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BIOAVAILABILITY OF ORGANIC SOLVENTS IN SOILS: INPUT INTO BIOLOGICALLY-BASED DOSE-RESPONSE MODELS FOR HUMAN RISK ASSESSMENT

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RESEARCH OBJECTIVE

The purpose of this study was to develop methods to expose rats and humans percutaneously and to use PBPK modeling to assess the percutaneous permeability of volatile compounds from aqueous or soil exposures. To estimate dermal absorption under realistic environmental exposure conditions, a patch system was developed that allowed for the volatilization of the compounds from the soil without contamination of inhaled or exhaled breath.

The end product for this research will be a tested framework for the rapid screening of real and potential exposures while simultaneously developing physiologically based pharmacokinetic (PBPK) models to comprehensively evaluate and compare exposures to volatile chemicals from either contaminated soil or water.

RESEARCH STATEMENT

Assessment of dermal exposures is an important component of risk assessment for any compound that may come into contact with the skin. The *a priori* assumption that oral and/or inhalation routes of exposures are the most important may be a gross oversimplification, as the dermal route may significantly contribute to total body burden {DOURSON1997, GUY1993, JO1990, ROY1998, WEISEL1996, WESTER1989}. Dermal exposure can occur from water and soil contact during normal every day activities, such as washing, swimming, recreation, and gardening, as well as through many work-related activities. The research described here focused on developing methods that would employ recent advances in atmospheric sampling mass spectroscopy (MS/MS) coupled with physiologically based pharmacokinetic (PBPK) modeling to evaluate the influence of exposure matrix (soil or water) on the real-time kinetics and bioavailability of volatile solvents.

The extent to which a chemical is absorbed through the skin varies greatly depending on multiple factors, including: chemical properties and concentration at the skin surface, temperature, location and surface area exposed, duration of exposure, exposure matrix, skin hydration, and rate of absorption {USEPA1992} . Quantitating the dermal penetration rate, or flux, is an integral part of dermal exposure risk assessment.

Exhaled breath measurements repeatedly collected during the rapid kinetic changes that occur during, and immediately post exposure, is an ideal non-invasive means for assessing bioavailability of volatile compounds. Breath measurements are useful in environmental exposure studies, particularly when repeated samples collected in real time allows for the tracking of trends in the changing matrix {GORDON1998, WALLACE1993, WEISEL1992}. Recently, a breath-inlet system has been developed that allows for direct, continuous real-time analysis of exhaled air from rats and humans {KENNY1991, THRALL1996}. The system uses a Teledyne 3DQ Discovery ion trap mass spectrometer (MS/MS) equipped with an atmospheric sampling glow discharge ionization source (ASGDI). The MS/MS system provides an appraisal of individual chemical components in the breath stream in the parts-per-billion (ppb) detectable range.

Breath analysis data has been analyzed for the dermal absorption of methyl chloroform (MC: 1,1,1-trichloroethane) and trichloroethylene (TCE) using PBPK models. PBPK modeling is frequently used to describe the biokinetics (absorption, distribution, metabolism and elimination) of a chemical in experimental animals and man. Physiologically based pharmacokinetic (PBPK) models are particularly well suited to describe the transdermal flux during non-steady state absorption. Since PBPK models

have a fundamental biological basis (i.e. tissue characteristics, blood flow, metabolism rates, etc.) they are ideal for estimating kinetic parameters from data following dermal exposure as a function of the exposure matrix and duration. Previous investigators have used PBPK models to integrate chemical absorption, distribution, metabolism, and elimination with transdermal flux equations to estimate the skin permeability coefficient (K_P) {CORLEY1999, JEPSON1997, MCDOUGAL1990, ROY1998}.

By combining dermal exposures to organic solvents in soil and water, the novel MS/MS breath analysis system, and PBPK modeling, this research will substantially improve risk assessment methodologies by providing an understanding of the effect of exposure matrix on pharmacokinetics and replace default assumptions of dermal bioavailability.

RESEARCH PROGRESS

Materials and Methods

Study Design

Significant levels of trichloroethylene (TCE), perchloroetheylene, benzene, and trichloroethane (methyl chloroform: MC) are found as soil contaminants at numerous sites within the DOE complex. Accordingly, these solvents have been chosen for examination in this study. Each chemical is being fully investigated sequentially across all specific aims. In this manner, analysis of each chemical will build upon the methodologies and results from previous chemicals. To date, rat and human exposures to MC and TCE, and Rhesus monkey exposures to benzene have been completed.

Human exposures to MC and TCE in water have been to concentrations of 0.1%, which is within the limit of solubility for these chemicals. Human and monkey soil exposures have been to 1.0%. Dermal exposures to benzene in Rhesus monkeys were to 0.18%, which is at the solubility limit.

A range of exposure times and concentrations have been compared in rats. Headspace gas chromatography (GC) analysis has been used to verify actual exposure concentrations. Rodent dermal soil exposures ranged from 0.03-0.6% MC and 0.05-1% TCE. Water concentrations were to $0.10 \pm 0.0051\%$ for MC and to 2 water concentrations for TCE, 0.29 ± 0.0013 % and $0.11 \pm 0.0031\%$.

PBPK Model Development

Previously established PBPK models for MC (Reitz et al., 1995) and TCE (Fisher et al., 1998) have been modified to include a separate skin compartment to describe the uptake of chemical following dermal exposure as described by Jepson and McDougal (1997). Generally the skin is assumed to act like a simple membrane, and Fick's law is used to describe transdermal flux:

$$J=K_PA\Delta C$$

where flux (J) is a function of the permeability constant (K_P), the area exposed (A) and the concentration gradient across the skin (ΔC). This flux equation assumes that the concentration inside the skin is uniform, which is only correct at steady state.

PBPK models can be used to assess K_p during non-steady state conditions. The dermal PBPK model also incorporates specific descriptions for richly and slowly perfused tissue, fat, liver, lung, and skin tissues (Fig 1.). MC- and TCE-specific parameters, including

blood:tissue partition coefficients and metabolism rates have been taken from the literature {FISHER1998, REITZ1988}, and dermal absorption rate constants have been experimentally determined by fitting constants to the data obtained from the expired air of rats and humans in MC. Preliminary K_p values have also been fit for rat exposures to TCE in water.

Application Techniques

Exposure systems were developed to expose rats and humans to water (occluded) or soil (non-occluded). The water exposure system consisted of a hand-blown glass cell (O.Z. Glass Co., Pinole, CA) with a sealable opening in the top to allow addition of the dosing solution. For rats, the glass cell was 2.5-cm diameter across and 1.2 cm deep. A larger glass cell was used for human exposures (4.0-cm x 2.5-cm). For non-occluded soil exposures, the glass exposure cell included an additional compartment for charcoal that was separated from the soil exposure compartment by a vapor permeable ceramic partition (Fig. 2). The glass cell was attached using a cyanoacrylate adhesive to either the forearm of volunteers or a clipper shaved area on the back of the rats.

Target exposure concentrations and the contents of the exposure systems (patch systems and charcoal) were analyzed by headspace gas chromatography to quantitate exposure doses, remaining chemical at the end of the exposure, and volatilized chemical. Charcoal from the non-occluded patch system was extracted using toluene and chemical concentrations measured by GC. TCE was use as an internal standard for MC studies, and MC was used as the internal standard for TCE studies.

To simulate a person working/gardening in soil or bathing, volunteers immersed a hand in containers with 0.1% MC or TCE in 4 kg of water or 4 kg of soil. The water or soil was prepared in a separate room, covered with plastic wrap and each subject placed their left hand in the container. A 4 minute background breath sample was collected from each subject before the exposures began and exhaled breath was monitored throughout the 2 hr exposure period and for an additional 2 hr post exposure.

Analytical Methods

Rats

Immediately following dermal application, the rats were individually placed in off-gassing chambers as described by Gargas et al. (1990). Intensity data from the MS/MS is converted to concentration (ppb) through the use of external standards and a calibration curve that was generated each day of experimentation.

Humans

Each volunteer was provided fresh breathing air on demand via a facemask with a two-way non-rebreathing valve to eliminate inhalation exposure. The facemask was connected to the inlet manifold of the Teledyne 3DQ MS/MS through a heated mixing chamber.

Results and Discussion

Rat water exposures

Representative examples of the exhaled breath concentrations of MC and TCE following dermal exposures (5 cm^2 surface area) to either chemical in 5 ml of water in a fully occluded patch is shown in Fig 3. In rats, peak exhaled breath concentrations of MC

after exposures to 0.1% were approximately 1400 ppb and were reached within 1 hr and declined slowly over the next 8 hr. TCE exhaled breath concentrations were much lower, most likely due in part to a higher rate of metabolism and differing tissue solubilities. The slow decline demonstrates that occlusion results in continuous exposure.

Table 1 shows the PBPK model estimations of the permeability coefficients (K_P) for dermal absorption of MC and TCE. The percentage of applied MC absorbed by the rat differed with exposure duration, with approximately 61% taken up by 4 hr and 85% after 8 hr of exposure. With the occluded water patch system, approximately 38% and 15% of the applied MC dose was recovered in the dosing media after 4 hr and 8 hr of exposure, respectively, confirming no loss of MC to the system (Fig. 4).

Rat Soil Exposures

The exhaled breath concentrations following dermal exposures in soil peaked at less than ½ hr for both chemicals and declined rapidly. At 0.1% exposures peak chamber concentrations were approximately 400 ppb and 500 ppb for MC and TCE, respectively. Peak chamber concentrations following exposures to 0.5% in soil were around 1500 ppb and 2500 ppb for MC and TCE, respectively. This may reflect either a lower rate of volatilization or a higher K_p for TCE than for MC. Fig. 5. Shows a dermal dose response for MC exposures in soil.

The rate of loss of MC to the system (k_1) was an integral factor in modeling the dermal uptake of MC from non-occluded soil (Fig. 4). Rates of loss were optimized to the final concentrations of MC recovered in the charcoal and other patch components as determined by GC analysis. The percentage of MC lost to the patch system was relatively constant for all samples at all concentrations, and k_1 was optimized for each data set. Roughly 65% of the original MC concentration was volatilized to the surrounding patch system and not available to be absorbed from the non-occluded soil. In 3 of the soil samples collected at the end of the 2.5 hr exposures, 0.02 - 0.1% of the original MC concentration still remained, no MC was detected in the other 5 soil samples. The K_P for the uptake of MC from the non-occluded soil patch was estimated using the PBPK model to be 0.15 (cm/hr), lower than that estimated for absorption of MC in water.

Human Subjects

MC and TCE were both found in the exhaled breath of human subjects following whole hand exposures in containers, with peak exhaled breath levels ranging from 1100 ppb to 1500 ppb following exposures to 855 - 1399 mg/kg MC (0.0855 - 0.1399%) in water. A lag time in the appearance of MC in exhaled breath occurred in all subjects and ranged from 0.7 - 1.3 hr. The lag time represents the initial partitioning into the stratum corneum and subsequent diffusion into the viable epidermis and capillary blood supply. Figure 6 shows the exhaled breath data from a representative volunteer exposed to MC in soil and water. Following exposures ranging from 606-1390 mg/kg TCE (0.0606-0.139%) in water, peak exhaled breath concentrations ranged from 500 - 100 ppb and the lag time for appearance was 0.1-0.4 hr. Figure 7 shows the exhaled breath data from a representative volunteer exposed to TCE in soil and water.

The exhaled breath data was analyzed using the PBPK model with physiological parameters set for humans. The concentration of MC in the container of water or soil was constant over the 2-hr exposure period as measured by headspace GC analysis and the concentration of TCE dropped by about 30%. For human exposures, the rate of

loss of MC from the exposure system (k_1) were set to zero and for TCE the rate of loss from the containers was about 0.2 hr^{-1}. For simplicity, this study assumed that all subjects had the same blood flow rates with only differences in the skin permeability coefficients accounting for inter-subject variability in exhalation of unmetabolized MC or TCE. The K_p for human dermal absorption of MC and TCE in soil and water estimated using the PBPK model are given in Table 1. Less than 0.2% of the amount of MC in the water was absorbed by human subjects exposed for 2 hr.

	Methyl Chloroform	*Trichloroethylene*	*Benzene*
Rat	K_p (cm/hr)	K_p (cm/hr)	K_p (cm/hr)
Water	0.25 ± 0.002	0.33 ± 0.057	In Progress
Soil	0.15 ± 0.006	In Progress	
Human			**Monkey**
Water	0.006 ± 0.0006	0.016 ± 0.002	In Progress
Soil	0.0015 ± 0.0005	0.007 ± 0.002	

Table 1. Skin permeability coefficients for MC and TCE estimated using PBPK models.

Rhesus Monkey Benzene Exposures

New methodologies were developed to sample exhaled air from an anesthetized monkey. A mask was placed over the nose and mouth and an endotracheal tube inserted. A ¼" teflon tube was fitted inside the endotracheal tube and inserted half way down. The MS/MS sampled air through the teflon tubing and excess exhaled air was allowed to escape around the sides. The monkey's right hand was placed into a container of water or soil up to the wrist and exposures carried out as for humans. Example exhaled breath data are given in Fig. 8.

Summary

Several studies have shown that rat skin permeability is generally greater than human skin permeability {JEPSON1997}. We have verified this to be the case for MC and TCE. In addition, the apparent k_p for MC is higher in water than from soil exposures in both rats and humans. Likewise, the apparent skin permeability of TCE is roughly double for human water exposures than for human soil exposures.

The validated PBPK model was used to simulate the relative contribution of dermal uptake to the total body burden of MC expected via drinking water consumption. Default EPA values for dermal contact with soil were used to estimate total body absorption following exposures to MC in water or soil (USEPA, 1991). In this simulation, the exposed surface area was fixed at 5700 cm^2 (approximately 30% of total body surface area) at a soil burden of 0.08 mg/cm^2, and the media MC concentration was assumed to be 0.01%. If a 70-kg human consumes 2 L of water/day and is exposed to MC in water or soil for 2 hr, the relative contribution of dermal absorption vs. oral is approximately 0.2, 1.1, and 3.2% of the total body burden for non-occluded soil, occluded soil, and

occluded water, respectively. This relative contribution increases for longer dermal exposure periods for occluded systems. For example, using the default EPA assumption of 24 hr dermal exposure, the percentages of dermal contribution increase to 9.3 and 16.2% for occluded soil and water, respectively. The loss rate estimated for the rodent studies was used to simulate a non-occluded exposure, in this case MC would volatilize and no significant increase in dermal obsorption occurs after 2 hr. In either scenario, the most significant route of exposure is by oral ingestion (Fig. 9).

Future Work

Work is in progress to analyze the soil and water samples from the monkey benzene exposures and to develop a PBPK model for the monkey. Rat exposures are currently underway. Once these objectives are completed, the exhaled breath data will be analyzed with the PBPK model to determine the skin permeability coefficient (k_p). In addition, perchlorethylene rat and human exposures and PBPK modeling will be completed in the next year.

The end result of this research will be a group of PBPK models that describe the dermal absorption of a cross-section of volatile organic chemicals. These data and models will be used to quantitatively compare and describe the effects of exposure matrices, soil or water, or percutaneous absorption of volatile organic chemicals.

Publications and Presentations

1) Thrall, KD, Poet, TS, and Corley, RA. 1998. An innovative method to determine percutaneous absorption: Real time breath analysis and physiologically based pharmacokinetic modeling. IN: *Percutaneous Absorption* (R Bronaugh and H Maibach, Eds.), Third Edition, Marcel Dekker, Inc. New York. 1999.

*2) Wester,RC, Hui,X, Maibach,HI, Poet,TS, Weitz,KK, Edwards,JE, Corley,RA, and Thrall,KD. 1999, An innovative method to detemrine dermal uptake of solvents from soil and water in vivo in humans. The Toxicologist, 48: 338,1999.

3) Poet,TS, Corley,RA, Thrall,KD, and Wester,RC. 1999, Assessing the dermal bioavailability of volatile organics in rats. The Toxicologist, 48:339, 1999.

4) Poet,TS, Thrall,KD, Corley,RA, Hui,X, Edwards, JE, Weitz, K, Maibach,HI, Wester,RC. Real time breath analysis and physiologically based pharmacokinetic modeling to determine the percutaneous absorption of methyl chloroform in rats and humans. Toxicol. Sci. Submitted.

5) Wester, RC, 1998. Presentation “Dermal Bioavailability” at NIOSH, Morgantown, W. Va. Sept., 1998.

6) Wester, RC. 1998. Chemical Manufactures Association Workshop of Research Planning. Research Triangle Park, N.C. May, 1998.

7) Wester, RC. Scheduled 10/99. 15th Annual International Conference on Contaminated Soils and Water. University of Massachusetts, Amherst.

* Society of Toxicology Risk Assessment Award, 1999.

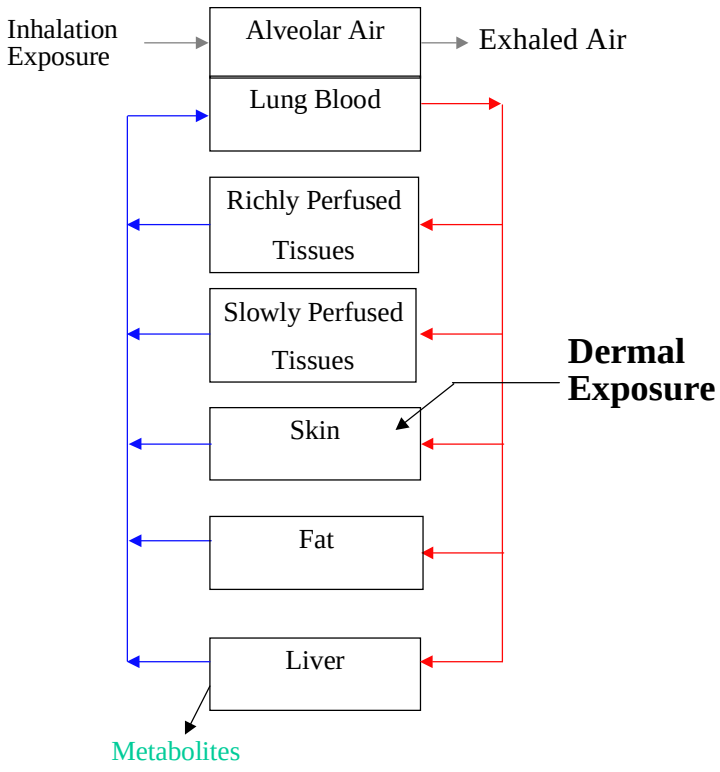

Fig. 1. Physiologically based pharmacokinetic model used to describe the pharmacokinetics of methyl chloroform and TCE in rats and humans during dermal exposures.

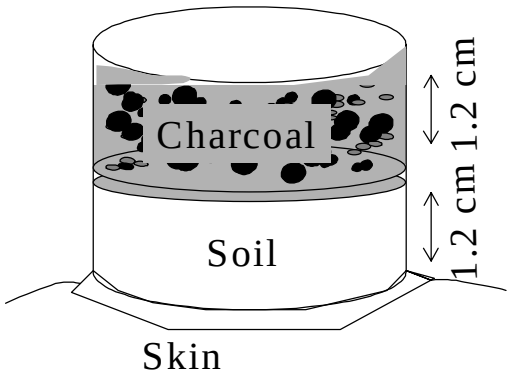

Fig. 2. Patch system for non-occluded exposures to chemical in soil, dimensions are for the rat exposure system.

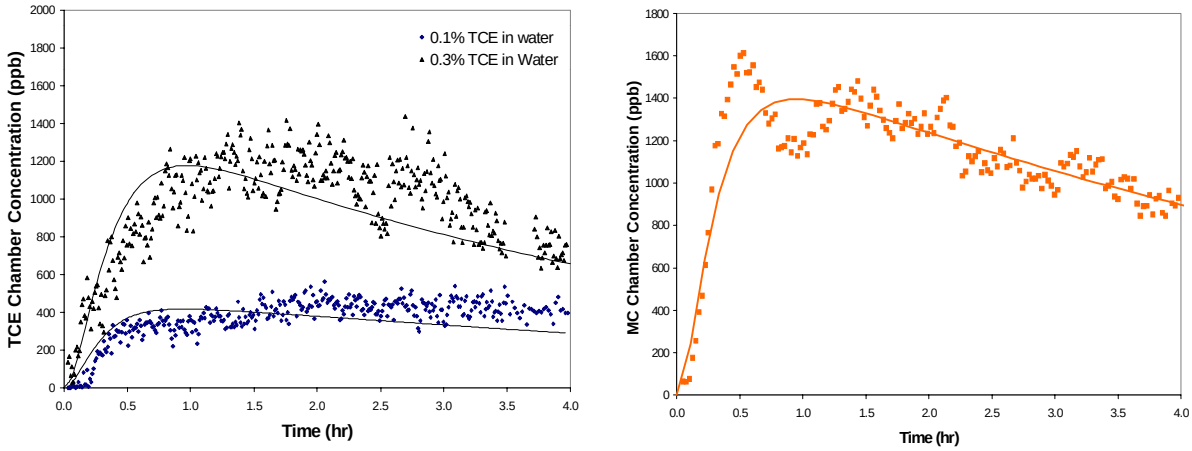

Fig. 3. Dermal exposures of rats to TCE (left) and MC (right) in water. Data points are chamber concentrations averaged approximately every minute. Lines represent PBPK predictions of exhaled breath profiles.

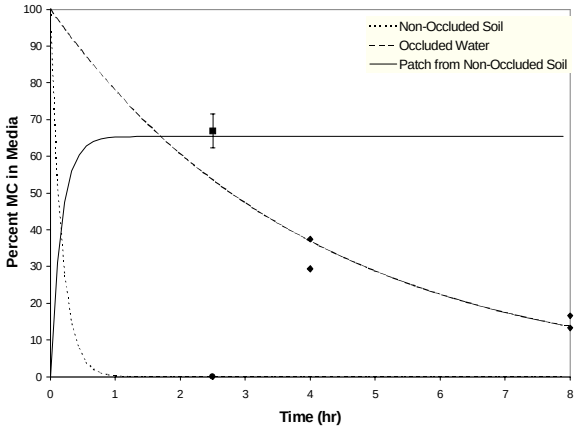

Fig. 4. Percentage of MC in the exposure media (soil or water) and volatilizing to the total patch system over time for soil exposures. Lines are PBPK estimations, points are concentrations measured by GC headspace analysis. For percentage in patch n = 6, for percentage remaining in soil n = 3, for water n = 2 at 4 hr, and n = 2 at 8 hr.

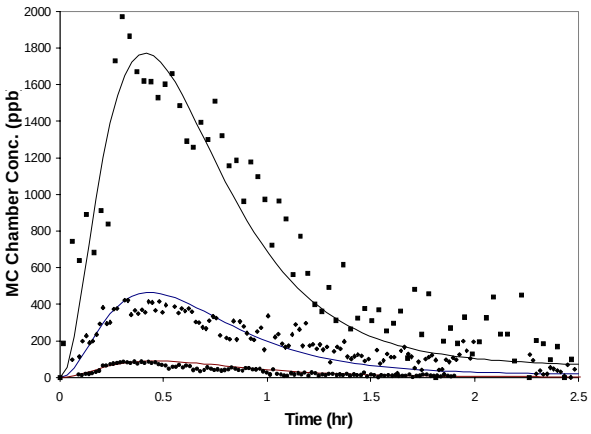

Fig. 5. Dermal exposures of rats to 0.03, 0.1, and 0.5% MC in soil. Data points are chamber concentrations averaged approximately every minute. Lines represent PBPK model predictions of exhaled breath profiles.

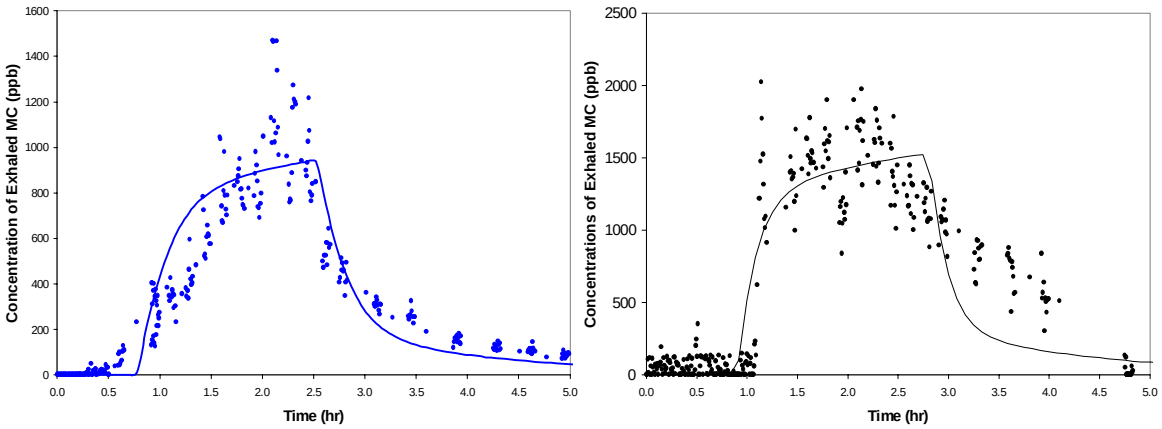

Fig. 6. Dermal exposures of a representative human volunteer to 0.1 % MC in water (left) and 0.75 % MC in soil (right). Data points are exhaled breath concentrations averaged approximately every minute. Lines represent PBPK model predictions of exhaled breath profiles.

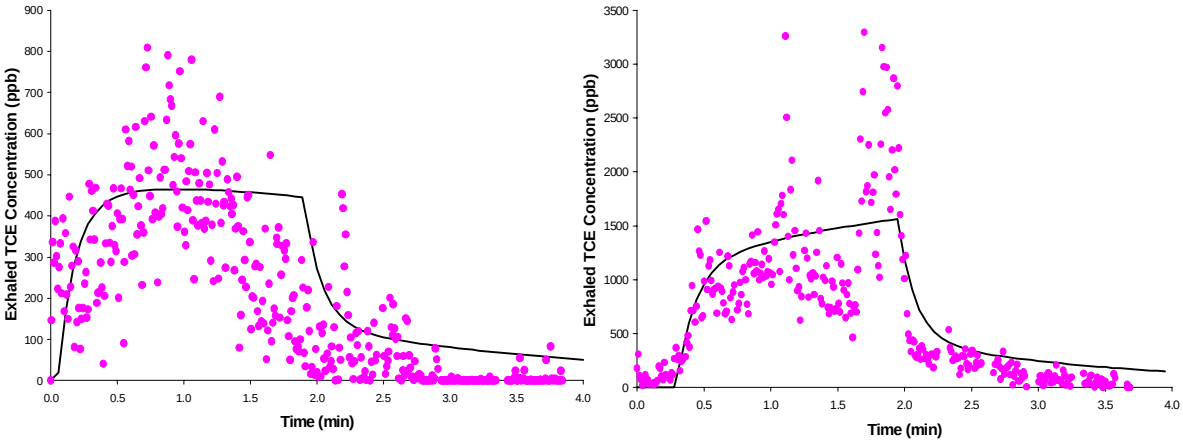

Fig. 7. Dermal exposures of a representative human volunteer to 0.1% TCE in water (left) and 0.4% TCE in soil (right). Lines represent PBPK model predictions of exhaled breath profiles.

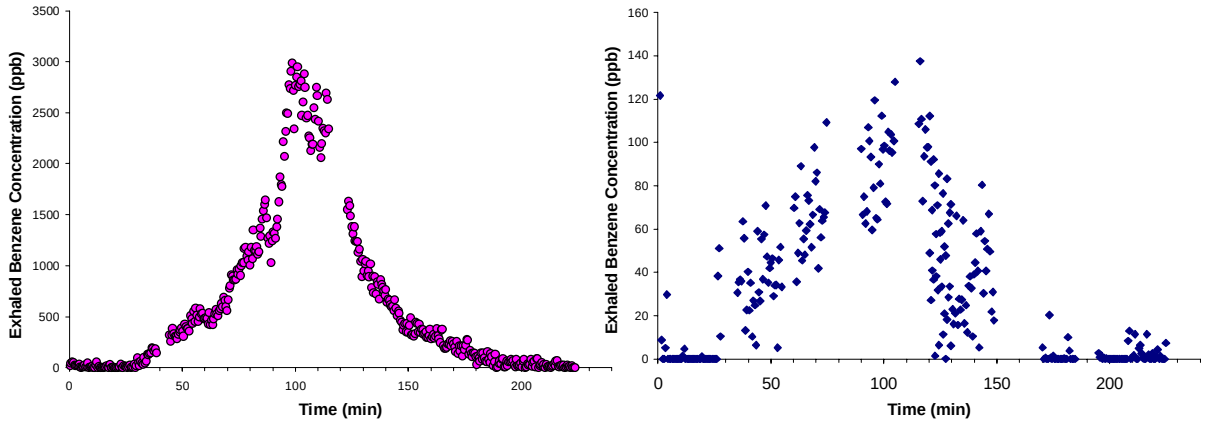

Fig. 8. Dermal exposures of representative female Rhesus monkeys to 0.18% benzene in water (left) and 1.% benzene in soil (right). Data points are exhaled breath concentrations averaged approximately every minute.

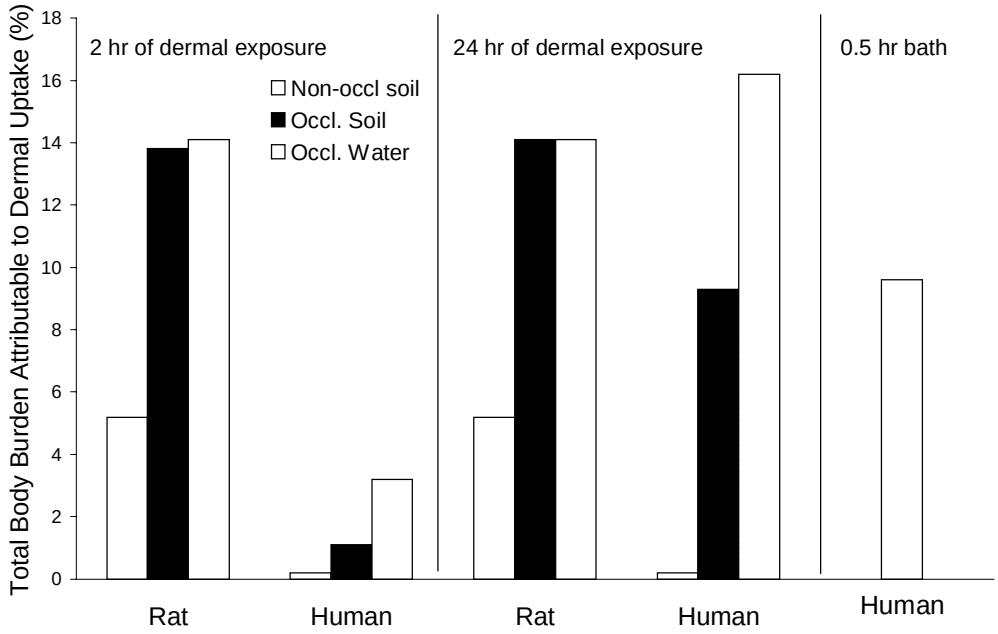

Fig. 9. The percentage of MC absorbed through dermal exposures is compared to model estimates of the amount absorbed for an oral equivalent dose calculated assuming 100% oral bioavailability and subjects consumed 2 L (human) or 40 ml (rat) of water/day. EPA default assumptions were used to assign the amount of media (soil or water) at 0.08 mg/cm^2 surface area exposed and exposure areas of 5700 cm^2 (human) and 81.8 cm^2 (rat). For bathing exposure the surface area was estimated from the total body surface area minus the head (9%).