpredicted, except for fairly precise predictions for the 3 mg/kg oral dose. These studies provide experimental evidence of the utility of the PBPK model for PCE in interspecies, route-to-route and dose extrapolations.

Key words: Physiological model, perchloroethylene, tetrachloroethylene, interspecies extrapolation, route of exposure, dose, pharmacokinetics

INTRODUCTION

Knowledge of the influence of different species, dose levels, and routes of administration on the kinetics of environmental chemicals has been considerably enhanced by the use of physiologically-based pharmacokinetic (PBPK) models (Clewell and Andersen, 1985; Reitz et al., 1988). Similarities in the anatomy and physiology of mammalian species make interspecies extrapolations (i.e., animal scale-up or-down) possible (Dedrick, 1988; Boxenbaum, 1984). Pharmacokinetic models developed in one species may be scaled, on the basis on allometric relationships, to allow prediction of chemical concentrations in other species. Model input parameters such as alveolar ventilation, tissue volumes and blood flows, and metabolism can be scaled as from one species to another. Changing the amount of chemical entering the animal allows for extrapolations between doses. Altering the point of entry of the chemical into the model enables pharmacokinetic comparisons between routes of administration.

PBPK models have been used to assess the effect of dose of PCE on the rate of urinary metabolite formation in mice and humans (Ward et al., 1988; Bois et al., 1990). The influence of different routes of exposure on PCE pharmacokinetics has also been evaluated using a PBPK model (Travis, 19&7). Thus far, mice, rats and humans have been the species employed in most interspecies comparisons and extrapolations employing PBPK models for PCE (Ward et al., 1988; Bois et al., 1990; Chen and Blancato, 1987). In most instances, model simulations of PCE exhalation and metabolism were compared to published data of other investigators. At this time, there have not been adequate data available on the time-course of blood PCE levels in rats to allow one to evaluate the accuracy PBPK model predictions. No one has published and validated a PBPK model

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for volatile organic chemicals (VOCs), including PCE, in the dog, despite the widespread use of this animal in pharmacokinetic and toxicology studies.

The primary objective of the current study, therefore, was to evaluate the ability of a PBPK model for PCE to forecast the kinetics of PCE in the rat and dog. The ability of the model to predict blood PCE levels in animals given different doses by different routes of exposure was also evaluated, by comparison of experimental blood concentration time courses with model simulations.

METHOD

Male beagle dogs (5-10 kg), obtained from Marshall Farms (North Rose, NY), and male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories (Raleigh, NC), were employed in these studies. The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 h. Rats 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 7-day acclimation period, at which time they were approximately 12 weeks old. Dogs were housed in runs and were fed Purina Dog Chow® during a 14-day acclimation period. Dogs were used when they were between 6-9 months of age and in a weight range of 6-15 kg. Solvent exposures were initiated between 1000 and 1200 h each day.

1,1,2,2-Perchloroethylene (PCE) (tetrachloroethylene) of 99%+ purity was obtained from Aldrich Chemical Co. (Milwaukee, WI). The purity of the chemical was verified by gas chromatography.

An indwelling carotid arterial cannula was surgically implanted into rats and dogs the day before ia administration of PCE. An indwelling jugular vein cannula was also implanted into all the test animals for serial blood sampling. 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) and xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). Dogs were anesthetized with 50 mg/ml Nembutol. The cannulated animals were allowed freedom of movement during the 24-h recovery period, with the cannulas protected from manipulation by exteriorization and taping at the back of the head. All animals employed in the kinetic studies were unanesthetized. PCE, in doses of 1, 3 or 10 mg/kg, was administered as a single bolus orally or ia using polyethylene glycol 400 (1 ml/kg bw) as a vehicle. The ia injections were conducted through the carotid arterial cannula. Oral doses were administered by use of a ball-tipped gavage needle for rats and a Teflon® tube for dogs. Food was withheld during the 18-h recovery period before dosing.

Serial 20-μl blood samples were collected from the indwelling jugular vein cannula at intervals up to 96 h following dosing. While rats exhibited only slight neurobehavioral effects following ia administration of PCE, dogs receiving the 10 mg/kg ia dose demonstrated pronounced central nervous system (CNS) depression. Dog ia data are therefore presented only for the 1 and 3 mg/kg doses. PCE concentrations in the blood of dogs given 1 mg/kg orally rapidly declined below the limit of detection. Therefore, the data for this group were not plotted nor used to calculate pharmacokinetic parameters.

PCE concentrations in the blood samples were analyzed by headspace gas chromatography (GC), according to the method developed by this laboratory (Chen et al., 1993). The blood samples were transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal. Each sample vial was then placed into the autosampler unit of a GC. The limit of detection for PCE was approximately 1 ng, or 6.7 parts of PCE per billion parts of air in the sample vial.

A Sigma model 300 GC equipped with an electron capture detector and a HS-6 headspace sampler (Perkin Elmer Co., Norwalk, CT) was used for the analysis of the halocarbon. Analyses were carried out on stainless-steel columns (182 cm x 0.317 cm) packed with 10% FFAP (Alltech Associates, Deerfield, IL). The GC operating conditions were: headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; flow rate for argon/methane carrier gas, 60 ml/min. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column. The aforementioned conditions resulted in vaporization of the halocarbon in the sample vial, since PCE was heated to a temperature slightly below its boiling point. The vial was subsequently pressurized and automatically vented into the GC.

The disposition of PCE in the rat and the dog was predicted using a physiologically-based pharmacokinetic (PBPK) model for po and ia administration (Fig 1). It was similar to PBPK models previously developed (Angelo and Pritchard, 1984; Ramsey and Andersen, 1984) for other VOCs, in that it provided for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood-flow-limited organ representations. The current model differed primarily in that it included a greater number of tissue compartments. For oral administration, the model had a gastrointestinal (GI) compartment, with its rate of blood flow and oral absorption rate (Ka) dictating uptake of the PCE. Values in our laboratory in the male Sprague-Dawley rat were employed for tissue volumes (Manning et al.,   ), blood flows (Delp et al., 1991) and alveolar ventilation (Dallas et al., 1991b). *In vivo* tissue:blood partition coefficients were calculated from tissue concentration-time course data following po

administration to rats and dogs (Dallas et al., 1993), using the area method (Gallo et al., 1987). The metabolic parameters K_m and V_{max}, and blood:air coefficients were estimated from the observed data by nonlinear regression analysis. The value for K_a was estimated from oral data by fitting of the blood and liver concentration versus time data for both species. Tissue volumes and blood flows for the dog were obtained from the literature. Sources of dog tissue volumes were as follows: liver, heart, lung, skeletal muscle, and blood (Andersen, 1970), kidney (Spector, 1956), and fat (Sheng and Huggins, 1971). Sources of blood flows in the dog were as follows: liver (Liang et al., 1982), kidney, muscle, and brain (Humphrey and Zins, 1983), cardiac output (Andersen, 1970), fat (Nagasaka et al., 1976), and heart (Liard et al., 1982). Values for alveolar ventilation in the dog were taken from the publication by Andersen (1970).

The blood concentration versus time data were evaluated by the Lagran computer program (Rocci and Jusko, 1983) for the assessment of the appropriate pharmacokinetic model and calculation of relevant pharmacokinetic parameters. The area under the blood concentration-time curve (AUC) was determined from the time of administration to infinity. Bioavailability was calculated by AUCpo/AUCia. Total body clearance was determined as the dose divided by the blood AUC in each species. The maximum concentration of PCE reached in blood and tissues (Cmax) and the time after dosing that it occurred (Tmax) were determined by observation of the available data points. The terminal elimination half-life (t½) was determined according to the formula: $0.693/\beta$, where β is the terminal elimination rate constant. The statistical significance of differences in pharmacokinetic parameters for different species, doses, and routes of administration was determined by Student's test.

Differential mass balance equations, incorporating the parameters listed in Table 1, that describe the transport of PCE in the rat and dog were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted PCE concentrations over time.

RESULTS

Pharmacokinetic parameter estimates for the different doses and routes of administration are shown in Table 2 for rats and Table 3 for dogs. Following oral administration of PCE to rats, maximum blood concentrations (Cmax) were achieved in approximately 20 to 40 min for all three doses. Increases in AUCs were slightly more than proportional to increases in dose for both the ia and po groups, although AUC did not vary as a function of route of administration. Clearance diminished somewhat with increase in dose in the ia- and po-dosed animals. The t½ was significantly longer in the po-dosed rats than in those receiving PCE by ia injection at the 3 ($p<0.05$) and 10 mg/kg ($p<0.01$) dosage-levels. Half-life increased significantly with increase in dose in both the ia and po groups. PCE was well absorbed from the GI tract, as manifest by high bioavailability values.

Unfortunately, it was difficult to evaluate the dose-dependency of PCE kinetics in dogs, due to the inability to obtain complete data sets for each exposure route over the range of doses given. There was a significant increase in the t½ with increase in dose from 1 to 3 mg/kg in dogs receiving PCE by ia injection, but no increase in t½ with increase in the po dose from 3 to 10 mg/kg. The t½ values at the one dose (3 mg/kg) for which ia and po data were available were not significantly different. As was seen in rats, clearance decreased as the increase in the ia dose given to dogs. Conversely, clearance increased with

oral dose increased from 3 to 10 mg/kg, as the AUC did not increase in proportion to dose in these two groups (Table 3).

Marked species differences in PCE pharmacokinetics were apparent upon comparison of kinetic parameters in Tables 2 and 3. For corresponding doses and routes of administration, the AUC in dogs was approximately twice that observed in rats at 1 and 3 mg/kg. Half-life values were significantly longer (p<0.001) in dogs, except at the highest (10 mg/kg) po dose. The Cmax achieved in dogs was approximately 3 times that in rats for equivalent ia and po doses, with the exception of the 10 mg/kg po dose. Bioavailability of PCE was essentially 100% in dogs receiving 3 mg/kg orally.

Predictions of PCE blood concentrations over the time following ia injection are compared to experimental (i.e., observed) values for the three doses of PCE in the rat in Fig 2. Over the 10-fold range of doses employed, concentrations of PCE in the venous blood were well predicted by the model. Blood PCE concentrations during the initial phase of rapid decline in the blood (i.e., redistribution phase) were slightly overpredicted. Model-generated concentrations were in close agreement, however, with the observed blood levels during the terminal elimination phase for all three doses.

Observed and predicted concentrations of PCE in the venous blood of dogs are shown after ia administration of PCE in Fig 3. Blood PCE concentrations were overpredicted during the initial rapid elimination phase following injection of 1 and 3 mg/kg. The difference between simulated and measured blood levels was consistent and relatively modest during the terminal elimination phase for the 3 mg/kg dose. The terminal elimination phase could not be accurately determined experimentally for the 1 mg/kg dose, as blood levels fell below the limit of detection after 20 h.

Model-generated and observed blood PCE concentrations were compared following oral administration of three doses of PCE to the rat in Fig 4. For all three doses, peak blood levels were slightly underpredicted. Simulated and observed values were in close agreement during the initial part of the rapid elimination phase. Blood concentrations during the latter portion of the rapid elimination phase and the initial part of the terminal elimination phase were somewhat underpredicted. There was generally good agreement during the remainder of the terminal phase in the 3 and 10 mg/kg groups. Unfortunately, blood PCE concentrations dropped below the limit of detection in the 1 mg/kg rats, so that the terminal elimination phase could not be defined experimentally.

Upon oral administration of PCE to the dog, model-predicted blood PCE concentrations were somewhat higher than the observed values during the rapid elimination phase (Fig 5). Blood PCE concentrations were overpredicted during the terminal elimination phase in the 10 mg/kg dogs, but well predicted in the 3 mg/kg animals.

DISCUSSION AND CONCLUSIONS

Limited information is available on the systemic absorption of PCE from the gastrointestinal (GI) tract (ATSDR, 1992). Pegg et al. (Pegg et al., 1979) gave male Sprague-Dawley rats 1 or 500 mg ^{14}C-PCE/kg bw in corn oil by gavage. Measurements of recovered radioactivity over 72 h following dosing indicated that absorption of both doses of PCE was virtually complete. Similar findings in mice (Schumann et al., 1980) and rats (Frantz and Watanabe, 1983) have been reported. High bioavailability (F) values were calculated for each dose of PCE given orally to rats in the current study (Table 2). Oral absorption was also extensive in the beagle dog, in that the F value for the 3 mg/kg dose was found to be about 1. Peak blood PCE levels were seen in rats 21 to 37 min post dosing. Pegg et

al. (1979) did not observe peak concentrations until approximately 1 h after oral dosing. These researchers, however, employed a corn oil vehicle. Corn oil has been shown to significantly delay oral absorption of VOCs, by acting as a reservoir for the lipophilic chemicals in the GI tract (Withey et al., 1983; Kim et al., 1990).

There is a paucity of blood concentration time-course data, which delineates the systemic uptake and elimination of PCE. Only one blood PCE concentration versus time profile was found in the literature (ATSDR, 1992). They took serial blood samples via a jugular cannula for up to 36 h from male Sprague-Dawley rats given 500 mg/kg of PCE orally. Limited analytical sensitivity apparently did not allow sampling at later time-points, or description of the blood profile in rats receiving a 1 mg/kg oral dose. It was reported that disappearance of PCE from the blood was appeared to be monophasic and followed first-order kinetics. Although they apparently did not monitor blood levels long enough to define the terminal elimination phase, their $t\frac{1}{2}$ values of 7.1 to 7.4 h are comparable to the $t\frac{1}{2}$ of 7.8 h for the 3 mg/kg po rats in the present study. The only other pharmacokinetic parameters estimated in this study were elimination rate constants for the blood and exhaled breath. Frantz and Watanabe (1983) reported a $t\frac{1}{2}$ of 7.1 h for pulmonary elimination of PCE by male Sprague-Dawley rats ingesting about 8.1 mg/kg of the chemical in drinking water over a 12-h period.

The data sets obtained in the current investigation allow a more comprehensive assessment of the pharmacokinetics of oral PCE than has previously been possible, since the sets include detailed time-course data for three doses and two routes of administration. Increases in AUCs were somewhat more than proportional to increases in dose in rats receiving PCE orally and by ia

injection (Tables 2 and 3). As would be expected, clearance diminished slightly with increase in dose in the rats. Half-life increased significantly with increase in dose in these animals. These findings are indicative of the onset of saturation of elimination processes in this dosage range (i.e., 1 to 10 mg/kg). It has been demonstrated previously that PCE metabolism was a saturable, dose-dependent process in male Sprague-Dawley rats, but the oral doses given (i.e., 1 and 500 mg/kg) varied markedly (Pegg et al., 1979). It has been theoretically proposed (Andersen, 1987) and demonstrated by observed measurements (Lee et al., 1991) that % elimination of VOCs via exhalation is independent of dose and blood concentration. Thus, respiratory elimination of PCE and other VOCs should not be saturable. Thus, the aforementioned findings in the present study, of decreasing clearance and increasing half-life in the 1 to 10 mg/kg dosage range, are apparently the result of the onset of metabolic saturation. Male Sprague-Dawley rats which ingested 8.1 mg/kg of ^{14}C-PCE in their drinking water over a 12-h period exhaled 88% of the dose as unchanged PCE (28). Only 7.2% and 1.7% of the dose of PCE was eliminated in the urine and feces, respectively. Thus, the rat appears to have a quite limited capacity to metabolize PCE. Unfortunately, it was not possible in our study to obtain data sets for the 1 mg/kg po and 10 mg/kg ia groups of dogs, so it is not possible at this time to make definitive statements about the influence of dose and exposure route on the pharmacokinetics of PCE in dogs.

There were pronounced differences in the kinetics of ingested PCE in rats and dogs. Rats exhibited substantially lower C_{max} and AUC values than dogs receiving the same dose of PCE by the same route. Clearance of PCE from the bloodstream was significantly slower in rats and t½s were longer (Tables 2 and 3). It has been predicted that clearance should be greater in smaller species,

since it increases as a fractional power of body weight (Andersen, 1987). The major route of PCE elimination by rats (Pegg et al., 1979; Frantz and Watanabe, 1983) and by humans (Ohtsuki et al., 1983) is exhalation of the parent compound. No data apparently exist for dogs, although a PBPK model was recently used to predict significantly greater PCE elimination by dogs than by rats (Dallas et al., 1991b). The blood:air partition coefficient is one important factor that governs PCE exhalation. The parameter is smaller in rats (19.8) than in dogs (40.5), which favors elimination in the expired air of rats (Table 1). Two other factors which enhance respiratory elimination of PCE and other VOCs, namely pulmonary blood flow and respiratory rate, are also markedly higher in the rat than the dog. Lastly, the extent of PCE metabolism also appears to be greater in rats. It has been shown that mice metabolize a substantially percentage of administered doses of PCE than do rats (Schumann et al., 1980). Ohtsuki et al. (1983) demonstrated that humans have a very limited capacity of metabolize PCE. Although there are apparently no direct measurement studies of PCE metabolism by dogs, a PBPK model employed by Dallas et al. (1993) forecast significantly less metabolism of PCE by dogs than rats. Thus, it is predictable that bioavailability of PCE is greater in the larger of the two species.

PBPK model simulation of the time-course of blood PCE concentrations in rats were in close agreement with direct measurements of the chemical for all 3 doses given by both routes of administration. 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 certain toxic effects. Others have developed PBPK models for PCE, but the models have primarily been used to forecast exhaled breath levels and metabolite formation (Ward et al., 1988; Bois et al., 1990; Travis, 1987; Chen and Blancato, 1987).

Simulations of blood and tissue concentrations of PCE over time have not conducted, largely due to a lack of empirical data to use in parameter determination and valid action of model predictions.

The accuracy of the present model, in predicting the uptake and elimination of PCE in the blood of the male Sprague-Dawley rat, we believe is due both to the model structure and to accurate *in vivo* model input parameters. The current model is similar to models previously developed by Angelo and Pritchard (Angelo and Pritchard, 1984) and Ramsey and Anderson (1984) for other VOCs. Each model provides for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood flow-limited organ representations. Our model differs from the others primarily in that it has a greater number of individual tissue compartments, and thus more closely represents the anatomy of the animal. In addition, the physiological and biochemical input parameters were not taken from the literature or *in vitro* assays, but measured in the test animal (i.e., the male Sprague-Dawley rat). As described in the Experimental section, values measured in our laboratory in Sprague-Dawley rats were utilized for tissue volumes, cardiac output and tissue blood flows, and alveolar ventilation. *In vivo* partition coefficients were determined in a prior investigation in which PCE was administered to rats by ia injection (Dallas et al., 1993), while metabolic rate constants were calculated from liver concentration versus time data from the same study. Hattis et al. (Hattis et al., 1990) found that there were appreciable differences in the prediction of PCE-PBPK models of different researchers, which was attributed largely to variance in input parameters, notably metabolic rate constants.

The present report apparently represents the first effort to simulate the kinetics of a VOC in the dog, by use of a PBPK model. The model generally

overpredicted blood PCE concentrations somewhat following po and ia administration of the halocarbon. This difference from measured blood levels was very likely due to the inaccuracy of certain input parameters, rather than incorrect model structure. As for the rat, it was possible to accurately determine some *in vivo* parameters from the dog blood concentration time-course data (i.e., blood:air and tissue:blood partition coefficients and metabolism constants). It was necessary to obtain other input parameters from the literature. Citations were found which spanned many years, a variety of techniques and different types of dogs. Where possible, values were taken from publications employing state-of-the-art techniques (e.g., measurement of tissue blood flows by a radiolabeled microsphere technique) in unanesthetized beagles. Where more than one investigator employed the same technique in beagles, the median value was used. Nevertheless, there was uncertainty about the validity of the literature values which were selected. Measurement and utilization of precisely determined physiological parameters in the dog should improve the accuracy of PBPK model predictions of the disposition of PCE and other VOCs in that species.

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Table 1
Parameters Used in the Physiologically Based
Pharmacokinetic Model for PCE in the Rat and the Dog

Parameter	Rat	Dog
Body Weight (kg)	0.340	10

Tissue volumes (ml)	*Percentage of Body Weight*	
liver	3.4	4.2
kidney	0.8	0.6
fat	5.0	15.2
heart	0.3	1.1
lung	0.4	0.7
muscle	35.4	46.8
brain	0.6	0.8
blood	7.4	8.2
rest of body	46.8	22.4

Alveolar ventilation	1.54 (ml/min·g) BW(g)$^{0.75}$	2.58 (ml/min·g) BW(g)$^{0.75}$
Cardiac output	1.57 (ml/min·g) BW(g)$^{0.75}$	1.05 (ml/min·g) BW(g)$^{0.75}$

Blood flows	*Percentage of Cardiac Output*	
liver	15.7	5.1
kidney	13.1	10.1
fat	6.6	5.0
heart	4.7	3.4
lung	100	100
muscle	26.1	40.6
brain	2.2	3.1
blood	100%=1.57 (ml/min·g) BW(g)$^{0.75}$	100%=2.05 (ml/min·g) BW(g)$^{0.75}$
rest of body	31.5	32.7

Partition Coefficients		
Blood:air	19.6	40.5
Fat:blood	152.5	63.2
Lung:blood	2.5	1.0
Liver:blood	5.2	1.6
Muscle:blood	3.0	1.9
Brain:blood	4.4	2.7
Heart:blood	2.7	1.7
Kidney:blood	4.4	1.5
Rest of body:blood	3.0	1.9

Metabolism constants		
Vmax (μg/min)	0.15	0.85
Km (μm/ml)	0.019	0.023

Absorption constant		
Ka(min^{-1})	0.025	0.34

TABLE 2

PHARMACOKINETIC PARAMETERS ESTIMATES FOR THE RAT[1]

Dose (mg/kg)	Route[2]	N	Area Under Curve (µg.min/ml)	Biological Half-Life (h)	Cmax (µg/ml)	Tmax (min)	Bioavailability (F)	Clearance (ml/min/kg)
1	PO	6	27.9 ± 1.7	3.2 ± 0.2	0.2 ± 0.0	20.8 ± 2.0	0.96	36.7 ± 2.8
1	IA	6	29.2 ± 2.3	3.8 ± 0.5	0.6 ± 0.2	1.2 ± 0.4		34.8 ± 2.4
3	PO	5	100.5 ± 10.8	7.8 ± 1.0	0.4 ± 0.1	37.0 ± 23.9	0.94	30.1 ± 2.9
3	IA	5	107.3 ± 16.8	6.0 ± 1.2	1.3 ± 0.2	2.8 ± 1.6		28.5 ± 4.3
10	PO	6	321.4 ± 27.6	15.5 ± 1.5	1.6 ± 0.2	22.5 ± 4.8	0.82	32.5 ± 3.3
10	IA	4	391.6 ± 36.4	7.5 ± 1.7	4.1 ± 0.4	6.0 ± 0.8		26.2 ± 2.5

[1]Each value represents the mean ± s.e.
[2]PO represents oral administration and IA intraarterial administration

TABLE 3

PHARMACOKINETIC PARAMETERS ESTIMATES FOR THE DOG[1]

Dose (mg/kg)	Route[2]	N	Area Under Curve (μg.min/ml)	Biological Half-Life (h)	Cmax (μg/ml)	Tmax (min)	Clearance (ml/min/kg)
1	IA	4	50.3 ± 11.3	6.4 ± 0.4	0.6 ± 0.2	5.5 ± 1.7	23.0 ± 4.7
3	PO	4	214.2 ± 25.4	20.8 ± 2.2	1.2 ± 0.2	15.0 ± 0.0	14.6 ± 1.8
3	IA	3	214.0 ± 17.4	21.6 ± 1.6	4.5 ± 1.0	2.3 ± 0.7	14.3 ± 1.1
10	PO	8	451.1 ± 51.2	16.6 ± 1.4	1.9 ± 0.1	31.0 ± 9.9	25.0 ± 3.9

[1]Each value represents the mean ± s.e.
[2]PO represents oral administration, and IA intraarterial administration

FIGURE LEGENDS

1. Diagram of the physiological pharmacokinetic model used to predict the uptake and elimination of PCE in venous blood following ia or oral administration to rats and dogs. The parameters used for input into the model are included in Table 1.

2. PCE concentrations in the venous blood of rats over time following ia administration of 1, 3 or 10 mg/kg of PCE. Each symbol represents the observed mean value for 4 rats, while the lines represent PBPK model-predicted values.

3. PCE concentrations in the venous blood of dogs over time following ia administration of 1 or 3 mg/kg of PCE. Each symbol represent the observed mean value for 3 dogs, while the lines represent PBPK model-predicted values.

4. PCE concentrations in the venous blood of rats over time following po administration of 1, 3 or 10 mg/kg of PCE. Each symbol represents the observed mean value for 5 rats, while the lines represent PBPK model-predicted values.

5. PCE concentrations in the venous blood of dogs over time following po administration of 3 or 10 mg/kg of PCE. Each symbol represents the observed mean value for 3 dogs, while the lines represent PBPK model-predicted values.
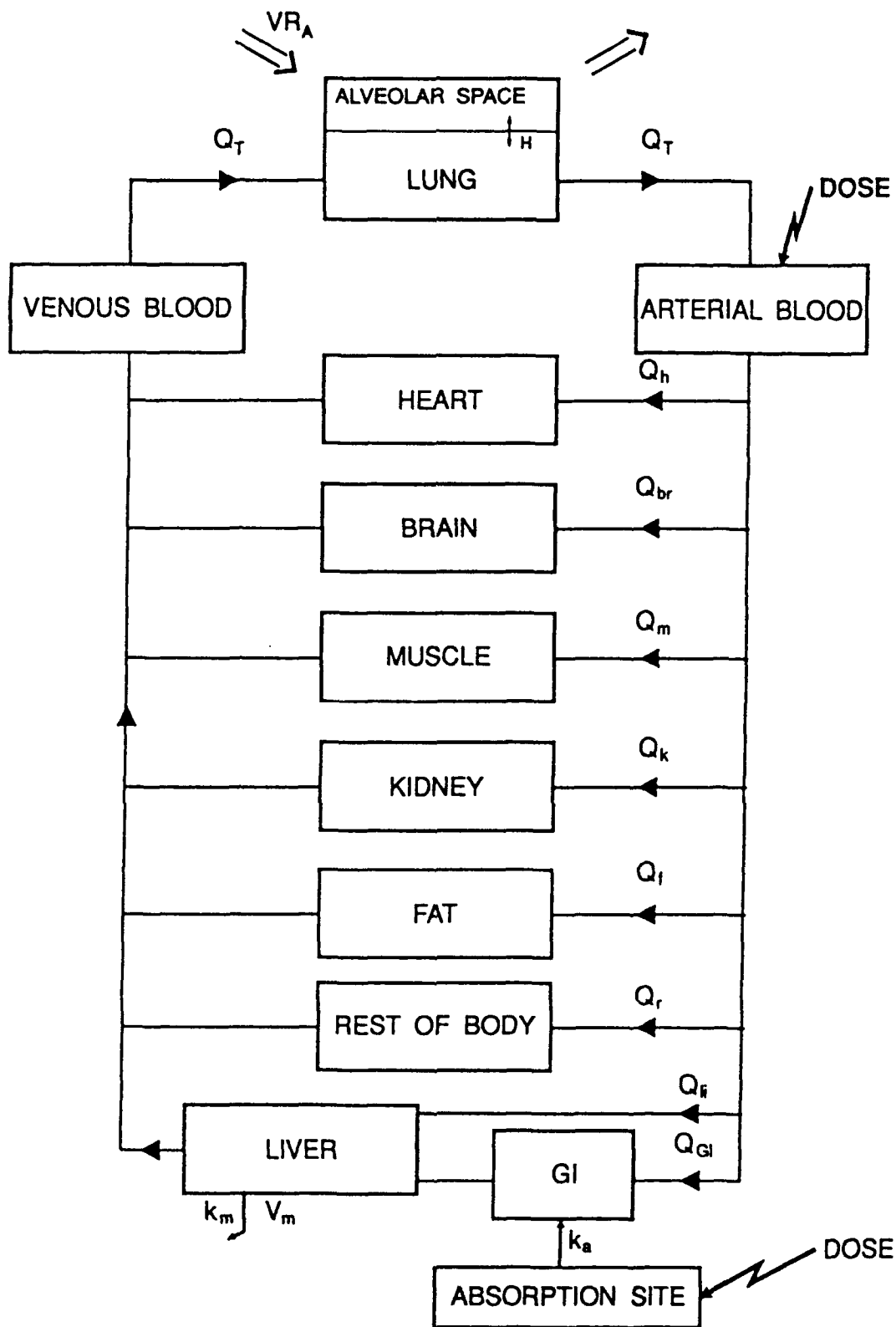
A Physiologically-Based Pharmacokinetic Model Useful in Prediction
of the Influence of Species, Dose and Exposure Route
on Perchloroethylene Pharmacokinetics

C.E. Dallas, X.M. Chen, S. Muralidhara, P. Varkonyi,
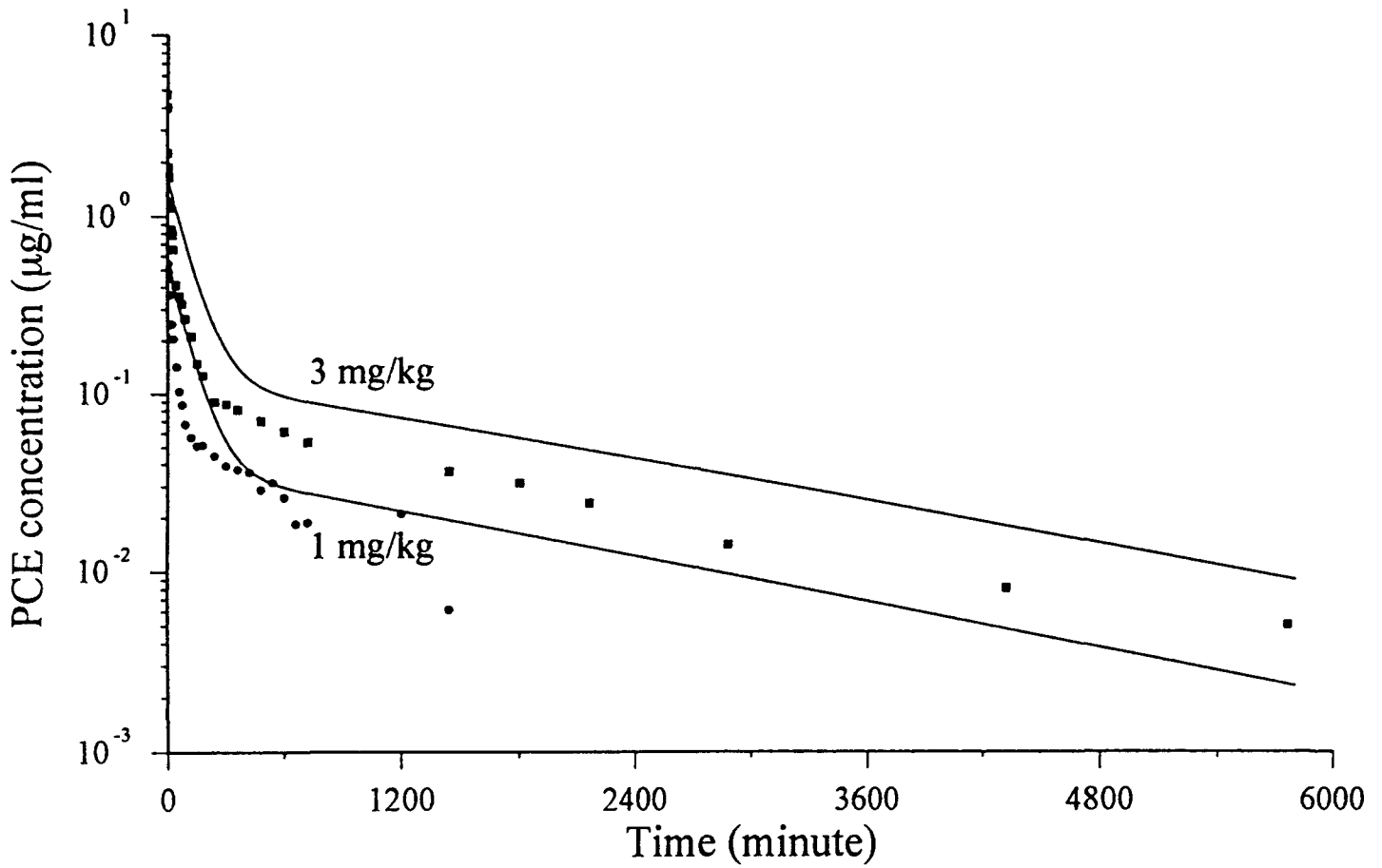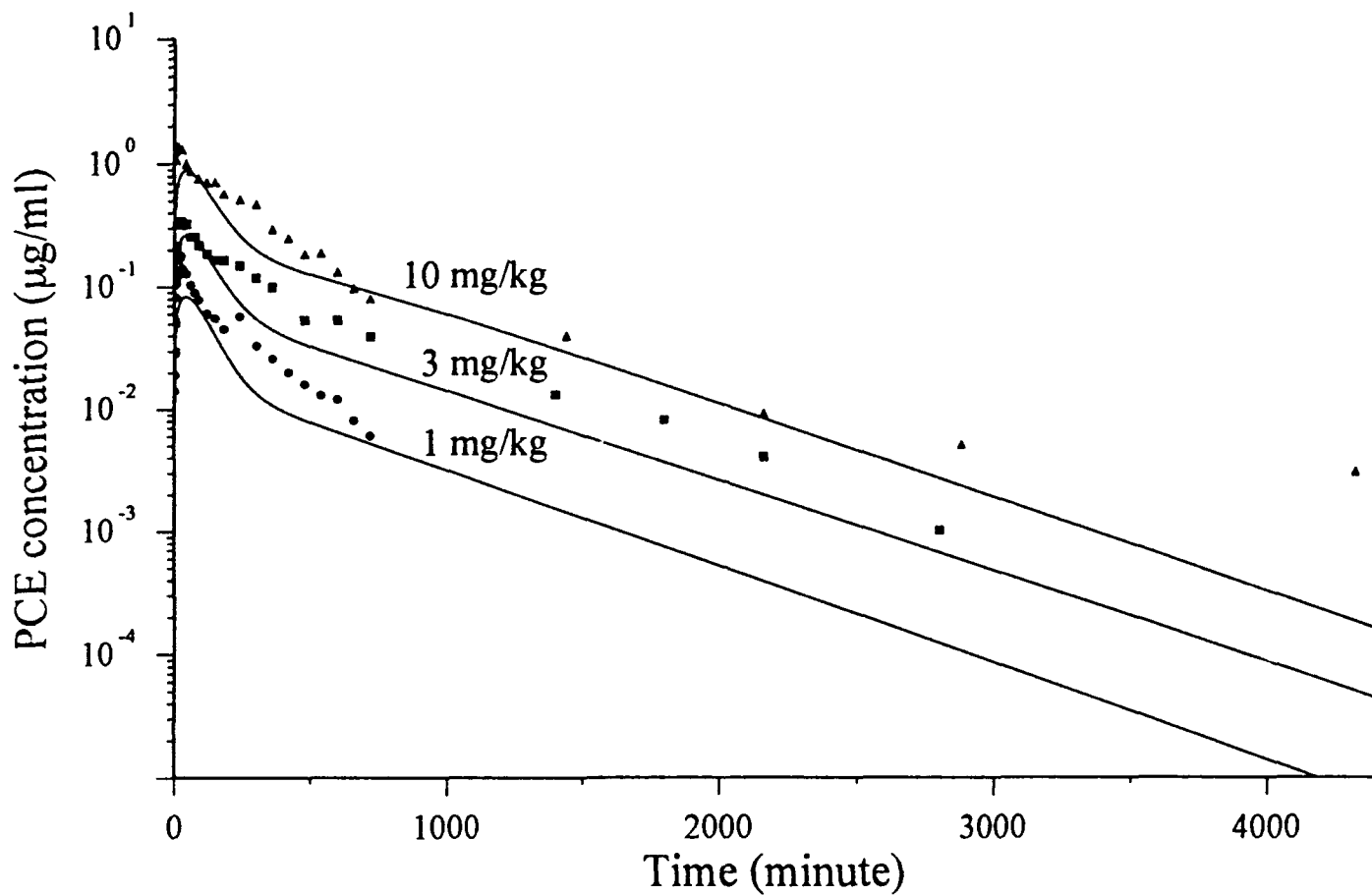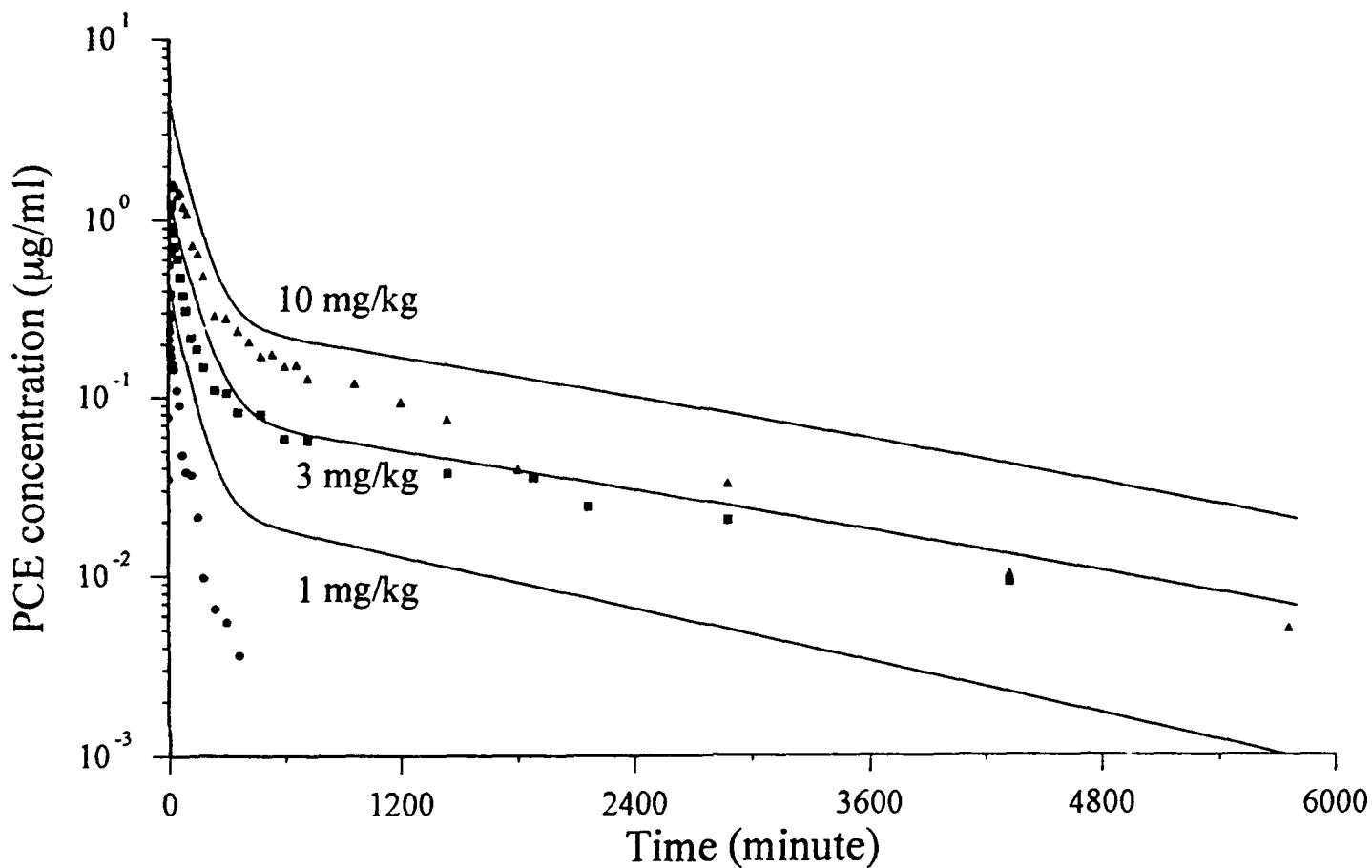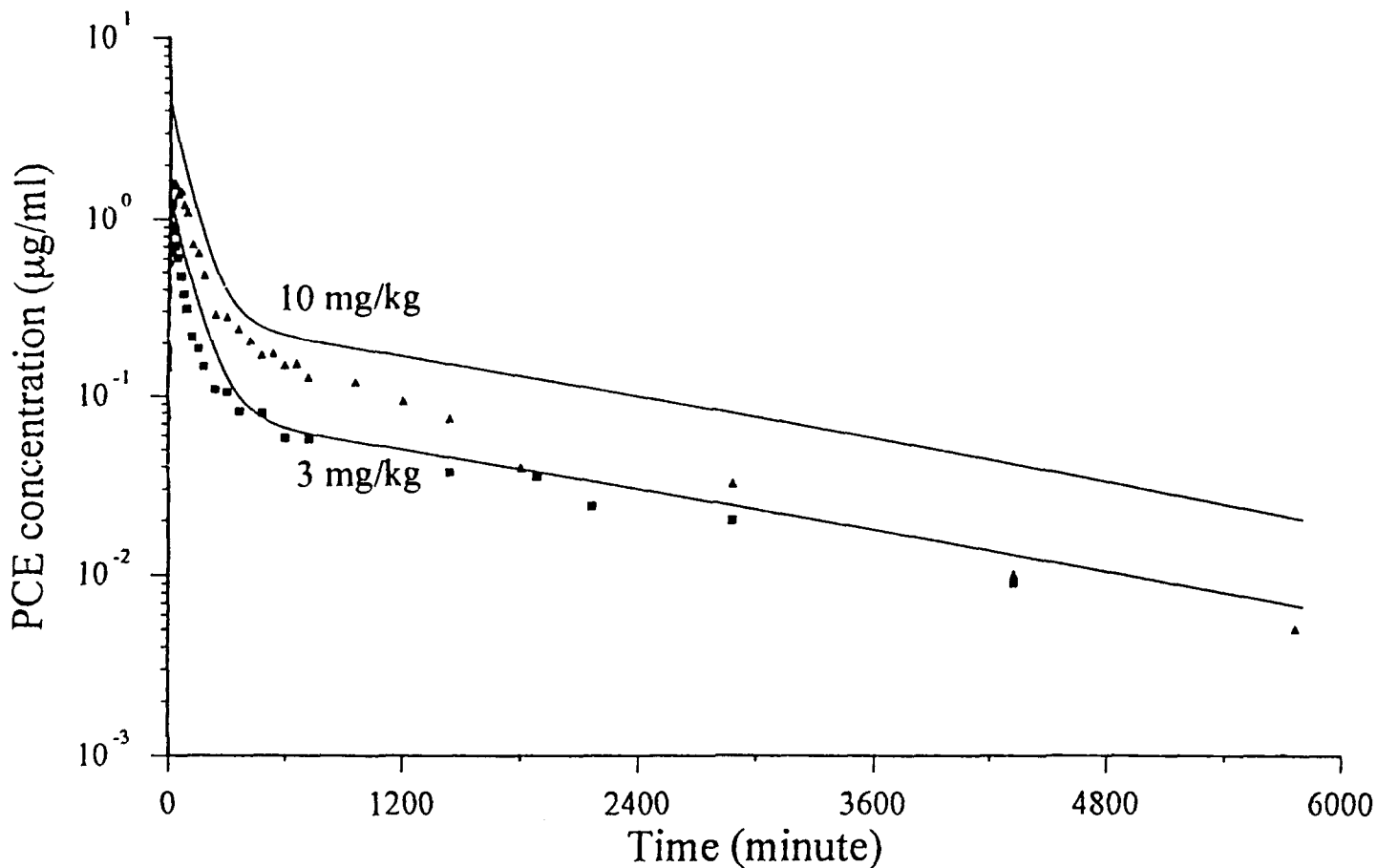R. Tackett, & J.V. Bruckner

FIGURE 1
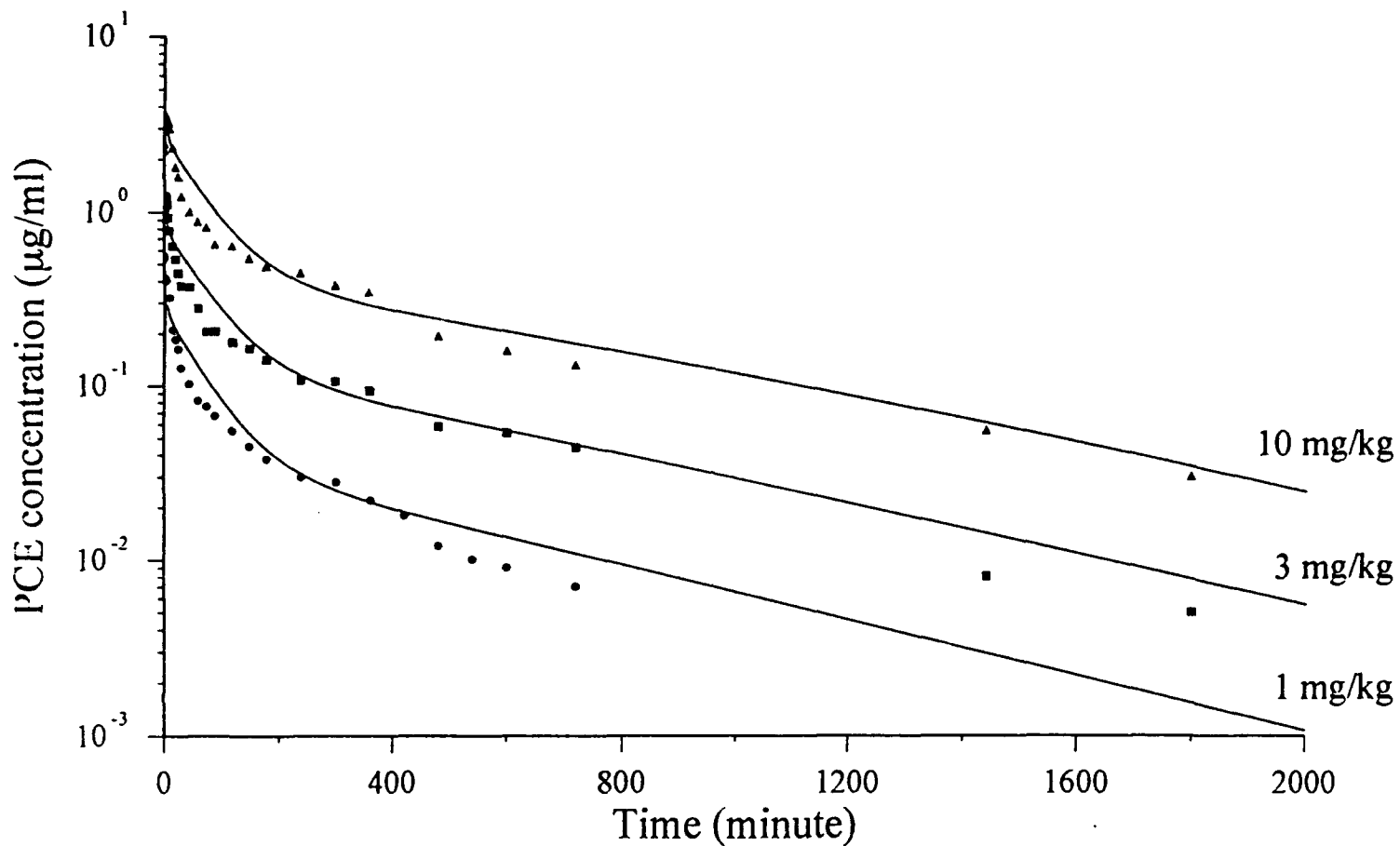
A Physiologically-Based Pharmacokinetic Model Useful in Prediction
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C.E. Dallas, X.M. Chen, S. Muralidhara, P. Varkonyi,
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FIGURE 2

A Physiologically-Based Pharmacokinetic Model Useful in Prediction
of the Influence of Species, Dose and Exposure Route
on Perchloroethylene Pharmacokinetics

C.E. Dallas, X.M. Chen, S. Muralidhara, P. Varkonyi,
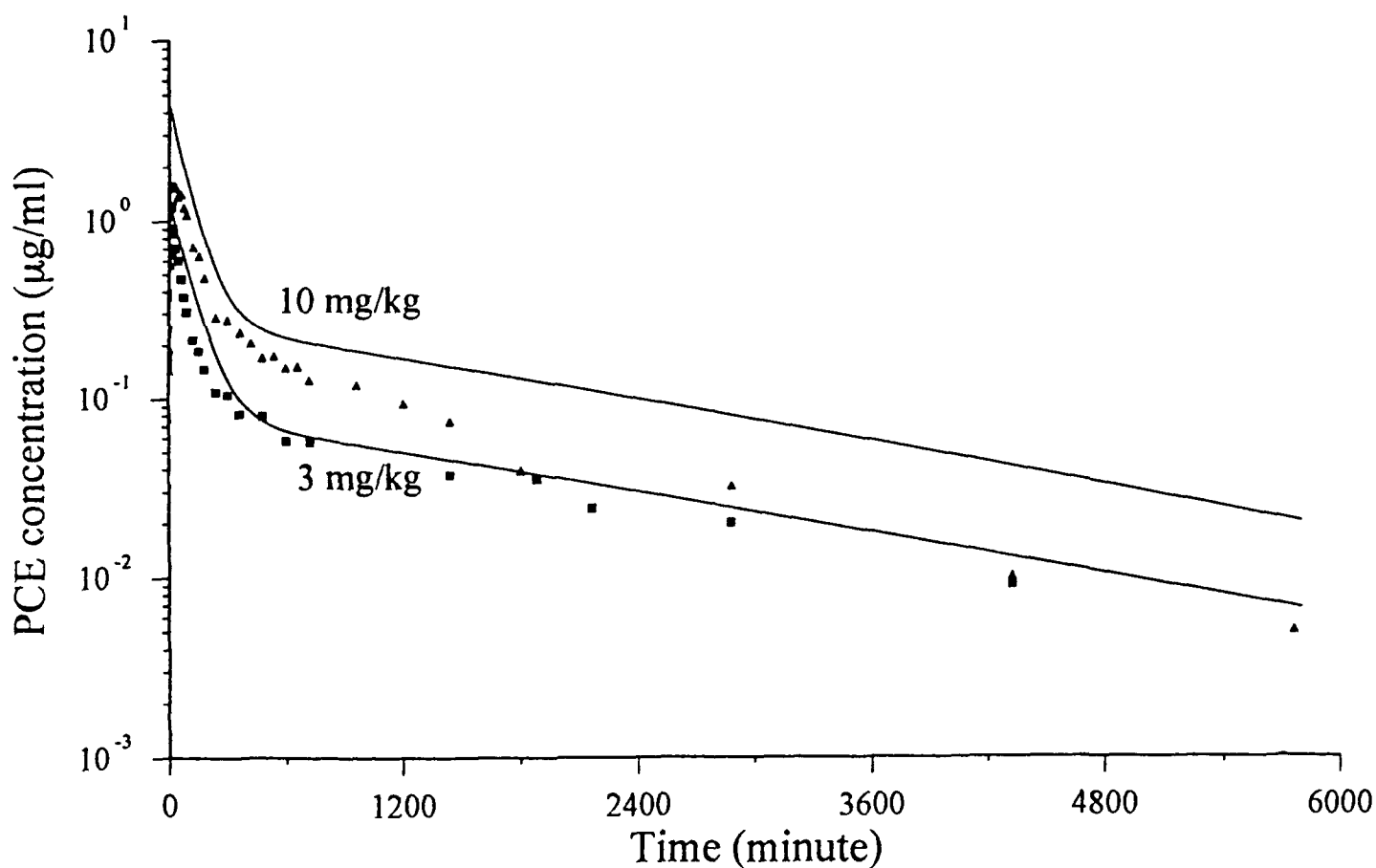R. Tackett, & J.V. Bruckner

FIGURE 3

A Physiologically-Based Pharmacokinetic Model Useful in Prediction
of the Influence of Species, Dose and Exposure Route
on Perchloroethylene Pharmacokinetics

C.E. Dallas, X.M. Chen, S. Muralidhara, P. Varkonyi,
R. Tackett, & J.V. Bruckner

FIGURE 4

A Physiologically-Based Pharmacokinetic Model Useful in Prediction
of the Influence of Species, Dose and Exposure Route
on Perchloroethylene Pharmacokinetics

C.E. Dallas, X.M. Chen, S. Muralidhara, P. Varkonyi,
R. Tackett, & J.V. Bruckner

FIGURE 5

A Physiologically-Based Pharmacokinetic Model Useful in Prediction
of the Influence of Species, Dose and Exposure Route
on Perchloroethylene Pharmacokinetics

C.E. Dallas, X.M. Chen, S. Muralidhara, P. Varkonyi,
R. Tackett, & J.V. Bruckner

FIGURE 5

A Physiologically-Based Pharmacokinetic Model Useful in Prediction
of the Influence of Species, Dose and Exposure Route
on Perchloroethylene Pharmacokinetics

C.E. Dallas, X.M. Chen, S. Muralidhara, P. Varkonyi,
R. Tackett, & J.V. Bruckner

FIGURE 5

APPENDIX D

Use of Tissue Disposition Data from Rats and Dogs to Determine Species
Differences in Input Parameters for a Physiological Model for Perchloreothylene.
In Press, *J. Toxicology and Environmental Health*, 1994.

Use of Tissue Disposition Data from Rats and Dogs

to Determine Species Differences in Input Parameters

for a Physiological Model for Perchloroethylene

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ABSTRACT

Use of Tissue Disposition Data from Rats and Dogs to Determine Species Differences in Input Parameters for a Physiological Model for Perchloroethylene. DALLAS, C.E., CHEN, X.M., MURALIDHARA, S., VARKONYI, P., TACKETT, R.L., AND BRUCKNER J.V. (1993) Environmental Research 0, 00-00.

Tissue disposition of perchloroethylene (PCE) was determined experimentally in two mammalian species of markedly different size, in order to derive input parameters for the development of a physiologically-based pharmacokinetic (PBPK) model, which could forecast the disposition of PCE in each species. Male Sprague-Dawley rats and male beagle dogs received a single bolus of 10 mg PCE/kg bw in polyethylene glycol 40C by gavage. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, perirenal fat and blood were taken for up to 72 hr following PCE administration. Blood and tissue PCE concentrations were analyzed using a gas chromatography headspace technique. Dogs exhibited considerably longer tissue and blood half-lives than did rats. The dogs also exhibited larger area under tissue concentration versus time curves (AUC) for all tissues except the liver. Whole body clearance of PCE in the rat was greater than in the dog. Model simulations indicated this could be attributed to more rapid and extensive PCE exhalation and metabolism by the rat. The *in vivo* blood:air partition coefficient determined for rats was similar to an *in vitro* value reported by Gargas *et al.* (1989). *In vivo* tissue:blood partition coefficients, however, were 1.4 to 2.8 times greater than published *in vitro* values. The PCE *in vivo* blood:air partition coefficient for the dog was twice that of the rat, but tissue:blood partition coefficients were 1.5 to 3.0 times greater in the rat than the dog. These results demonstrated the existence of significant differences in partition coefficients in two species commonly used in toxicity testing. The

PBPK model was shown to have utility in predicting the impact of metabolism and exhalation on pharmacokinetics of PCE in different species of widely differing size.

Introduction

Physiologically-based pharmacokinetic (PBPK) models are being increasingly used in health risk assessments of chemicals, particularly in interspecies extrapolations of pharmacokinetic and toxicologic data. By use of physiological and metabolic parameters for a particular species, accurate predictions of chemical concentrations in the blood and tissues of different that species are feasible with PBPK models. Similarities in the anatomy and physiology of mammals make scaling from one species to another possible, when experimentally derived physiological data are not available for a species of interest (Dedrick, 1973; Boxenbaum, 1984; Travis, 1987).

Tetrachloroethylene, or perchloroethylene (PCE), a commonly used volatile organic compound (VOC), has frequently received the attention of PBPK modelers. Ward *et al*. (1988) developed a PBPK model for PCE with four tissue compartments. This model adequately predicted empirical data other researchers compiled in studies of mice (Schumann *et al*., 1980; Buben and O'Flaherty, 1985), rats (Pegg *et al*., 1979), and humans (Stewart *et al*., 1961; Ikeda *et al*., 1972; Fernandez *et al*., 1976; Monster *et al*., 1979). The observed data consisted of PCE concentrations measured in exhaled breath over time and various measures of PCE metabolism. A similar approach was used by Travis (1987) for forecasts of PCE kinetics in rats and humans, and by Chen and Blancato (1987) for mice, rats and humans. There have been descriptions of the utility of PBPK models in predicting metabolite formation following PCE exposure of mice, rats, and humans (Bogen and McKone, 1988; Bois *et al*., 1990; Hattis *et al*., 1990). The focus of these studies was not to verify the accuracy of model predictions of concentrations of PCE or its metabolites in target tissues, but to evaluate the models' potential to simulate PCE metabolism, for use in cancer risk assessments. Tissue

concentrations were not employed in the development or validation of any of the aforementioned PBPK models for PCE. There have not been any PBPK models for PCE or other volatile organic chemicals (VOCs) published, in which the dog was a test species.

Tissue:blood partition coefficients are an important input parameter for PBPK models. These parameters describe the transfer of chemical between the blood and each of the tissue compartments included in the model. Ward *et al.* (1988), Bois *et al.* (1990) and Travis *et al.* (1987) all utilized partition coefficients derived using an *in vitro* vial equilibration technique (Gargas *et al.*, 1989). An *in vivo* approach to derive tissue:blood partition coefficients for PBPK models has been described (Gallo *et al.*, 1987), in which the tissue-concentration time-courses of the test chemical are employed. There has been little opportunity for the use of this procedure, because of the paucity of detailed tissue concentration versus time data for most VOCs, including PCE.

Therefore, the time-course of uptake, deposition and elimination of PCE in blood and seven tissues was determined in two species, and the data utilized to derive partition coefficients for a PBPK model for PCE. The rat and the dog were selected, in order to utilize dissimilar species commonly employed in toxicological and pharmacological testing. A PBPK model was used to account for differences in the kinetics of PCE in two species, by simulating metabolism and exhalation of PCE in each animal.

Methods

Male beagle dogs (6-15 kg) obtained from Marshall Farms (North Rose, NY), and male Sprague-Dawley rats (325-375 g) obtained from Charles River Laboratories (Raleigh, NC), were employed in these studies. The animals were maintained on

a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. Rats 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 7-day acclimation period, at which time they were approximately 12 weeks old. Dogs were housed in dog runs, allowed a 14-day acclimation period, and fed Purina Dog Chow®. Dogs used were between 6-9 months of age and in a weight range of 6-15 kg. Solvent exposures were initiated between 1000 and 1200 hr each day.

1,1,2,2-Perchloroethylene (PCE) (tetrachloroethylene) of 99%+ purity was obtained from Aldrich Chemical Co. (Milwaukee, WI). The purity of the chemical was verified by gas chromatography.

All animals employed in the tissue kinetic studies were unrestrained and unanesthetized. PCE was administered as a single bolus by gavage in a dose of 10 mg/kg, using polyethylene glycol 400 (1 ml/kg bw) as a vehicle. Groups of 3 dogs and 4 rats each were serially sacrificed (using pentobarbital and CO_2 to terminate dogs and rats, respectively) at the following times post dosing: 1, 5, 10, 15, 30 and 60 min, and 2, 4, 6, 12, 18, 36, 24, 30, 48 and 72 hr for rats, and 1, 4, 12, 24, 48 and 72 hr for dogs. Blood samples were obtained by cardiac puncture. Approximately 1-g samples of liver, kidney, brain, lung, heart, perirenal fat, and skeletal muscle were then quickly removed and placed into previously chilled 20-ml glass vials containing 2 ml of ice-cold saline and 8 ml of isooctane. These samples were analyzed by headspace gas chromatography using the method developed by Chen *et al.* (1993). Each tissue was homogenized for an established time-interval with a Ultra-Turrax SDT homogenizer (Tekmar Co., Cincinnati, OH). These times were kept as brief as possible, in order to minimize loss of the halocarbon by volatilization. Brain, liver and fat were the

most easily homogenized, requiring only 3-4 sec. Kidney, lung and heart required 5-8 sec. Skeletal muscle was the most difficult to homogenize, as it required 20 sec. The homogenates were then centrifuged at 1800 x g for 10 min at 4°C in capped vials. An aliquot of the isooctane layer was transferred to 8-ml headspace vials. These sample vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal.

A Sigma model 300 gas chromatograph (GC) equipped with a HS-6 head space sampler (Perkin Elmer Co., Norwalk, CT) was used for the analysis of the halocarbon. The GC was equipped with an electron capture detector. Analyses were carried out on stainless-steel columns (182 cm x 0.317 cm) packed with 10% FFAP (Alltech Associates, Deerfield, IL). The GC operating conditions were: headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; flow rate for argon/methane carrier gas, 60 ml/min. All analyses were conducted using a 20-μl aliquot of isooctane in the 8-ml headspace vials. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column. These conditions resulted in vaporization of the halocarbons in the sample vials, since PCE was heated to a temperature slightly below its boiling point. The vial was subsequently pressurized and vented into the GC. The limit of detection for PCE was approximately 1 ng, or 6.7 parts of PCE per billion parts of air in the sample vial.

Areas under concentration-time curves (AUCs) for blood and tissues were determined from the time of administration to infinity. Total body clearance in each species was calculated by dividing the dose of PCE by the blood AUC. The maximum PCE concentration reached in blood and tissues (Cmax) and the time after

dosing that it occurred (Tmax) were determined by observation of the available data points. The terminal elimination half-life ($t\frac{1}{2}$) was determined according to the formula: $0.693/\beta$, where β is the terminal elimination rate constant.

Metabolism and exhalation of PCE in the rat and the dog were predicted using a physiological-based pharmacokinetic (PBPK) model for oral administration of PCE (Fig. 1). The model was similar to PBPK models previously developed by Angelo and Pritchard (1984) and Ramsey and Andersen (1984) for other VOCs, in that it provided for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood-flow-limited organ representations. The current model differed primarily in that it included a greater number of tissue compartments. Values determined experimentally in this laboratory were employed for tissue volumes (Manning *et al.*, 1991), blood flows (Delp *et al.*, 1991) and alveolar ventilation (Dallas *et al.*, 1991) for the male Sprague-Dawley rat. *In vivo* tissue:blood partition coefficients were calculated from tissue and blood concentration-time data for the rat and the dog by the area method of Gallo *et al.* (1987). For the eliminating organs, nonlinear regression analysis was used to determine: (a) the blood:air and lung:blood partition coefficients from the measurements of PCE in the blood and lung; and (b) the liver:blood partition coefficient, K_m, and V_{max} from the liver and blood PCE data. The absorption rate constant (K_a) was estimated from the data by fitting of the blood and liver concentration versus time data for both species. Tissue volumes and blood flows for the dog were obtained from the literature. Sources of dog tissue volumes were as follows: liver, heart, lung, skeletal muscle, and blood (Andersen 1970), kidney (Spector, 1956), and fat (Sheng and Huggins, 1971). Sources of blood flows were as follows: liver (Liang *et al.*, 1982), kidney, muscle, and brain (Humphrey and Zins, 1983), cardiac output (Andersen, 1970), fat (Nagasaka *et al.*,

1976), and heart (Liard *et al.*, 1982). Values for alveolar ventilation in the dog were taken from the publication by Andersen (1970).

Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of PCE in the rat and the dog were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted PCE concentrations over time.

Results

Pharmacokinetic parameter estimates for oral administration of PCE to rats are included in Table 2, while blood and tissue PCE concentration versus time profiles are presented in Figures 2 and 3. After oral dosing, the Cmax was reached in 10 to 15 min for each non-lipoidal tissue, other than lung and muscle, which required 60 min. Uptake into the adipose tissue was quite slow (Fig. 3C), in that its Tmax was 360 min. As would be anticipated for a chemical as highly lipophilic as PCE, the Cmax, AUC and t½ values for adipose tissue were substantially greater than for other tissues. The t½ for nonfat tissues were relatively consistent, though liver and muscle exhibited somewhat shortest t½s.

The rate of blood perfusion and lipid content of individual tissues of rats had a significant influence on PCE deposition in organs. Highly perfused, lipid-rich organs such as the liver and brain had quite high Cmax and AUC values. Although not lipid-rich, the highly perfused kidneys also exhibited relatively high Cmax and AUC values. Poorly perfused, nonlipoidal tissues, such as skeletal muscle (Fig. 3B), had relatively low Cmax and AUC values.

Pharmacokinetic parameter estimates for dogs following oral administration of PCE are presented in Table 3, while blood and tissue level time-courses are

shown in Figs. 4 and 5. The observed Tmax for all nonlipoidal tissues was at the first sampling point (i.e., 60 min). The actual Tmaxs for these tissues were probably at shorter, though this cannot be ascertained from the present study, since no earlier sampling times were employed. The Tmax for fat in the dog was also twice as long as in the rat, though the Cmaxs in the two species were comparable. As in the rat, much greater deposition of PCE was observed in the fat of the dog than in the other tissues (Fig. 5C). Except for the brain, Cmax and AUC values for the fat were at least 8 and 33 times higher, respectively, than in nonfat tissues. PCE accumulation in the dog brain was considerably higher than in the other nonfat tissues (Table 3). Unlike the rat, the other highly perfused tissues (i.e., kidney and liver) of the dog did not show Cmax and AUC values that were different from less well perfused, lean tissues. For the Cmax values, this is likely due to the lack of available time points during the first hour. The $t_{\frac{1}{2}}$ for the dog brain was significantly longer than for other tissues, whereas the $t_{\frac{1}{2}}$ for the rat brain similar to most other tissues. The liver $t_{\frac{1}{2}}$, which was relatively short in rats, was comparable to a number of other tissues in the dog.

PCE tissue dosimetry varied substantially in the dog and the rat. AUCs were larger for dog tissues than for corresponding rat tissues, except for liver. The larger AUCs in dog tissues can be attributed primarily to prolonged elimination, since Cmax values were generally comparable in each species for most tissues. The $t_{\frac{1}{2}}$s in the nonfat dog tissues were 4-12 times longer than in corresponding rat tissues. The apparently short $t_{\frac{1}{2}}$ value for dog fat was due to a lack of time-points after 72 hr, such that an accurate $t_{\frac{1}{2}}$ could not be determined. Following oral administration, whole body clearance of PCE was 30.1 and 12.8 ml min/kg for rats and dogs, respectively.

The time-courses of exhalation and metabolism of PCE were predicted by the PBPK model for the rat (Fig. 6A) and dog (Fig. 6B) following oral administration of the chemical. For each species, the fraction of PCE exhaled was substantially greater than the fraction metabolized, with the difference steadily increasing during the period following oral dosing. The rate and magnitude of exhalation and metabolism were markedly higher in the rat than in the dog.

DISCUSSION

Tissue concentration-time data were obtained in two species in this study, in order to derive *in vivo* partition coefficients to utilize in development of a PBPK model for PCE. Previously, such detailed tissue concentration-time data sets have not been available for VOCs, including PCE. The most logical measure of target organ exposure to toxicants is the area under the tissue concentration versus time curve (AUC) of the biologically-active form of a chemical (Andersen, 1987). The AUCs measured in the present study, following oral administration of PCE to rats and dogs, have proven useful in verifying assumptions concerning the interspecies scaling of tissue deposition. Interspecies scaling has been described as the determination of how target tissue exposure is affected by the size of a species for a particular administered dose (Andersen, 1987). By scaling metabolic and physiological clearance as a power of body weight and tissue volumes in proportion to body weight, the NRC (1986) predicted that greater AUCs for parent compounds would be manifest in larger animals. Findings in the current study support this concept. AUCs were greater in dogs than in rats in 6 of 7 tissues following ingestion of an equal oral dose of PCE. Andersen (1987) concluded that since clearance increases as a fractional power of body weight, it will be greater in smaller species. Indeed, clearance of PCE

much greater in rats than in dogs in the present investigation. Blood and tissue t½ values were much shorter in rats than dogs (Tables 2 and 3). The major route of PCE elimination is exhalation (Pegg et al., 1979; Schumann et al., 1980). The blood:air partition coefficient is smaller in rats (19.8) than in dogs (40.5), so PCE will more readily diffuse from the pulmonary blood into the alveolar air of the rat. The rate of PCE exhalation is also dependent upon the rates of pulmonary (blood) perfusion and alveolar ventilation, both of which are significantly higher in the rat. Although metabolism plays a limited role in systemic clearance of PCE, metabolism would be expected to be greater in rats. Both exhalation and metabolism of PCE were predicted to be higher in the rat than in the dog by the PBPK model employed in the current investigation.

The primary method that has been employed to date to derive partition coefficients for VOCs has been the vial equilibration technique (Gargas et al., 1989). This in vitro technique is based on a similar method previously used to estimate partition coefficients for blood, oil and water (Sato and Nakajima, 1979) and tissue homogenates (Fiserova-Bergerova et al., 1984). The blood:air partition coefficient for PCE reported by Gargas et al. (1989) (18.9) was quite similar to that calculated from in vivo data for rats in the current study (19.8). Our in vivo tissue:blood partition coefficients, however, were consistently higher than the limited number of in vitro values published by Gargas et al. (1989) for rats. Corresponding in vivo and in vitro tissue:blood partition coefficients were as follows: liver:blood - 5.0 and 3.71; muscle:blood - 2.4 and 1.06; and fat:blood - 150.5 and 86.7. In contrast, the tissue:blood partition coefficients were 1.5 to 3.0 times higher in the rat than the dog. As the in vivo partition coefficients are derived from the tissue AUCs, it should be recognized that errors in determination of these values will be reflected in

the accuracy of the coefficients. For example, since PCE levels were relatively high at the last sampling time (i.e., 72 hr), an inaccurate estimation of the elimination rate constant could compromise the validity of the fat:blood partition coefficient. Although Gargas *et al.* (1989) reported similar PCE blood:air partition coefficients for mice and rats, the values were more than 75% higher than that for humans. In the current study, the blood:air coefficient for the dog was more than twice that of the rat. These results demonstrate the importance of accurately determining and recognizing species differences in partition coefficients.

In an evaluation of the uncertainties involved in PBPK models for PCE, Hattis *et al.* (1990) found that there was appreciable variance in predictions of models of different researchers. Of the many potential sources of uncertainty, it was concluded that the primary cause of differences in model predictions of risk were the approaches employed in estimating the metabolic parameters. There were 20- and 60-fold differences in estimates of K_m for mouse and rat models, respectively. V_{max} estimates differed by 8- and 15-fold for the mouse and rat models, respectively. The values for K_m and V_{max} in the present study were estimated by nonlinear regression, fitting the model predictions to the observed blood and liver concentration versus time data. Our V_{max} value (2.9 nM/ml Kg) for rats, when expressed in the same units, is 10 times lower than the range of values evaluated by Hattis *et al.* (1990). The range of estimates of V_{max} reported by Hattis *et al.* (1990) were not based on liver concentration-time data. Due to the dependence of PBPK models on a number of variables, there are other potential sources of error including model structure and other input parameters.

It has frequently been necessary during the development of the PBPK models for VOCs to manipulate anatomical, physiological and physicochemical input

parameters, in order to obtain adequate simulations of observed/experimental data. In an analysis of the precision of PBPK models for PCE in risk assessment, Bois *et al*. (1990) quantified the uncertainties associated with parameter variability. The kinetic parameter defining metabolic rate was seen as the most important variable in assessing cancer risk. Changes in key input parameters have often had to be made arbitrarily because of incorrect or inadequate information. Ramsey and Andersen (1984), for example, had to make the following changes in their PBPK model for styrene to obtain good agreement between observed and predicted values: increase in the fat tissue volume and blood perfusion rate; decrease in the fat:blood partition coefficient; increase of the blood flow rate to the metabolizing tissue group; and offsetting alterations in the muscle and richly perfused tissue groups. Reitz *et al*. (1988) found that the pharmacokinetics of trichloroethane in older rats could be simulated by increasing the size of the fat compartment in their model. It has frequently been necessary to make such assumptions and utilize unverified values for input parameters in the absence of data. Determination and utilization of accurate parameters should significantly improve the accuracy of PBPK model simulations. Therefore, *in vivo* studies that provide species-specific physiological and physicochemical parameters, as was done for dogs and rats in the present investigation, can significantly reduce uncertainty of PBPK models predictions of the systemic disposition of VOCs.

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ACKNOWLEDGEMENTS

Table 1
Parameters Used in the Physiologically Based
Pharmacokinetic Model for PCE in the Rat and the Dog

Parameter	Rat	Dog
Body Weight (kg)	0.340	10

Tissue volumes[a] (ml)	*Percentage of Body Weight*	
liver	3.4	4.2
kidney	0.8	0.6
fat	5.0	15.2
heart	0.3	1.1
lung	0.4	0.7
muscle	35.4	46.8
brain	0.6	0.8
blood	7.4	8.2
rest of body	46.8	22.4
Alveolar ventilation[b]	1.54 (ml/min·g) BW(g)$^{0.75}$	2.58 (ml/min·g) BW(g)$^{0.75}$
Cardiac output[c]	1.57 (ml/min·g) BW(g)$^{0.75}$	1.05 (ml/min·g) BW(g)$^{0.75}$

Blood flows[c]	*Percentage of Cardiac Output*	
liver	15.7	5.1
kidney	13.1	10.1
fat	6.6	5.0
heart	4.7	3.4
lung	100	100
muscle	26.1	40.6
brain	2.2	3.1
blood	100%=1.57 (ml/min·g) BW(g)$^{0.75}$	100%=2.05 (ml/min·g) BW(g)$^{0.75}$
rest of body	31.5	32.7

Partition Coefficients		
Blood:air	19.6	40.5
Fat:blood	150.5	71.4
Lung:blood	1.9	1.3
Liver:blood	5.0	2.4
Muscle:blood	2.4	2.4
Brain:blood	4.1	4.1
Heart:blood	2.4	2.4
Kidney:blood	3.2	2.1
Rest of body:blood	3.0	1.9

Metabolism constants		
Vmax (μg/min)	0.15	0.85
Km (μm/ml)	0.019	0.023

Absorption constant		
Ka(min^{-1})	0.025	0.34

[a]Tissue volumes for rats were determined by Manning *et al.* (1991); tissue volumes for dogs were obtained from Andersen (1970) for liver, heart, lung, skeletal muscle and blood, from Spector (1956) for kidney, and from Sheng and Huggins (1971) for fat.
[b]Alveolar ventilation values for rats and dogs were obtained from Dallas *et al.* (1991) and from Andersen (1970), respectively.
[c]Tissue blood flows for rats were obtained from Delp *et al.*, 1991; blood flows for dogs were obtained from Liang *et al.* (1982) for liver, from Humphrey and Zins (1983) for kidney, muscle and brain, from Andersen (1970) for cardiac output, from Nagasaka *et al.* (1976) for fat, and from Liard *et al.* (1982) for the heart.

Table 2
Pharmacokinetic Parameters in the Rat Following
Oral Administration of 10 mg PCE/kg bw[1]

Tissue	Area under curve$^\infty$. (μg·min/ml)	Half-life (min)	Cmax (μg/g)	Tmax (min)
Liver	1673 ± 345	331 ± 2	12.3 ± 7.6	10
Kidney	1057 ± 139	395 ± 60	5.5 ± 3.1	10
Fat	49964 ± 9745	695 ± 154	36.0 ± 17.4	360
Heart	806 ± 145	396 ± 73	2.9 ± 0.4	15
Lung	627 ± 167	342 ± 69	1.6 ± 0.8	60
Muscle	798 ± 428	310 ± 37	2.1 ± 0.8	60
Brain	1377 ± 230	327 ± 35	5.1 ± 0.8	15
Blood	332 ± 145	384 ± 145	1.0 ± 0.2	15

[1]Each value represents the mean ± S.D. for 4 rats at 14 time-points, ranging from
1 min to 72 hr.

Table 3
Pharmacokinetic Parameters in the Dog Following
Oral Administration of 10 mg PCE/kg bw[1]

Tissue	Area under curve[c]. (μg·min/ml)	Half-life (min)	Cmax (μg/g)	Tmax (min)
Liver	1851 ± 757	2448 ± 922	6.3 ± 0.6	60
Kidney	1606 ± 621	1572 ± 262	4.9 ± 0.4	60
Fat	55838 ± 9640	494 ± 77	42.8 ± 3.5	720
Heart	1849 ± 620	1775 ± 464	5.7 ± 1.6	60
Lung	1001 ± 681	2289 ± 863	2.4 ± 0.5	60
Muscle	1907 ± 1564	1625 ± 886	3.1 ± 0.1	60
Brain	3238 ± 1153	4641 ± 1547	11.4 ± 8.2	60
Blood	782 ± 146	865 ± 385	1.5 ± 0.3	60

[1]Each value represents the mean ± S.D. for 3 dogs at 6 time-points ranging from 1 to 72 hr.

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FIGURE LEGENDS

1. Diagram of the physiological pharmacokinetic model used to simulate the metabolism and exhalation of PCE, following its oral administration to rats and dogs. The parameters used for input into the model are included in Table 1.

2. PCE concentrations measured in the liver (A), kidney (B), brain (C), and lung (C) of rats following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean ± SD for 4 rats.

3. PCE concentrations measured in the heart (A), muscle (B), fat (C) and blood (D) of rats following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean ± SD for 4 rats.

4. PCE concentrations measured in the liver (A), kidney (B), brain (C), and lung (D) of dogs following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean ± SD for 3 dogs.

5. PCE concentrations measured in the heart (A), muscle (B), fat (C) and blood (D) of dogs following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean ± SD for 3 dogs.

6. Model predictions of the exhalation and metabolism of PCE over time, following po dosing of (A) rats and (B) dogs, with a single 10 mg/kg bolus dose of PCE.
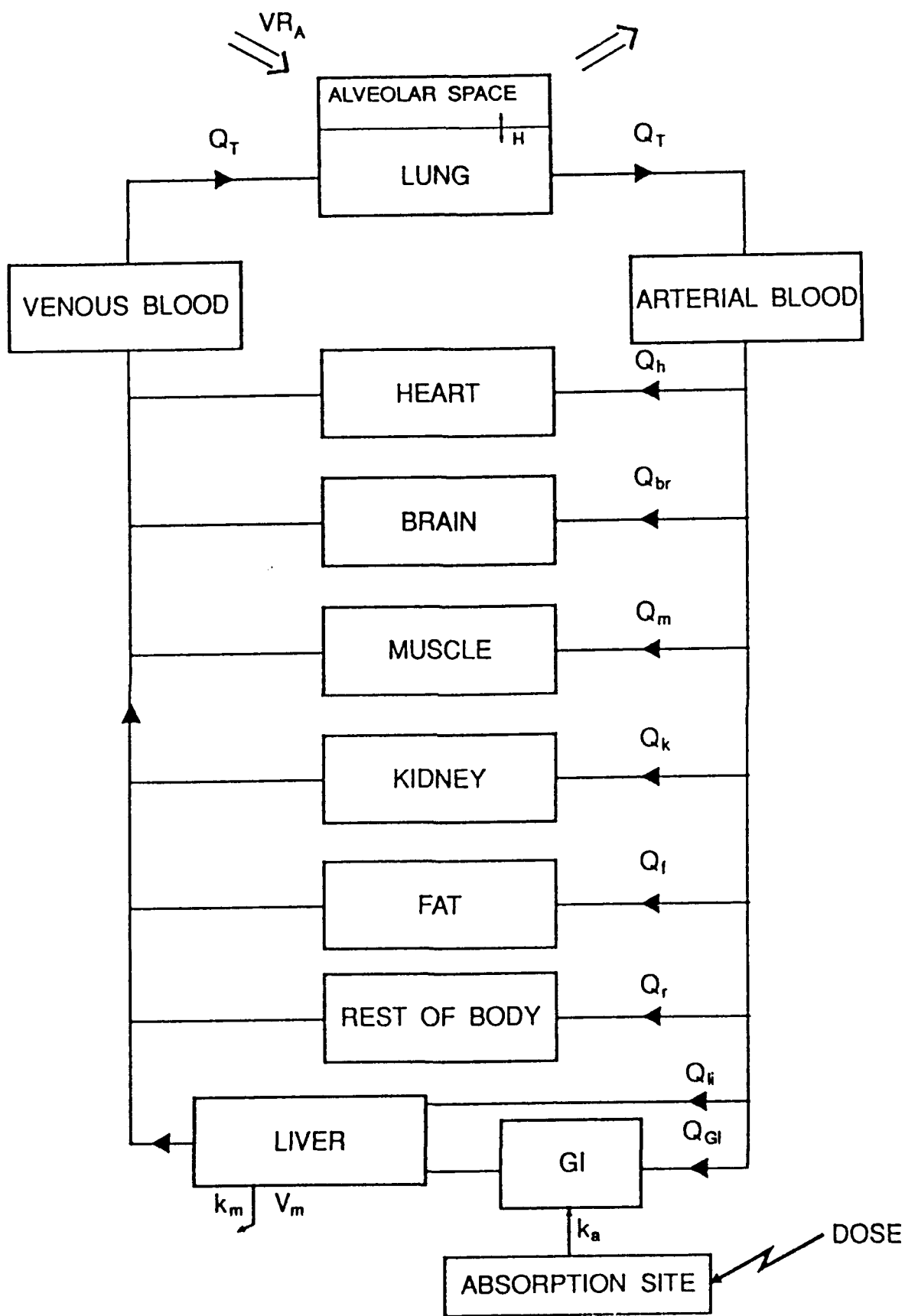
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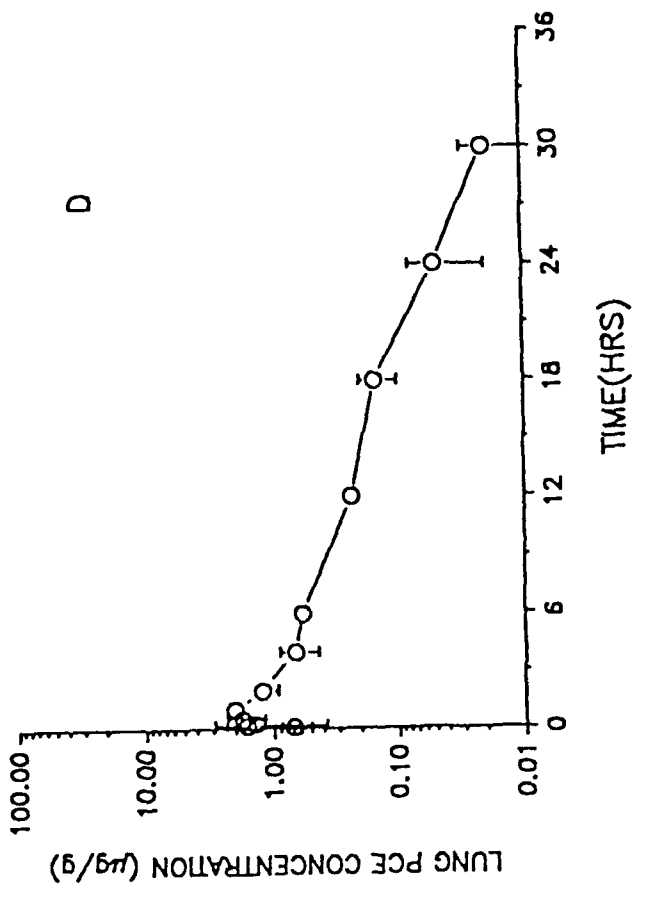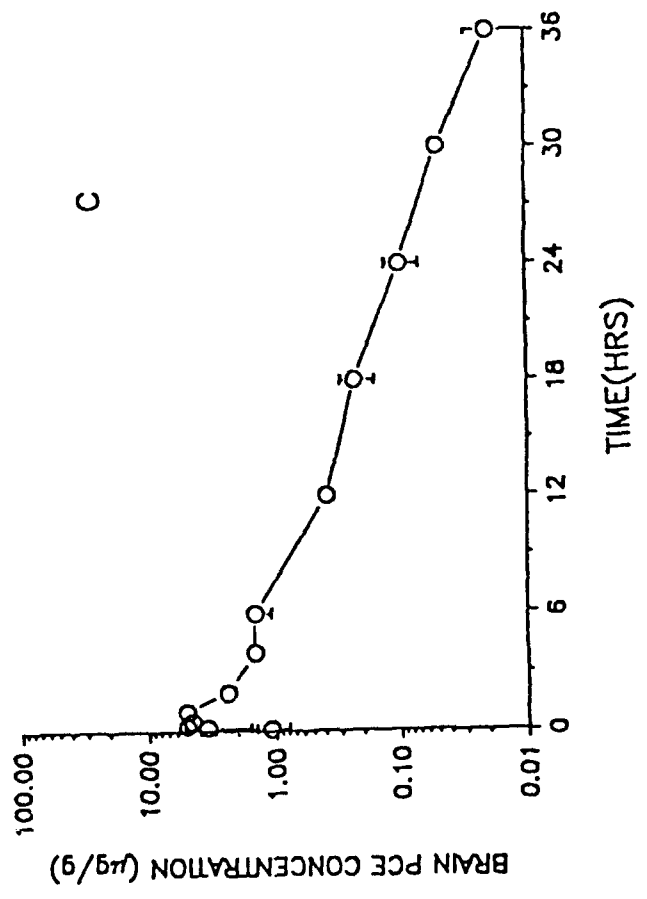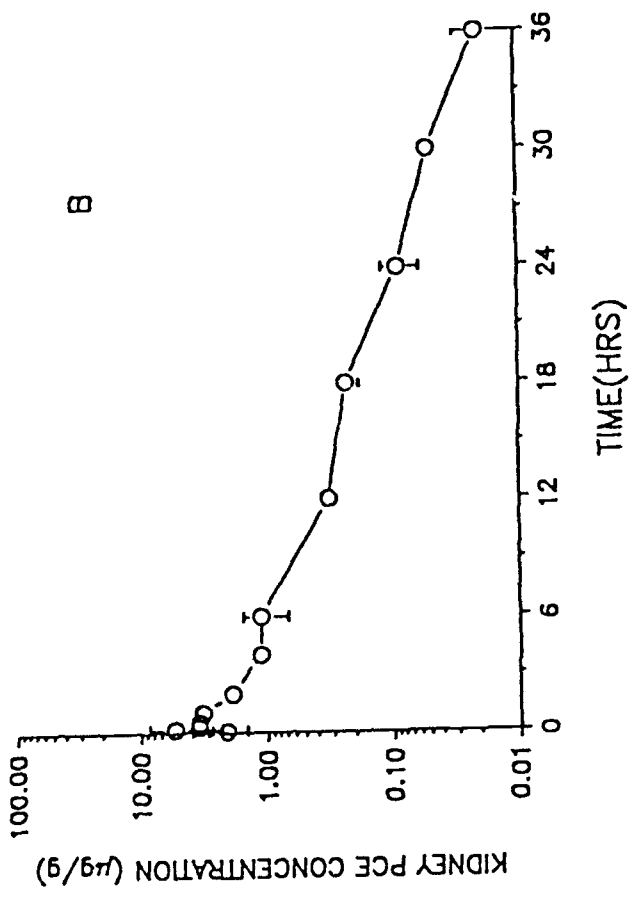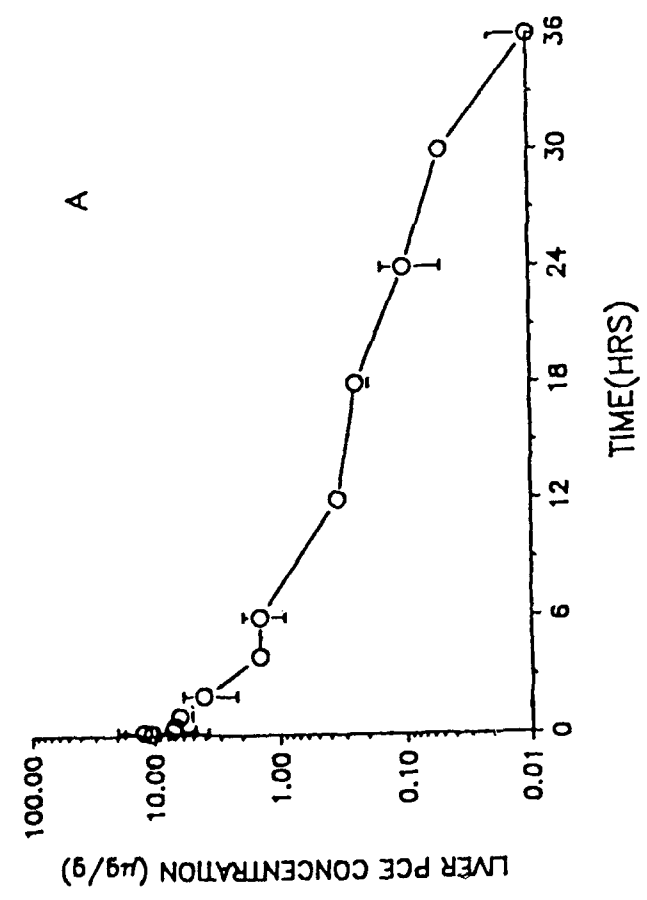
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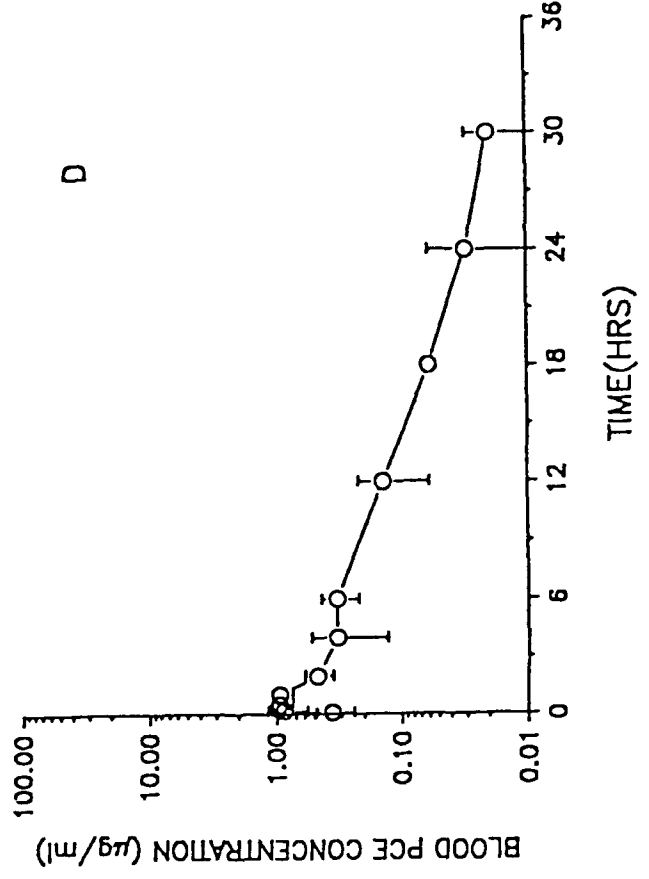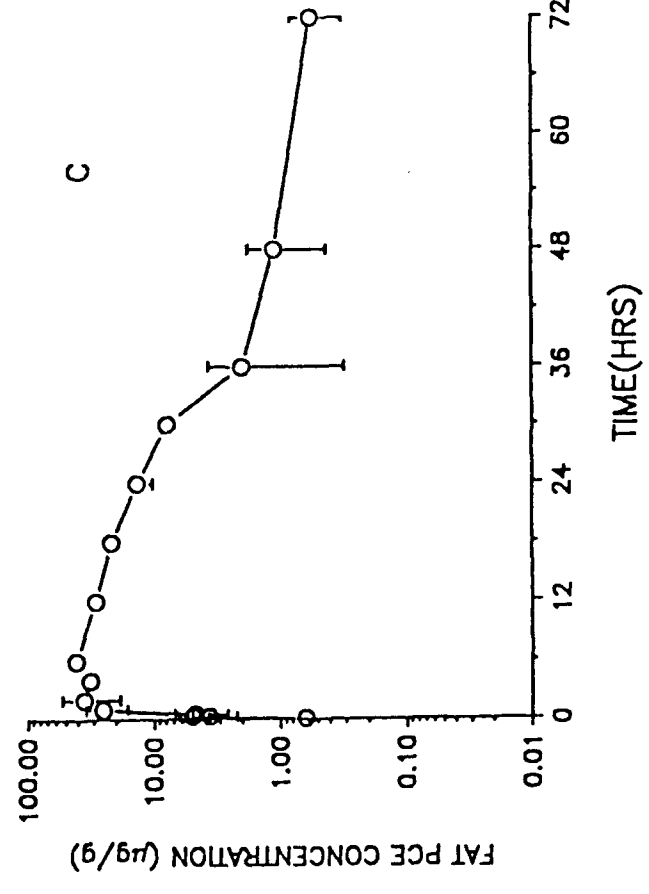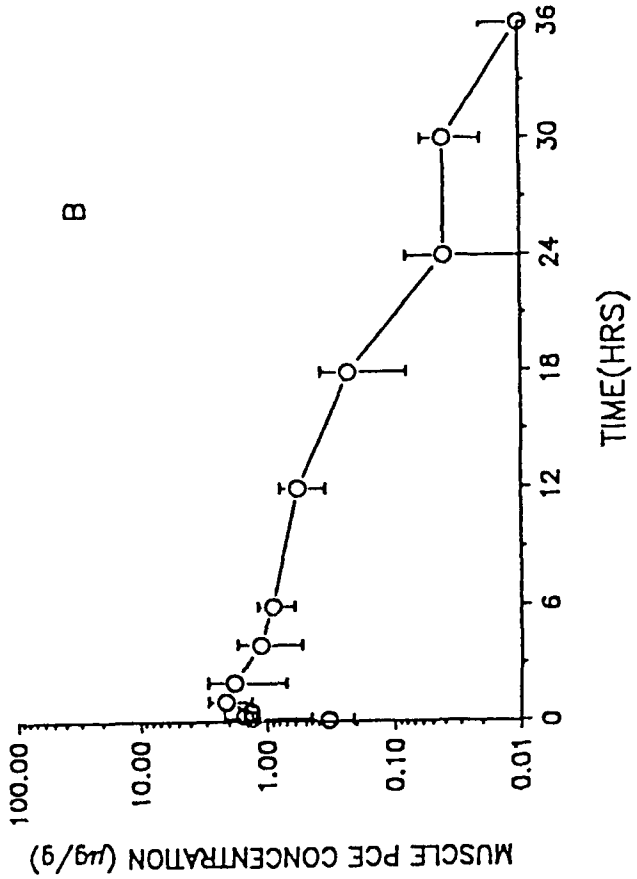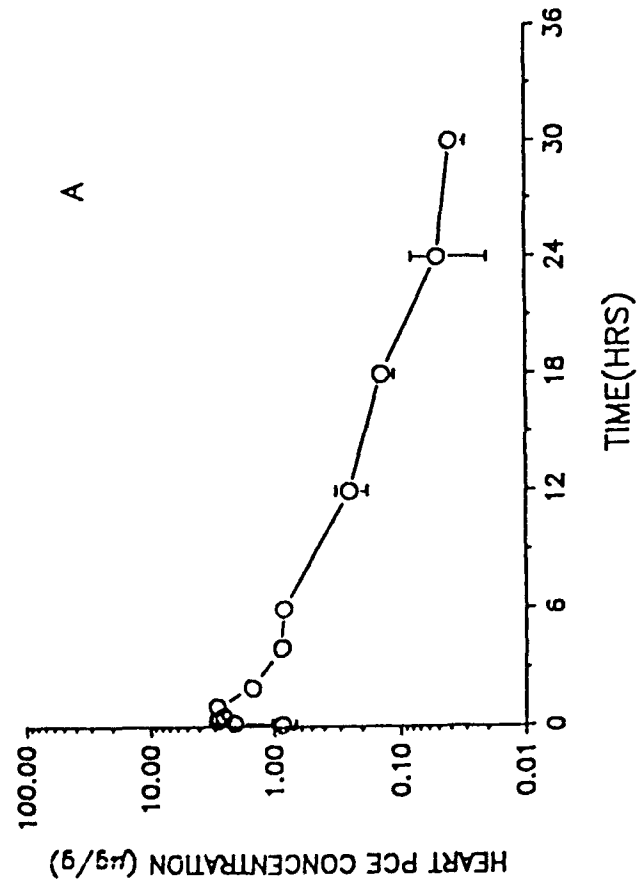
FIG. 3
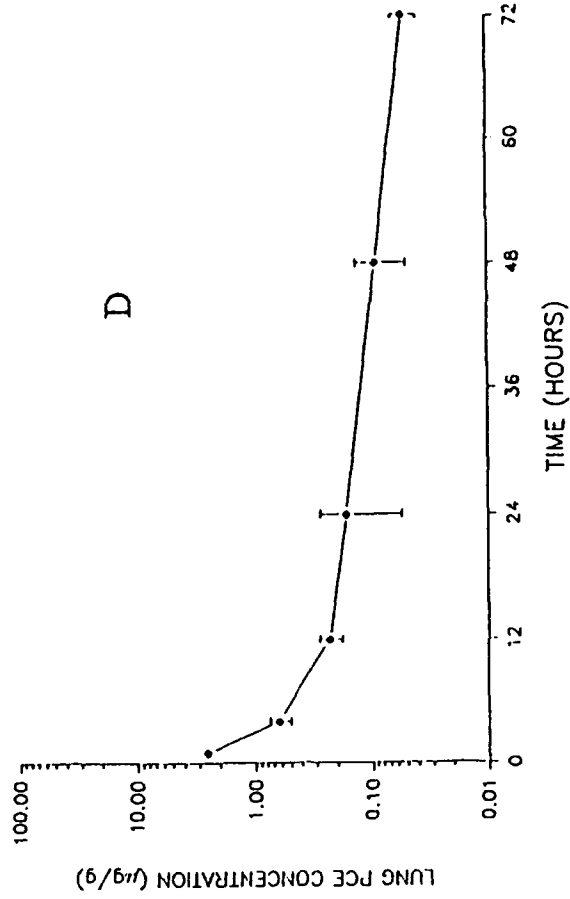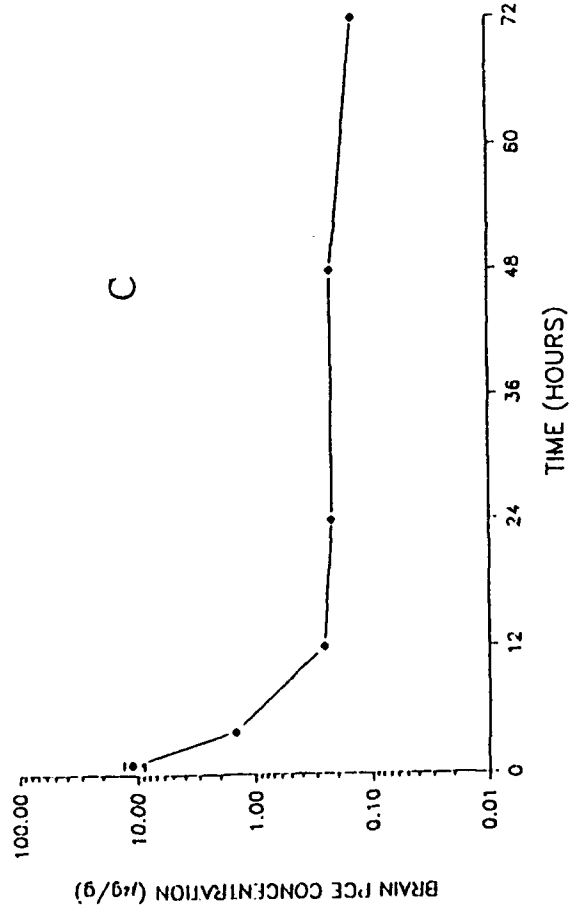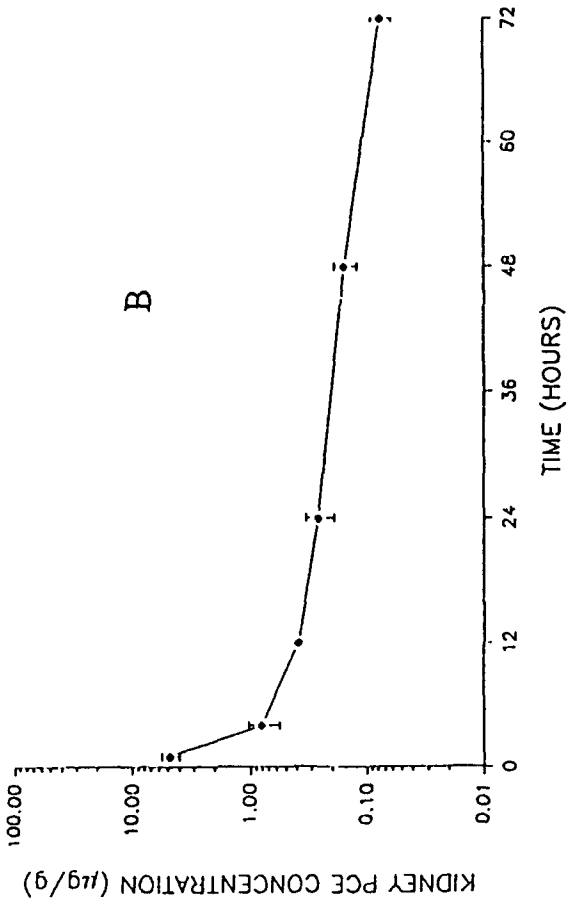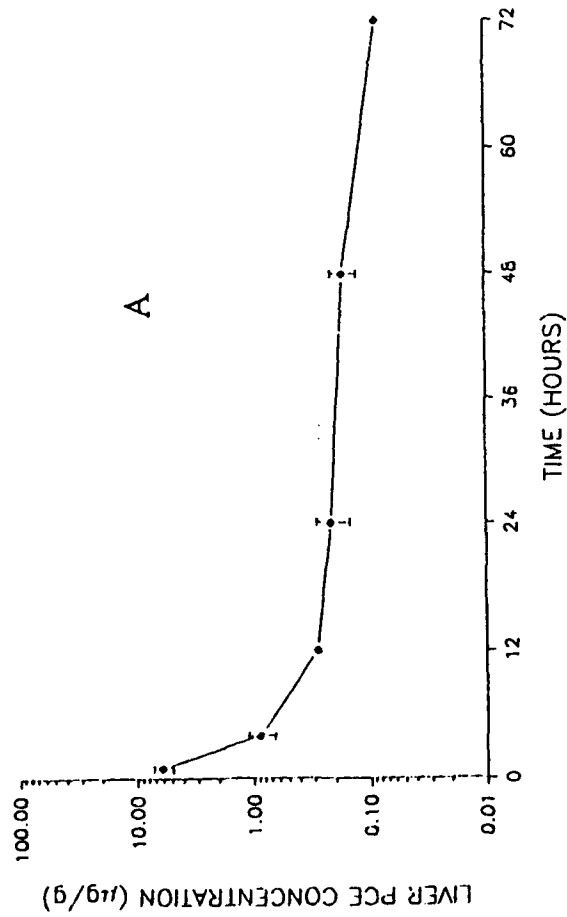
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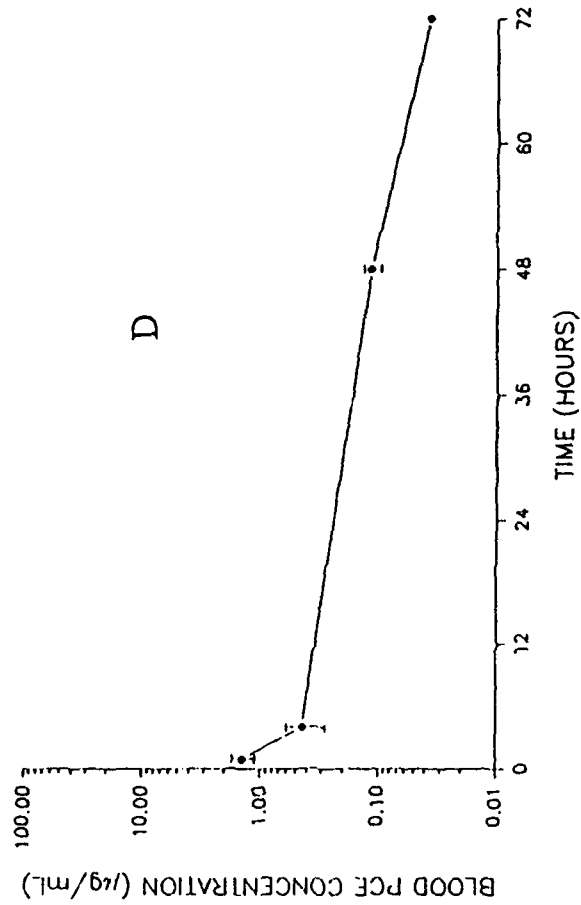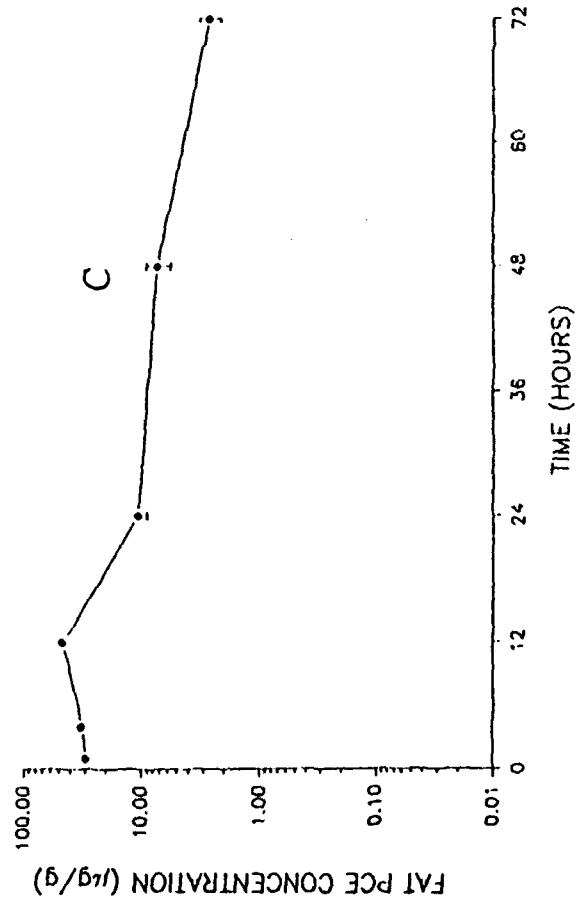
FIG. 5

FIG. 6

APPENDIX E

Comparisons between Operant Response and 1,1,1-Trichloroethane Toxicokinetics in Mouse Blood and Brain.   In Press, *Toxicology*, 1994.

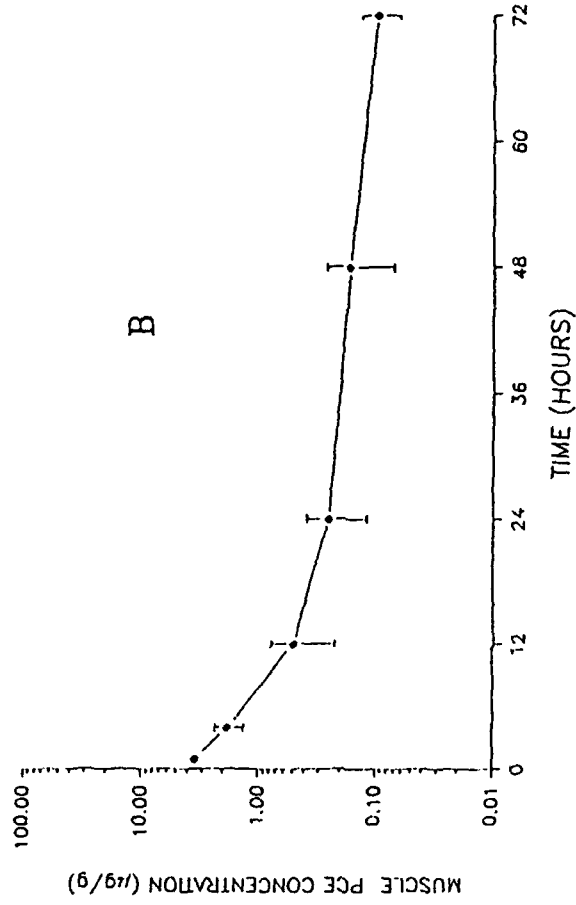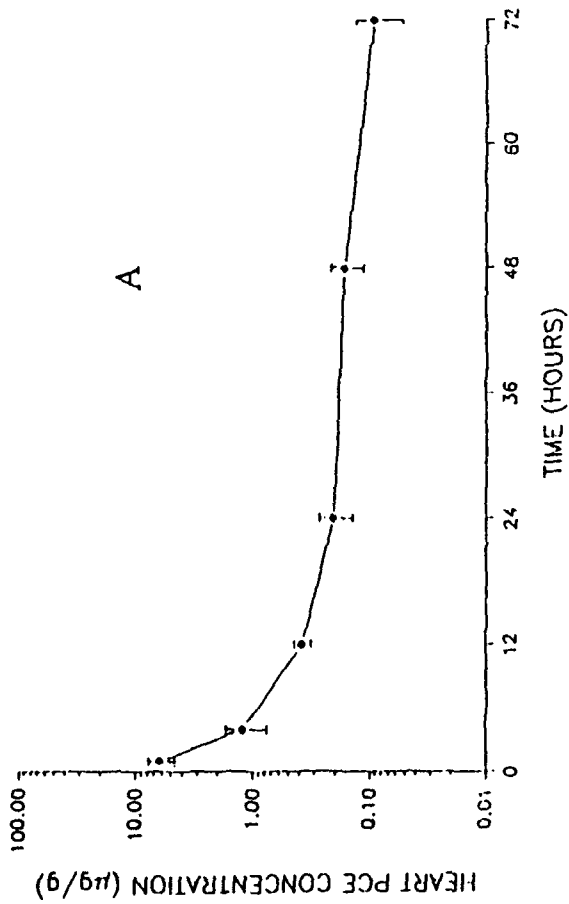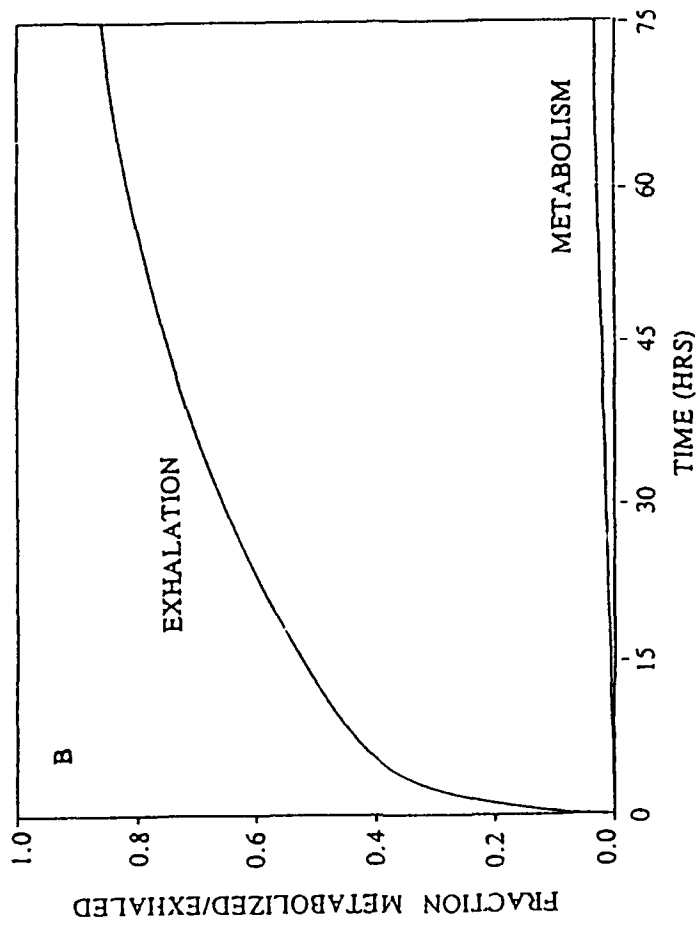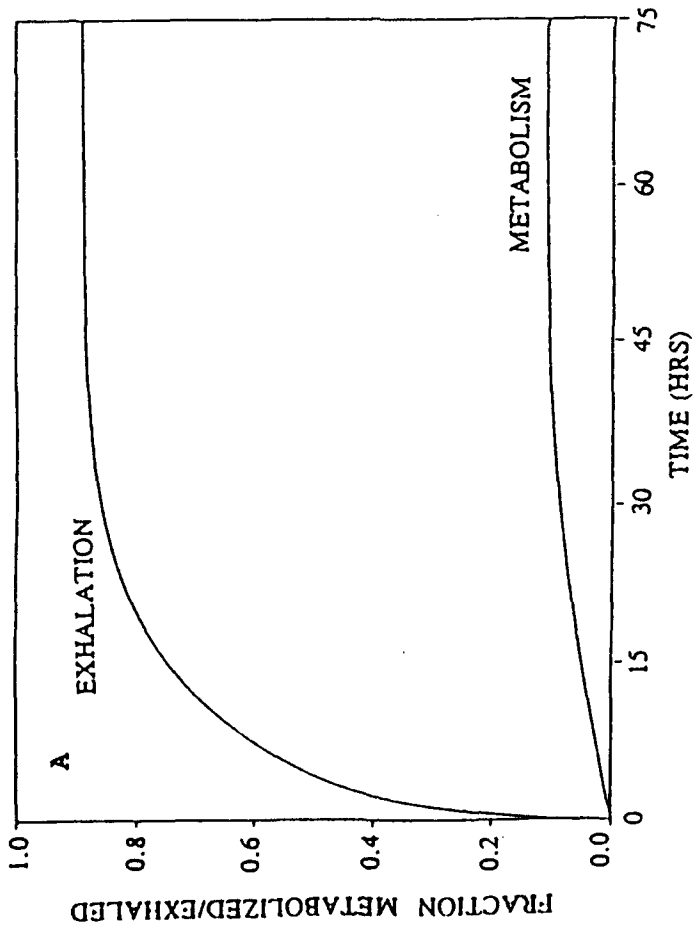# Comparisons between operant response and 1,1,1-trichloroethane toxicokinetics in mouse blood and brain

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Abstract

The effect of 1,1,1-trichloroethane (TRI) inhalation on operant response was evaluated in relation to the concentration of TRI in blood and brain tissue in mice during exposure. Male CD-1 mice were trained to lever-press for an evaporated milk reinforcer on a variable interval (VI 60) schedule for 2 h. Trained mice were then exposed to either 3500 or 5000 ppm TRI for 100 min, and the changes in the schedule-controlled performance were measured. Additional groups of mice were exposed under the same conditions as those used in the behavioral study and sacrificed at various times during exposure, and the blood and brain samples were collected and subsequently analyzed for TRI content by headspace gas chromatography. Uptake of TRI into blood and brain was rapid, with near steady-state levels reached after approximately 40–60 min of exposure. Inhalation of 5000 ppm, but not 3500 ppm TRI was seen to cause inhibition of operant response, starting −30 min following the initiation of inhalation exposure and beginning to recover after 80 min of exposure. The threshold concentrations for the maximal behavioral inhibition were −110 μg/g and 130 μg/ml in mouse brain and blood, respectively. It appears that in addition to TRI concentrations in blood and brain tissue, the time it takes to reach the apparent threshold TRI concentration was also a determinant for the onset of TRI neurobehavioral depression.

Keywords: 1,1,1-Trichloroethane; Operant responding; Neurobehavioral depression; Toxicokinetics

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1. Introduction

1,1,1-Trichloroethane (TRI), also known as methyl chloroform, is a chlorinated aliphatic hydrocarbon used as a solvent in a large number of industrial and commercial products. The world production of TRI was ~680/000 tons in 1988, of which ~50% was produced in the US. (Arlien-Søborg, 1992; Dobson and Jensen, 1992). TRI is considered to have a relatively low degree of toxicity, with central nervous system (CNS) depression as the reported primary effect after high levels of exposure in humans (Kleinfeld and Feiner, 1966; Stewart, 1968; Torkelson and Rowe, 1981). Following animal studies of very high doses of TRI, cardiac arrhythmias (Reinhardt et al., 1973; Herd et al., 1974) and hepatic and renal toxicity (Plaa and Larson, 1965; Klaassen and Plaa, 1966, 1967) have also been reported.

Because of their high volatility, inhalation is the primary route of exposure to most volatile organic compounds (VOCs). Inhalation exposure to TRI occurs frequently in occupational settings as well as through intentional intoxication with commercial products. Previous studies have shown that TRI is able to affect CNS functions in several experimental models, and its toxicity has been expressed as a disturbance of neurobehavioral functions similar to that of depressant drugs (Rees et al., 1987; Evans and Balster, 1991, 1993). It is important to assess changes in CNS basal activity in a valid quantitative manner (Horwath and Frantik, 1973; Filov et al., 1979). Repetitive, on-line determinations of neurobehavioral response concurrent with solvent exposure are of significant utility in elucidation of the time course of CNS effects of these compounds (Balster et al., 1982). Operant performance measurements have been shown to be useful in the detection of subtle CNS effects of low VOC exposure concentrations prior to reaching a level which would result in irreversible neuropathological changes (Geller et al., 1979). CNS-depressant effects of VOCs have been demonstrated in animals by operant tests at doses (Wood et al., 1983) similar to those that have been shown to alter human performance (Gamberale and Hultengren, 1972). TRI has been found in several animal models to reduce response rates in operant behavior of different reinforcing schedules (Balster et al., 1982; Geller et al., 1982; De Ceaurriz et al., 1983).

It has been shown in the case of toluene that the magnitude of the solvent-induced performance inhibition was correlated with the concentration of parent compound in the CNS of mice (Bruckner and Peterson, 1981). Although the highest degree of correlation was consistently seen between the extent of CNS depression and brain toluene concentration, blood levels were also a reasonably reliable index of the depth of narcosis. However, there is a general paucity of data for most VOCs that links toxicokinetics of solvents and their neurobehavioral effects. The purpose of this study therefore, was to demonstrate the effects of TRI on variable-interval response in mice and examine the relationship between the neurobehavioral effect and the chemical concentration in the blood and brain tissue.

2. Methods

2.1. Animals

CD-1 male mice from Charles River Breeding Laboratories (Raleigh, NC), weighing 30-35 g, were used in the study. The mice were housed on a constant light-dark cycle, with light from 07:00-19:00 h, and darkness from 19:00-07:00 h, in standard mouse cages. The mice were acclimated to the animal facility for at least 1 week, during which tap water and commercial rodent chow were provided ad libitum. The mice were then housed individually and restricted in food intake so that their body weight would be reduced over 1 week to and maintained at 80% of the original weight. Water access was unlimited. In the kinetic study, the mice were fed in the same way as in the behavioral study for 3 weeks. The mice were maintained at this weight until used in the kinetic and behavioral studies.

2.2. Chemicals

1,1,1-Trichloroethane, of 99% purity and iso-octane, of 99.98% purity, were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ)

2.3. Inhalation exposures

Inhalation exposures were conducted in 1.0 m³ Rochester-type dynamic flow chambers. Test atmospheres of TRI were generated by vaporization of the compound under different combinations of air flow- and temperature-controlled schedules. A flow of nitrogen gas carried the vaporized TRI from a flask into the exposure chamber influent air stream. The entire TRI generation system was enclosed in a specially fabricated safety box, which was maintained under constant negative pressure during inhalation exposures in the same manner as the inhalation chamber. Exhaust air from the vapor-generation box and the inhalation chamber were vented to HEPA and charcoal filters, so that all air with any chemical was scrubbed before release to the environment. The exposure chamber was operated at flow rates of 0.2-0.42 m³/min. The concentration of TRI in the exposure chamber was monitored by an on-line Miran 1B2 IR spectrophotometer (Foxboro Analytical, South Norwalk, CT) on the level of the bottom of the operant cage. Clean air was pumped through the chamber following the termination of the inhalation exposure.

In the behavioral study, modular operant cages for mice (Coulbourn Instruments, Allentown, PA) were positioned inside the inhalation chamber throughout the training and exposure sessions. The operant cages had been adapted so that TRI vapors could move freely through the cages during operant sessions. In the kinetic study, mice were exposed to TRI at the same inhalation exposure concentrations as in the behavioral study.

2.4. Operant study

With slight modifications, operant testing in the present study was conducted according to the method described by Balster et al. (1982). Briefly, the mice were food restricted during the period in which they were shaped to lever-press for 0.01 ml evaporated milk presentation, and trained to a variable-interval 60 s (VI 60) sche-

date in 2-h daily sessions in the modular operant cages. The baseline response rates were determined from operant sessions spaced 24 h apart. Output of the operant cage was analyzed by a modular behavioral analysis instrument panel (Coulbourn Instruments, Allentown, PA). The schedule contingencies and data recording were carried out by a computer program, COSMOS (Coulbourn Instruments, Allentown, PA), that was run on an IBM-compatible 386 computer. The number of lever presses in each 5-min period of the operant session was recorded for both baseline and exposure sessions. A stable performance was judged by two criteria: (i) the standard errors of the operant response rates in each of those three behavioral sessions immediately before the inhalation exposure fell within 10% of their means; (ii) the variations in means of each session became less than 10% of their grand mean. After the schedule-controlled response became stable, the mice were exposed to clean air for 20 min followed by either 3500 or 5000 ppm TRI for 100 min. Response ratios were calculated by dividing the number of responses during exposure by the average number of responses during the three control sessions immediately preceding exposure.

2.5. Kinetic study

The prepared mice were exposed to either 3500 or 5000 ppm under the same conditions as the behavioral study for either 10, 20, 40, 60, 80, or 100 min at which time blood samples (0.5 ml) were withdrawn by closed chest cardiac puncture immediately after the mice were sacrificed by cervical dislocation. Samples of brain were quickly removed and were immediately placed into ice-chilled scintillation vials containing 4 ml of iso-octane and 1 ml of 0.9% NaCl and tightly capped. The tissues were homogenized using the Ultra-Turrax SDT homogenizer (Tekmar, Cincinnati, OH) for 5 s. The vials were then vortex-mixed for 30 s and centrifuged at 1800 × g for 10 min at 4°C in capped vials. Aliquots of the iso-octane supernatant layer were taken into 20-ml vials, which were capped immediately with teflon-lined rubber septa and crimped to insure an air-tight seal. The capped vials were then placed into a headspace auto-sampler unit of a Perkin-Elmer Model 8500 gas chromatograph (GC) (Perkin-Elmer, Norwalk, CT). Analyses were carried out using a stainless-steel column (182 cm × 0.317 cm) packed with 3% OV-17 (Alltech Associates, Deerfield, Il). The GC operating conditions were headspace sampler temperature, 70°C; injection port temperature, 150°C; column temperature, 80°C; detector temperature, 360°C. Each sample vial was heated thermostatically for ~~10 min~~ 30 min in the GC autosampler unit, pressurized for 30 s with the carrier gas, and a fixed volume of the iso-octane supernatant injected automatically into the GC column. The limit of detection for TRI by GC was less than 1 ng in 20 ml air, or 8.4 ppb TRI in the sample vial. The recovery rates of TRI in blood and tissue from the procedure were determined prior to the kinetic study using the method described by Chen et al. (1993). Briefly, samples of blood and brain tissue were taken from chemical-naive mice (n = 10) immediately after they were terminated by cervical dislocation. Each sample was spiked with TRI by injection of 4 µl of a solution containing 1 mg/ml TRI in iso-octane. All of the spiked samples were then processed as described above. The % recovery was derived by dividing the amount of TRI resulting from the GC analy-

ses by 4 μg, the amount injected into the samples.

Animal training was arranged between 09:00–17:00 h, 7 days per week. Each animal was trained in the same period of time each day. The training and exposure sessions were carried out at a same time period throughout the study. All the inhalation exposures for the kinetic study were conducted between 09:00–12:00 h. The collected samples were processed and analyzed immediately following exposure.

2.6. Statistics

A repeated measures analysis of variance was employed to determine the difference between the pre-TRI response rates and the response rates during the treatment sessions as well as to examine the effects overtime. An independent t-test was used to detect differences in TRI tissue concentration of 3500 ppm and 5000 ppm groups and between the baseline operant response rates of the two groups. The level of significance was set at $P < 0.05$ in all tests.

3. Results

It took less than 3 min for TRI concentrations in the inhalation exposure chamber to reach the target concentrations of 3500 and 5000 ppm TRI after the initiation of exposure. TRI concentrations were maintained at the level of the target concentration $\pm$ 5%. The values ($n = 10$) of % recovery of TRI from the spiked blood and brain tissue samples were 94.7 $\pm$ 2.4 (mean $\pm$ S.E.) and 90.5 $\pm$ 1.9 for blood and brain, respectively. These values were subsequently factored into the calculation of TRI concentrations.

In the behavioral study, all animals that were subsequently tested had achieved a consistent performance that was maintained for at least 3 days before the TRI exposures were conducted. The standard errors of the baseline operant response rates within each operant session ranged from 3.4% to 9.6% of their means. The baseline performance was also maintained during the initial 20 min of the clean air exposure before the chemical was introduced. The baseline response rates across animals in each group of mice were 153 $\pm$ 13.6 and 142 $\pm$ 14.4 (Mean $\pm$ S.E.) for each 5-min period in the 3500 and 5000 ppm groups, respectively. An independent t-test revealed no statistically significant difference between the baseline response rates for the two groups of mice. The repeated measures analysis of variance was applied to both dose levels. At 3500 ppm level inhalation exposure, the mice tested ($n = 7$) showed no statistically significant decrease in operant response rates comparing to the baseline values throughout the 100 min exposure session, although there appeared to be a small attenuation in response rate following the introduction of TRI. The response rates stayed at ~85% of the base rate after the initiation of exposure with the highest at 88% and the lowest of 81% (Fig. 1a). At 5000 ppm level, the mice ($n = 5$) showed a trend of reduced response rates starting at ~25 min of inhalation exposure, although this reduction was not statistically significant until after 45 min of exposure. This depression of response lasted ~40 min (except at 50 min), and afterward the depression gradually recovered and the operant response rates returned to baseline level (Fig. 1b). It was also revealed that changes in response rates over time were

significant and there was an interaction between treatment and exposure time in the 5000 ppm group.

The uptake of TRI into blood and brain tissues was rapid, and the concentrations in both blood and brain increased steadily over time (Fig. 2a,b). Concentrations in blood samples reached near steady-state within 40 min of inhalation exposure, whereas it took ~60 min in the brain tissue. The highest TRI concentrations measured at 100 min in blood were 141 and 198 μg/ml for 3500 and 5000 ppm levels, respectively; while the highest concentrations (also at 100 min) measured in brain were 134 and 189 μg/g tissue. In blood, TRI concentrations of the 3500 and 5000 ppm groups were significantly different at 60, 80 and 100 min. There were significant differences in brain concentrations between the two exposure groups at 40, 60, 80 and 100 min.

The TRI brain concentration was 114 μg/g at 40 min in the 5000 ppm group, when the operant response ratio showed a significant decrease relative to baseline response. However, when the TRI brain concentration in the 3500 ppm group reached approximately the same level at 80 min (113 μg/g) and surpassed it at 100 min with 134 μg/g, there was no significant decrease in the operant response rate. Also, when the depressed operant response rate in the 5000 ppm group returned to baseline level after chemical exposure for 80 min, the brain and blood TRI concentrations continued to increase.

Pooling the data for both inhalation concentration exposure groups, the mean response rates of the operant tests were plotted against brain (Fig. 3a) and blood (Fig. 3b) TRI concentrations. For the blood, mean response ratios remained between 80–90% until 120 μg/ml TRI was achieved after which there was a general decline. The same was true of mean response ratios when mean brain concentrations were below 110 μg/g. Above 150 μg/ml concentrations in blood and 160 μg/g in brain the mean response ratios plummeted to 30–40% of control levels. However, in both cases, data points were distributed as two clusters, and the second degree regression models only displayed a moderate fit.

4. Discussion

The toxicokinetic relationship between the uptake and disposition of chemicals and their toxic effects has been recognized as an increasingly important factor in risk assessment (Clark and Smith, 1984; Clewell and Andersen, 1985). Among the studies conducted on the pharmacokinetics of VOCs, there has been a particular paucity of data obtained during inhalation exposure for most VOCs. Likewise, there have been few studies that measured blood and tissue TRI concentrations during inhalation exposure. Uptake of TRI into the arterial blood of rats during inhalation exposure was found to be rapid, with a near steady-state equilibrium achieved after ~30–40 min (Dallas et al., 1991). In the current study with mice, blood TRI levels also reached near steady-state ~40 min into exposure at both dose levels, whereas TRI brain concentrations achieved near steady-state within 60 min of exposure. This rapid equilibration demonstrates the importance of the characteristic volatility of TRI and other VOCs in determining the rate of uptake following inhalation expo-

...sure. Using a comparison for which data are available, this rapid uptake of TRI in the current study for mice and previously in rats (Dallas et al., 1989) is considerably faster than that of toluene, which reached steady blood and brain concentrations only after >2 h of inhalation exposure at 4000 ppm level (Bruckner and Peterson, 1981). The difference in pharmacokinetics of the two chemicals can be largely attributed to differences in their partition coefficients, and particularly the blood:air partition coefficient, which is 15.6 for toluene (Sato and Nakajima, 1979a) and 3.3 for TRI (Sato and Nakajima, 1979b). It was shown by Reitz et al. (1988) that after inhalation exposure to TRI at 150 and 1500 ppm levels for 6 h, mice blood concentrations of the chemical were 9.27 and 111 μg/ml, respectively. A study conducted by Schumann et al. (1982) demonstrated similar results of TRI blood concentrations in mice with 12.6 and 105.3 μg/g for 150 and 1500 ppm, respectively, following inhalation exposure for 6 h. If one assumes a linear scale-up to the 3500 and 5000 ppm exposures used in the current study, the blood levels would be 233 and 333 μg/ml, respectively at the end of the 6 h exposure. These values would be 66% and 68% higher than the levels measured at 100 min in the present study, as we detected 141 and 198 μg/ml of blood TRI at 100 min exposure for 3500 ppm and 5000 ppm, respectively. Because of saturation kinetics in the current study after 40–60 min exposure, the aforementioned difference cannot be completely attributed to longer exposures in previous studies. Therefore, a simultaneous scale-up on dimensions of both exposure period and dose seems inappropriate. It was found, however that the kinetics of TRI in mice was linear over the high doses employed. TRI concentrations in the blood and brain after 100 min of exposure both increased 1.41-fold, with a 1.43-fold increase in the inhalation exposure concentration (from 3500 to 5000 ppm). Similar findings have been found following TRI inhalation exposures in mice and rats (Schumann et al., 1982; Dallas et al., 1991), and in humans (Nolan et al., 1984). It was reported in these studies that concentrations in blood and tissues, exhaled breath, and body burden of TRI were each proportional to dose in these species.

The effects of TRI on the CNS, as determined by measurements of schedule-controlled behavior, have been demonstrated by several studies (Balster et al., 1982; Moser et al., 1985; Warren et al., 1993). Using different exposure protocols, operant schedules, and two species (mice and rats), the experimental animals generally showed depression in their operant response within minutes of the initiation of exposure to concentrations higher than 2000 ppm. In the current study this inhibition effect was not observed in the 3500 ppm group, and at 5000 ppm it was not until 45 min into the exposure that the operant response began to be significantly depressed. All of the previous studies in mice used a fixed-ratio performance schedule, which probably accounts for differences in the time of onset of CNS depression to TRI inhalation as well as different sensitivity to exposure concentration. It is known that the use of different schedules to which operant response is reinforced often cause different sensitivities to toxicological insult (Laties and Wood, 1986; Cory-Slechta, 1992). These differential effects of reinforcement schedules have been shown for fixed-ratio and variable-interval as well as other types of reinforcement (Davey, 1981). While the underlying mechanism has been subject to various behavioral interpretations, the rates and patterns of operant response are thought to be among the

primary determinants of the ability of an agent to enhance or inhibit the established operant behavior (Davey, 1981; Glowa, 1990). It has been shown that CNS depressants like pentobarbital and some other VOCs (e.g. toluene) can cause rate-dependent effects on operant behavior using different schedules. Because a lower rate of response is associated with variable-interval relative to fixed-ratio schedule, the former may be less sensitive to the neurobehavioral effects of TRI in mice.

An interesting toxicokinetic finding is that there was recovery from the CNS behavioral inhibition during the latter part of the 100-min exposure to 5000 ppm TRI, despite the continued near steady-state blood and brain concentrations of TRI during the period. At 80 min of exposure, where recovery started, the brain and blood concentrations of TRI were 178 μg/g and 167 μg/ml, respectively, and there was a slight trend to increasing concentration. Because there have not been any reported studies in mice that have had operant sessions concurrent with TRI exposure lasting as long as 80 min, comparison with other studies in this respect are not available. For example, Balster et al. (1982) monitored operant behavior during 20 min of exposure to TRI. It might be feasible that there was a development of acute tolerance as an adaptive change during exposure, due to exposure length and operant schedule employed. When a behavioral phenomenon has been the end point of evaluation, it is known that tolerance may develop more readily when the effect of the agent has a behavioral 'cost' to experimental animals such as when it reduces the capability to obtain a reward or to avoid punishment (Jaffe, 1990). In order to achieve a more complete understanding of behavior as a function of CNS processes, behavioral endpoints need to be co-ordinated with physiological and biochemical variables, of which pharmacokinetics is a particularly valuable candidate (Weiss, 1985). Most co-ordinated assessments of behavior and pharmacokinetics have had to overcome formidable technical limitations. For example, Gallaher et al. (1982) had to assay ethanol in blood samples taken from a snipped tail vein of mice for a single time point for comparison with ataxic effects of ethanol. Geller et al. (1982) pointed out the difficulties inherent in measuring blood and tissue levels of the inhaled compound in conjunction with operant sessions, and had to make assumptions of the pharmacokinetic behavior of TRI in the absence of data to co-ordinate with their neurobehavioral measurements. Blood and brain levels of toluene were compared to the neurobehavioral effects of toluene during and following inhalation exposure (Bruckner and Peterson, 1981). Toxicity was measured in a battery of tests of unconditioned performance and reflex to assess CNS effects in mice and rats. There was a good correlation between degree of behavioral depression and both blood and brain concentrations during and following inhalation exposure. In the current study, blood and brain concentrations of TRI were related to behavioral depression, but were certainly not the only factors that dictated the resulting behavioral effect over time. The length of time that it took to reach certain brain concentrations associated with behavioral depression seemed to also be an important factor. The brain TRI concentration at 5000 ppm dose level was 114 μg/g at 40 min when a significant inhibition of operant response was observed. Brain concentrations in the mouse inhaling 3500 ppm reached a similar level at 80 min (113 μg/g) and exceeded it at 100 min with 134 μg/g. It might be postulated that the use of the

variable-interval schedule only allowed the detection of CNS depression at the higher dose, since the TRI concentration necessary to elicit a response was achieved considerably earlier in the operant session at the higher dose. Indeed, when time is eliminated in pooled comparison of blood or brain TRI concentrations and behavioral deficit (Fig. 3), only a moderate correlation is available from second degree regression curves. Because of the possibility of the effect of time of exposure on the onset of behavioral depression, singular use of blood or brain concentration to determine the behavioral toxicity of TRI inhalation in mice may result in inaccurate predictions.

5. Acknowledgements

6. References

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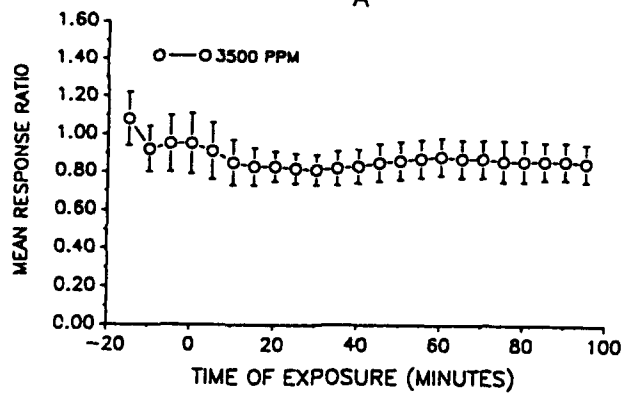Wood, R.W., Rees, D.C. and Laties, V.G. (1983) Behavioral effects of toluene are modulated by stimulus control. Toxicol. Appl. Pharmacol. 68, 462–472.

Fig. 1. Mean response ratios ($\pm$ S.E.) for a 20-min exposure to clean air following by 100 min of exposure to (a) 3500 ppm or (b) 5000 ppm TRI. Mice were trained to perform under a variable-interval 60 s schedule of milk presentation until attaining a stable response.

Fig. 2. Concentrations of TRI in the (a) blood and (b) brain of mice following exposure to either 3500 ppm or 5000 ppm TRI for up to 100 min. Serial sacrifices were conducted at 10, 20, 40, 60, 80 and 100 min and TRI analyzed by headspace GC. Values are expressed as the mean $\pm$ S.E. of 5 mice at each point. *Statistically significant difference with 3500 ppm group, $P < 0.05$.

Fig. 3. The relationship between operant response rates and (a) blood TRI concentrations, and (b) brain TRI concentrations. The values were pooled from both the 3500 and 5000 ppm groups. Curves for the relationship are generated by a second order regression. The equation for the blood and mean response ratio is $y = 0.514 + 0.01x - 6.81 \ x^2 \cdot 10^{-5}$ and $r = 0.799$; and the equation for the brain and mean response ratio is $y = 0.689 + 5.25x \cdot 10^{-3} - 4.12 \ x^2 \cdot 10^{-5}$ and the $r = 0.775$.

Fig. 1. TOX2825

Fig. 2. TOX2825

A

Y-axis: BLOOD CONCENTRATION (µg/mL)
X-axis: TIME OF EXPOSURE (MINUTES)
Legend: ●——● 3500 PPM TRI; △——△ 5000 PPM TRI

B

Y-axis: BRAIN CONCENTRATION (µg/g)
X-axis: TIME OF EXPOSURE (MINUTES)
Legend: ●——● 3500 PPM TRI; △——△ 5000 PPM TRI

Fig. 3 TOX2825

A

Y-axis: MEAN RESPONSE RATIO (TRI/CONTROL)
X-axis: MEAN BLOOD CONCENTRATION (µg/mL)

B

Y-axis: MEAN RESPONSE RATIO (TRI/CONTROL)
X-axis: MEAN BRAIN CONCENTRATION (µg/g)
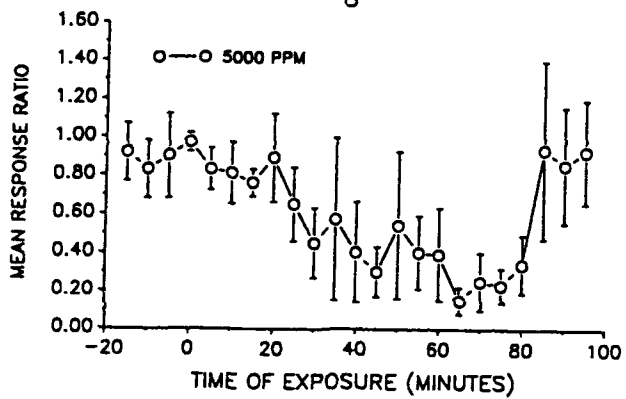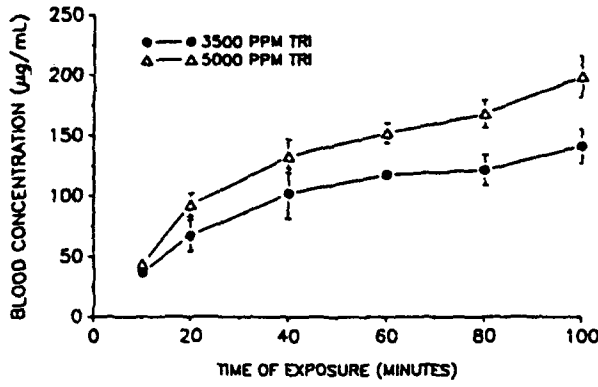
APPENDIX F

The Pharmacokinetics of Inhaled 1,1,1-Trichloroethane Following High Milk Intake in Mice. In Press, *Drug and Chemical Toxicology*, 1994.

THE PHARMACOKINETICS OF INHALED 1,1,1-TRICHLOROETHANE

FOLLOWING HIGH MILK INTAKE IN MICE
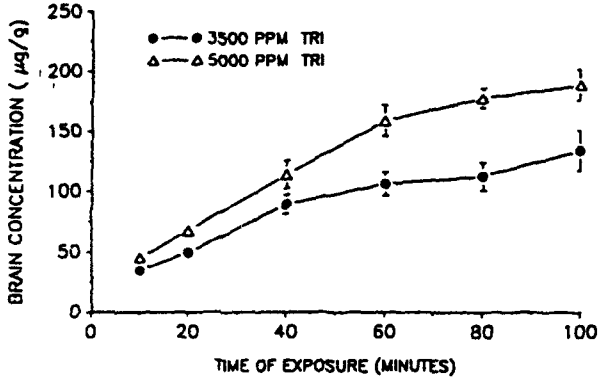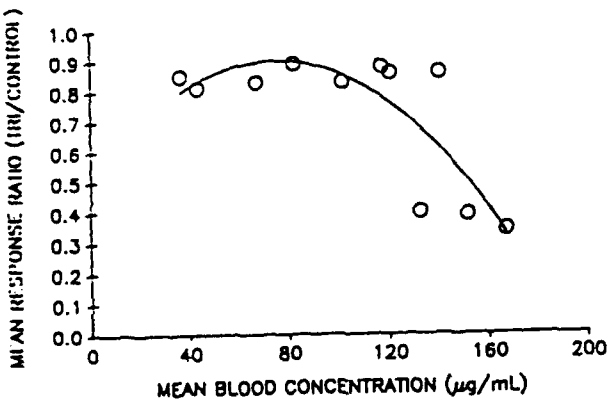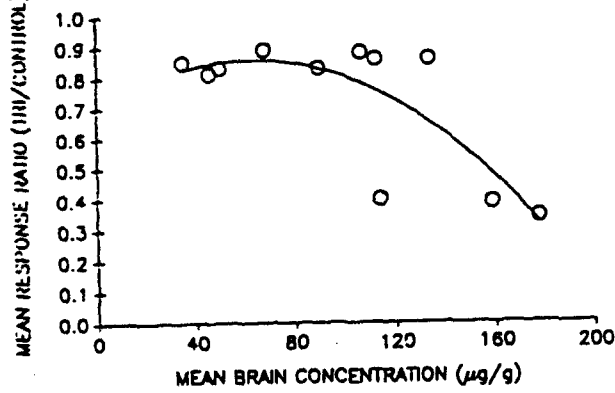
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ABSTRACT

In the evaluation of lipophilic halocarbons for neurobehavioral toxicity in operant testing, animals often receive large amounts of milk as a behavioral reinforcer over time. If this increase of fat in the diet sufficiently impacted the lipid depots of the animal, the pharmacokinetics of lipophilic test compounds might be significantly affected and thus obscure the accompanying neurobehavioral effects. The effects of milk intake, comparable to what was consumed as behavioral reinforcer during operant behavioral sessions, on the pharmacokinetics of inhaled 1,1,1-trichloroethane (TRI) were therefore examined in the blood and nine organ tissues of mice. Male CD-1 mice were food restricted so that their body weights would be reduced to and maintained at 80% of their original, and received a single gavage dose of 1.0 ml evaporated milk daily for three weeks. A control group with similar food restrictions was dosed with the same volume of water. Inhalation exposures to 3500 ppm TRI for 100 minutes were conducted at the end of the treatment period. Blood and nine organ tissues were sampled at a

series of time points, and their TRI contents were analyzed by headspace gas chromatography. The uptake of TRI was rapid, with near steady state approached in blood and most tissues after 40-60 minutes of exposure. All of the tissues except fat had similar TRI time-concentration profiles, while TRI concentrations in fat tissue were about 20-30 times higher than in other tissues. There was no statistically significant difference in the tissue concentrations between the milk-dosed group and water-dosed group at all of the time points for all tissues measured. Therefore, it appears unlikely that this level of milk intake as a reinforcer in behavioral studies will affect the results of operant testing evaluations by altering the pharmacokinetics of lipophilic halocarbons such as TRI.

INTRODUCTION

1,1,1-Trichloroethane (TRI) is a volatile organic compound (VOC) widely used in many industrial and commercial products. Regarded as of relatively low toxicity , TRI has nonetheless been shown to be associated with some adverse health effects in animals as well as in humans when administered at high dose levels[1]. Because inhalation is the primary route of exposure for many VOCs in both occupational settings and intentional intoxication with commercial products, it is frequently employed in investigations of TRI toxicity. Previous studies have shown that, in addition to cardiac arrhythmias[2,3] and hepatic and renal toxicity[4-6], the acute toxicity of TRI at high doses is most often characterized by central nervous system (CNS) depression[7-9].

In the studies on the CNS effects of VOCs, schedule-controlled behavior has been frequently employed as a quantitative endpoint to measure neurobehavioral toxicity[10,11]. The inhalation of TRI by test animals has been associated with

reductions in the responding rates of operant behavior using various reinforcing schedules[12-14]. Frequently, milk presentation has been used as a reinforcer to establish the operant behavior in those studies on solvent neurobehavioral toxicity[15-19]. Depending on the animal model and experimental protocol of the study, shaping animals to perform reliably on a reinforcing schedule to be used in operant measurements has usually been a lengthy process (often taking weeks), and the amount of the milk consumed by the animals over the period of the study may be considerable.

It has been recognized that diet is one of the factors that can alter the pharmacokinetics and toxicity of a compound. The pattern, quantity, and content of dietary intake is able to change the responsiveness of a biological system to a toxicant, as well as to influence the bioavailability of the chemical in the system[20,21]. In the case of VOCs, it has been demonstrated that pharmacokinetics are directly correlated with the subsequent neurobehavioral toxicity for TRI[15] and toluene[22]. During inhalation exposure to TRI in rats, Warren et al.[15] demonstrated a correlation between the area-under-the-tissue-concentration time curve in the brain and the extent of depression of operant responding. However, the consequence of high milk intake on VOC pharmacokinetics that would occur during the behavioral sessions of neurobehavioral toxicity studies has not been examined previously. Since an effect on the pharmacokinetics of a toxicant often results in an alteration in toxicity, this study was undertaken to determine the potential impact of high milk intake during operant training on the pharmacokinetics of inhaled VOCs.

METHODS

Animals

CD-1 male mice from Charles River Breeding Laboratories (Raleigh, NC), weighing 30-35 g, were used in the study. The mice were housed on a constant light-dark cycle, with light from 0700 to 1900 hr, and darkness from 1900 to 0700 hr, in standard mouse cages and acclimated to the animal facility for at least one week, during which tap water and commercial rodent chow were provided ad libitum. The mice were then housed individually and restricted in food intake so that their body weight would be reduced over one week to and maintained at 80% of the original weight. Water access was not limited. Starting with the food restriction, the mice were administered a single bolus dose (1.0 ml) of evaporated milk, which has a fat content of 85 mg/ml, by gavage on a daily basis. A control group with similar food restrictions was gavaged with same volume of water. This was done between 1100 to 1200 hr every day, seven days a week, and the mice were maintained at this feeding protocol for three weeks until the chemical exposure.

Chemicals

1,1-1-Trichloroethane, of 99% purity and isooctane, of 99.98% purity, was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ).

Inhalation Exposures

Inhalation exposures were conducted in 1.0 M^3 Rochester-type dynamic flow chambers. Test atmospheres of TRI were generated by vaporization of the compound under different combinations of air flow- and temperature-controlled schedules. A steady flow of nitrogen gas carried the vaporized TRI from a flask into the exposure chamber influent air stream. The entire TRI generation system was enclosed in a specially fabricated safety box, which was maintained under

constant negative pressure during inhalation exposures in the same manner as the inhalation chamber. Exhaust air from the vapor-generation box and the inhalation chamber were vented to HEPA and charcoal filters, so that all air with any chemical was scrubbed before release to the environment. The exposure chamber was operated at flow rates of 7 to 15 ft^3 per minute. The concentration of TRI in the exposure chamber was monitored by an on-line Miran 1B2 infrared spectrophotometer (Foxboro Analytical, South Norwalk, CT) on the level of the mouse cage. Clean air was pumped through the chamber following the termination of the inhalation exposure.

Determination of percentage of recovery

The recovery rates of TRI in blood and tissues from the sample processing procedure were determined prior to the kinetic study, using the method described previously for rats by Chen et al.[23]. Ten chemical-naive mice were terminated by cervical dislocation. Blood samples of 0.5 ml were immediately withdrawn by closed chest cardiac puncture. The whole organ of spleen and portions (0.2-0.4 g) of liver, kidney, fat, muscle, heart, lung, gastro-intestinal tract (GI), and brain were removed and placed on an ice-chilled glass plate. Each sample was spiked with TRI by injection of 4 μl of a solution containing 1 mg TRI per 1 ml isooctane. All of the spiked samples were then processed immediately, and their TRI contents were analyzed as described in the following section.

Kinetic study

The mice that had been food restricted and gavaged with either milk or water for three weeks were exposed to 3500 ppm for either 10, 20, 40, 60, 80, or 100 minutes. At each time point blood samples (0.5 ml) were withdrawn by closed chest cardiac puncture immediately after the mice were sacrificed by cervical dislocation. Samples of liver, kidney, fat, muscle, heart, lung, gastro-intestinal tract (GI), spleen and brain were quickly removed and were

immediately placed into ice-chilled scintillation vials containing 4 ml of isooctane and 1 ml of 0.9% NaCl and tightly capped. The tissues were homogenized using a Ultra-Turrax SDT homogenizer (Tekmar, Cincinnati, OH) for 5-15 seconds. The vials were then vortex-mixed for 30 seconds and centrifuged at 1800 x g for 10 min at 4°C in capped vials. Aliquots of the isooctane supernatant layer were transferred into 20 ml headspace vials, which were capped immediately with teflon lined rubber septa and crimped to insure an airtight seal. The capped vials were then placed into a headspace autosampler unit of a Perkin Elmer Model 8500 gas chromatograph (GC) (Perkin-Elmer, Norwalk, CT). Analyses were carried out using a stainless-steel column (182 cm x 0.317 cm) packed with 3% OV-17 (Alltech Associates, Deerfield, Il). The GC operating conditions were headspace sampler temperature, 70°C; injection port temperature, 150°C; column temperature, 80°C; detector temperature, 360°C. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas, and a fixed volume of the isooctane supernatant injected automatically into the GC column. The limit of detection for TRI by the GC was less than 1 ng in 20 ml, or 8.4 ppb TRI in the air of the sample vial. All of the inhalation exposures for the kinetic study were conducted between 0900 to 1200. The collected samples were processed and analyzed immediately following the exposure.

Statistics

An independent t-test was used to detect the difference in TRI tissue concentrations between the milk-dosed and water-dosed groups. The level of significance was set at $p < 0.05$.

RESULTS

The mean values (N=10) of % recovery of TRI from the spiked blood and tissue samples are presented in Table 1. The lowest recovery rate (86.6%) was found in spleen, which had the smallest tissue mass (about 0.1 g) available for the processing procedure. The highest recovery (95.5%) was from lung tissue, which was followed closely by recovery from blood (94.7%). Indeed, all tissues but spleen and fat had greater than 90% recovery. The ten tissues showed an average of 91.45 ± 2.7% (mean ± SD) recovery of TRI from the sample processing procedure. Those recovery values were subsequently factored into the calculation of TRI tissue concentrations.

TABLE 1

Recovery of 1,1,1-Trichloroethane from
Mouse Blood and Tissues of Different Organs

Samples	Recovery (%)[*]
Liver	92.5 ± 1.7
Kidney	91.8 ± 1.8
Fat	88.1 ± 2.1
Muscle	92.4 ± 1.2
Heart	90.6 ± 1.0
Lung	95.5 ± 1.4
GI	91.8 ± 2.4
Spleen	86.6 ± 1.1
Brain	90.5 ± 1.9
Blood	94.7 ± 2.4

[*]Values represent the mean ± SE for recovery of TRI from blood and tissues of ten mice.

It took less than 3 minutes for TRI concentrations in the inhalation exposure chamber to reach the target level of 3500 ppm after the start of

introducing the chemical. The TRI concentrations were maintained at this level throughout the exposure period, with fluctuations within ± 5%.

The uptake of TRI into blood was rapid, with the TRI concentration increasing steadily over time and approaching near steady state within 40 minutes following the initiation of inhalation exposure in both milk- and water-dosed groups (Fig. 1A). The accumulation of TRI in the brain tissue increased steadily over the exposure period (Figure 1B). The highest TRI concentrations measured at 100 minutes in blood were 126.54 and 140.55 $\mu g/ml$ for treatment and control group, respectively, and in brain were 143.23 and 133.93 $\mu g/g$ tissue, respectively. In blood and in brain tissues, the two parameters of greatest interest relative to neurobehavioral toxicity with VOCs, TRI exhibited similar pharmacokinetic characteristics for both the treatment and the control groups, and there was no statistically significant difference in TRI concentrations of the two groups at any of the time points measured.

TRI concentrations in liver approached a near steady state equilibria for both milk- and water-dosed mice after approximately 60 minutes (Fig. 2A), as did the kidney TRI levels for the milk-dosed animals (Fig. 2C). The kidney concentrations of TRI in water-dosed animals appeared to be continually increasing over the exposure period, but there was no statistical difference between the concentrations of the treatment and control groups for the kidney at any time point. TRI concentrations increased in a similar pattern over time in lung (Fig. 2B), heart (Fig. 2D), and spleen (Fig. 3C) for both milk- and water-dosed mice. There appeared to be a pattern of a higher TRI concentration in muscle (Fig. 3A) and GI (Fig. 3B) in the water-dosed animals relative to the mice receiving milk, especially at the terminal time point at 100 min. However, there was no statistically significant difference at any time point.

TRI showed a very different kinetic profile in fat tissue (Fig. 3D) than in the blood and other tissues. Fat TRI concentrations continued to rise considerably over the course of the whole exposure period, with the highest concentrations measured at 100 minutes as 3326.8 and 3461.7 μg/g for the treatment and control group, respectively. TRI concentrations in fat were about 20 to 30 times higher than the corresponding concentrations in the other tissues. It does appear that fat TRI concentrations in both of the milk- and water-dosed mice had approached a near-steady state after about 60 minutes. There were no statistically significant differences between TRI concentrations in fat tissues of milk- and water-dosed mice at any time point, though. The areas-under-the-curve (AUCs) for the blood and tissue concentration-time courses were calculated and plotted in Fig. 4. The blood and all nonfat tissues had similar AUC values for both milk- and water-dosed mice of approximately 10 mg.min/g, while the AUCs for fat tissue were close to 200 mg.min/g.

DISCUSSION

Studies of the pharmacokinetics of inhaled chemicals, especially VOCs, are playing an increasingly important role in toxicology[24,25]. Because chemical toxicity is a dynamic process which depends on absorption, tissue uptake, interaction with cellular components, and elimination, it has been recognized that the results of toxicity studies need to be evaluated in terms of the characteristic pharmacokinetics of the agents[26]. It has also been suggested that the evaluation of endpoints in behavioral toxicology needs to be coordinated with pharmacokinetics as a sensible integration of behavior, physiology, and biochemical variables for VOCs[27]. Operant performance tests have been one of the most useful neurobehavioral tests in detecting behavioral changes in laboratory

animals following exposure to many VOCs, including TRI[11,18]. However, since there has been a paucity of data concerning tissue concentrations of VOCs, it has been difficult to evaluate the relative impact of the disposition of these neuroactive compounds in the brain as a target organ of interest in the conjunction with neurobehavioral studies of VOCs.

In conducting operant performance measurements, the use of milk presentation as a reinforcer in establishing operant behavior has proven very effective in the evaluation of the neurobehavioral effects of VOCs[16-19]. In many of these studies, the repeated administration of milk over time has resulted in the animals receiving a considerable cumulative dose of milk over the course of the behavioral study. In operant measurements in rats conducted to evaluate the neurobehavioral effects of TRI, Warren et al.[15] typically administered more than 200 ml of milk to the test animals during the training periods. This would result in a body burden of about 660 g milk per kg of body weight (bw) of these animals as a result of the training protocol. In the current study, mice received approximately 21 ml over a three week period, to result in an administered dose of about 840 g milk/kg bw in mice.

Such a large addition of lipophilic components in the diet from this high degree of milk dosage would be expected to result in an increase in adipose tissue and lipid content in various organs. It has been found that the increase in body fat in humans and animals that is concurrent with the aging process has been a very important factor in altering chemical distribution[28,29]. A greater volume of distribution for chemical disposition would be expected to result for lipophilic compounds when there is an increase in adipose tissue. At the sites of action of these agents, this could mean that there could be a transiently lower concentration of lipophilic chemical like the great majority of VOCs.

However, the most important factor in chemical distribution of these lipophilic compounds concurrent with an increase in body fat would be a pronounced increase in the body burden of these VOCs. Following repeated exposures of mice and rats to TRI, it is possible that the reported increased body burden of the compound was due to the concurrent increase in the volume of distribution of the fat compartment during that time period[30], as the high volatility and short half-life of the compound is known to typically result in its rapid elimination[31]. It was shown that physiologically-based pharmacokinetic model simulations of TRI disposition were dramatically improved in comparison to experimentally observed data when the volume of the animals's body fat compartment was increased from 7 to 18% of body weight by computer optimization[32].

In the current investigation, though, there were not any significant differences in the pharmacokinetics of TRI in the blood and nine tissues over a 100 minute time course of inhalation in the treatment and control groups of mice. In the blood and the brain, which would be expected to be the most important body compartments in relation to the neurobehavioral toxicity of VOCs, the time course of TRI following inhalation was remarkably similar between the mice that received water and those that were administered high doses of milk. The area under the blood and tissue concentration versus time curves have been proposed as very feasible measures of target organ dose for experimental comparisons[33], and there were only negligible differences between the two treatment groups for these parameters.

It might have been expected that the most vivid effect of the milk dietary addition would have appeared in the fat compartment, and there both the milk- and water-dosed animals appeared to reach a near steady-state in TRI fat concentrations after approximately 60 minutes of TRI inhalation. As for

individual nonfat organs, it has been proposed that a high lipid intake would increase lipid levels in the liver, which would enhance the target organ disposition there of lipophilic halocarbons[34]. However, there were no significant differences between the milk- and water-dosed mice in liver TRI concentrations over the time course of the inhalation exposures employed in the current study.

There has been a paucity of tissue time course data published for VOCs to be used in comparison with toxicity data, such as the neurobehavioral depression associated with most of these compounds. From blood and exhaled breath data during inhalation exposures, it has been proposed that short-chain aliphatic VOCs (such as TRI) would behave kinetically like other relatively water-insoluble gases[31,35]. The results of the current study indicated that this was also the case for uptake and disposition in the tissues following inhalation exposure in mice. TRI is a small, uncharged lipophilic molecule so it was readily absorbed across membranes of the pulmonary capillary bed into the systemic circulation, as evidenced by the rapid appearance of the compound in all nine tissues monitored within 10 minutes of the initiation of inhalation exposure. As the chemical accumulated in the blood and tissues, the uptake became progressively slower. This was reflected in the achievement of a near steady state equilibrium in most tissues after approximately 40-60 minutes of inhalation exposure to TRI.

It has been reported that the uptake and disposition of toluene[22] and TRI[15] in the blood and brain of rats was closely associated with the degree of neurobehavioral depression observed. It is reasonable to conclude, then, that the lack of the effect of the increased milk diet in mice on blood and tissue pharmacokinetics would preclude a pharmacokinetically related effect of milk diet on the neurobehavioral tests conducted in those animals. This would lend additional credibility to the continued use of milk as a reinforcer in neurobehavioral studies of lipophilic compounds such as VOCs.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

FIGURE 1

Concentrations of TRI in the A) blood and B) brain of mice following inhalation exposure to 3500 ppm for up to 100 minutes. The animals received a single gavage dose of either evaporated milk or water daily for 21 days. Blood and brain tissue were sampled at a time series of 10, 20, 40, 60, 80 and 100 minutes, and their TRI contents were analyzed by headspace gas chromatography. Values are expressed as the mean ± SE of 5 mice at each time point.

FIGURE 2

Concentrations of TRI in the A) liver, B) lung, C) kidney, and D) heart of mice following inhalation exposure to 3500 ppm for up to 100 minutes. The animals received a single gavage dose of either evaporated milk or water daily for 21 days. The tissues were sampled at a time series of 10, 20, 40, 60, 80 and 100 minutes, and their TRI contents were analyzed by headspace gas chromatography. Values are expressed as the mean ± SE of 5 mice at each time point.

FIGURE 3

Concentrations of TRI in the a) muscle, b) GI, c) spleen, and d) fat of mice following inhalation exposure to 3500 ppm for up to 100 minutes. The animals received a single gavage dose of either evaporated milk or water daily for 21 days. The tissue were sampled at a time series of 10, 20, 40, 60, 80 and 100 minutes, and their TRI contents were analyzed by headspace gas chromatography. Values are expressed as the mean ± SE of 5 mice at each time point.

FIGURE 4

The areas under the blood and tissue concentration versus time curves for animals that inhaled 3500 ppm TRI for up to 100 minutes. The animals received a single gavage dose of either evaporated milk or water daily for 21 days. The time interval is from 0 to 100 minutes.