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EVALUATION OF THE DERMAL ABSORPTION OF AQUEOUS TOLUENE IN F344 RATS USING REAL-TIME BREATH ANALYSIS AND PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING

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Toluene is a ubiquitous chemical that is commonly used for its solvent properties in industry and manufacturing, and is a component of many paint products. Because of its widespread use, there is potential for both occupational and nonoccupational dermal exposure to toluene. To understand the significance of these exposures, the dermal bioavailability of toluene was assessed in F344 male rats using a combination of real-time exhaled breath analysis and physiologically based pharmacokinetic (PBPK) modeling. Animals were exposed to toluene at 0.5 or 0.2 mg/ml aqueous concentration (0.05% or 0.02%) using a 2.5-cm-diameter occluded glass patch system attached to a clipper-shaved area on the back of the rat. Immediately following exposure, individual animals were placed in a glass off-gassing chamber and exhaled breath was monitored as chamber concentration in real time using an ion-trap mass spectrometer (MS/MS). The real-time exhaled breath profile clearly demonstrated the rapid absorption of toluene, with peak chamber concentrations observed within 1 h from the start of exposure. The PBPK model describing the exposure and off-gassing chamber was used to estimate a dermal permeability coefficient (K_p) to describe each set of exhaled breath data. Regardless of exposure level, a single K_p value of 0.074 ± 0.005 cm/h provided a good fit to all data sets. These rat studies using aqueous toluene will form the basis for comparing the dermal bioavailability of toluene in various paint products and may ultimately aid in understanding human health risk under a variety of exposure scenarios.

From the 1950s through the 1970s, the rate of uptake of a chemical through the skin was generally estimated based on studies of humans (Paustenbach & Leung, 1993). More recently, evaluations of dermal uptake of a chemical are made using animal skin (in vivo or in vitro) or human skin in vitro. In vivo, the rate of uptake of a chemical through the skin has been estimated using radiolabeled compounds and tracking the radioactivity in blood and excreta following topical application (Paustenbach & Leung, 1993). While this method for determining percutaneous absorption provides an estimate of the total absorbed dose, it often fails to reveal information of the uptake, distribution, and clearance phases of dermal absorption kinetics.

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Further, since blood levels may be very low in these situations, this practice is often restricted by sensitivity limits of the assay or analysis. An additional drawback with this methodology is that the nature of the radioactivity, whether it represents the parent or metabolites, is often undefined, and thus kinetic interpretation is also limited.

Recent studies have illustrated that exhaled breath presents a useful alternative to radiotracer studies by providing a noninvasive methodology for assessing bioavailability of volatile compounds (Corley et al., 2000; Thrall et al., 2000). Breath measurements are particularly useful in studies where repeated samples collected in real time allows for the tracking of trends. Since breath concentrations reflect blood concentrations, continual analysis of exhaled breath provides a unique opportunity to evaluate differences in the rapid exponential emptying of the blood compartment that occurs immediately following peak exposure. Furthermore, the noninvasive nature of breath analysis improves the participation rate in controlled human exposure and environmental or occupational biomonitoring studies.

For exhaled breath measurements to be useful, they must be evaluated using some form of a kinetic model. Physiologically based pharmacokinetic (PBPK) models are particularly useful for integrating a variety of data, including breath analysis, to determine the penetration rates of chemicals through the skin. A PBPK model is particularly well suited for assessing dermal exposures under non-steady-state conditions (Jepson & McDougal, 1997) where the transdermal flux is a function of the permeability coefficient (K_p), the area exposed, and the changing concentration gradient across the skin. The integration of real-time exhaled breath measurements with a PBPK model to determine dermal absorption has been successfully used for a number of compounds, including methyl chloroform, trichloroethylene, and benzene (Thrall et al., 2000).

The objective of the study presented here was to evaluate the dermal absorption of toluene in rats using exhaled breath and PBPK modeling. Toluene is a clear, colorless liquid with a sweet odor used in making paints, paint thinners, fingernail polish, lacquers, adhesives and rubber, and is added to gasoline (U.S. EPA, 1990). Because of its widespread use, there is potential for both occupational and nonoccupational exposure to toluene. Exposure to toluene is most likely to be through inhalation; however, an understanding of the dermal contribution to total uptake is useful for predicting realistic human exposures. Furthermore, although these studies were conducted using aqueous toluene, the resulting permeability estimates will form the basis for comparing relative dermal bioavailability of toluene in the organic matrices of occupational and consumer products.

METHODS AND MATERIALS

Animals

Male F344 rats (200–250 g) were obtained from Charles River Breeding Laboratory (Raleigh, NC). Animals were housed in solid-bottom cages with

hardwood chips, and were acclimated in a humidity- and temperature-controlled room with a 12-h light/dark cycle for at least 5 d prior to use. Certified Purina rodent chow (Ralston Purina Co., St. Louis, MO) and water were provided ad libitum throughout the acclimation period.

Rat Dermal Exposure Conditions

Dermal exposures were conducted as described previously (Thrall et al., 2000) with modifications. In brief, animals were prepared for application of the dermal patch the day prior to experimentation by lightly clipper shaving the hair on the lower back under gentle restraint. The aqueous exposure patch consisted of a 2.5-cm inner diameter hand-blown glass cell (O.Z. Glass Co., Pinole, CA) with a needle hole opening in the top to allow addition of the dosing solution. The cell was attached to the shaved area on the lower back of the animal using a cyanoacrylate adhesive and allowed to dry overnight. On the day of exposure, approximately 2 ml aqueous toluene was added to the exposure cell by passing a 23-g blunt-tip needle on a gas-tight syringe through the needle hole, which was then sealed using silicone. Actual dosing volume was determined by weighing the syringe before and after dosing. The surface area of skin exposed was 4.9 cm^2.

Animals were exposed dermally to aqueous toluene at 1 of 2 target concentrations of 0.5 mg/ml (0.05%; $n = 3$) or approximately half that value at 0.25 mg/ml (0.025%, $n = 3$). Dermal dosing solutions were prepared fresh on the day of the experiment in a small volume with shaking to ensure the solution was well mixed. Target concentrations were selected to stay below the solubility limit of toluene (0.07%) and still achieve measurable toluene levels in the exhaled breath of the animal exposed. To quantitate total absorbed dose, a weighed aliquot (100 µl) was collected from the original dosing solution and from the remaining solution at the end of exposure. These samples were analyzed by a gas chromatographic (GC) headspace method using a Hewlett-Packard model 5890 Series II (Avondale, PA). The GC used a hydrogen flame ionization detector (FID) with nitrogen as the carrier gas. The column was a DB-Wax, 30 m, 1.5 µm thickness (Restek, Bellefonte, PA). The detector was operated at 275°C, the inlet at 180°C, and the final oven temperature was 180°C. Under these conditions, toluene had a retention time of approximately 0.6 min.

Real-Time Breath Analysis System

The exhaled breath monitoring system utilized during the animal studies consisted of a small glass off-gassing chamber connected directly with a Teledyne Discovery II (LGC, Inc., San Jose, CA) tandem ion trap mass spectrometer (MS/MS) equipped with an atmospheric sampling glow discharge ionization (ASGDI) source. The animals were individually placed in the small glass off-gassing chambers immediately following dermal application. Animals were awake and could move freely while in the off-gassing chamber. Certified "Grade-D" breathing air was supplied to the animal through the lid of the off-gassing chamber at a calibrated rate of approximately 12 L/h. The

ASGDI-MS/MS system continually withdrew air samples from the off-gassing chamber through a port in the lid at the same rate of approximately 12 L/h to provide a new data point every 1.6 s. The concentration of toluene in the chamber was used to represent exhalation from the animal using PBPK model equations as described by Gargas (1990):

$$\frac{dA_{Ch}}{dt} = (Q_{Alv} \times C_{Exh} - Q_{Alv} \times C_{Ch}) - F_{Ch} \times C_{Ch}$$

where A_{Ch} is the amount of toluene in the chamber (μg), Q_{Alv} is the alveolar breathing rate (ml/h), C_{Exh} is the concentration exhaled (μg/ml), C_{Ch} is the concentration in the chamber (μg/ml), and F_{Ch} is the air flow through the chamber (ml/h).

Intensity data from the mass spectrometer were converted to concentration (ppb) using external gas standards prepared in Tedlar bags and a calibration curve. A new calibration curve was generated every day of operation. The ASGDI–MS/MS methodology had detection limits in the 2–10 ppb range for toluene.

At the end of the monitoring period, animals were humanely sacrificed by CO_2 asphyxiation and returned to the off-gassing chamber for several minutes to verify that chamber concentration reflected toluene eliminated via the exhaled breath, and not leakage from the exposure patch system.

PBPK Model

The toluene PBPK model was adapted from Tardif et al. (1993) and modified by the addition of a skin compartment (Figure 1). Anatomical compartments in the model were used to describe the distribution of toluene into the rapidly perfused, slowly perfused, fat, liver, and skin compartments. The skin compartment in the model represented exposed skin; nonexposed skin was lumped into the slowly perfused compartment. Total skin, with a volume of 10% of the body weight, is assumed to receive 5% of the cardiac output. The exposed skin volume and blood flow rate were calculated as described by Jepson and McDougal (1997). For toluene, metabolism has been shown to occur primarily in the liver, and to be independent of the route of exposure (Cohr & Stokholm, 1979). Although metabolism at the site of entry is considered possible, this loss is assumed to be insignificant in comparison to liver metabolism. Therefore, metabolism was described as occurring only in the liver. Model parameters specific to the physiology, partition coefficient, and metabolism were taken from the literature (Table 1). To simulate dermal exposures, the rate of change in the concentration of toluene in the skin compartment (C_{sk} μg/ml) was related to the rate of penetration through the skin (the flux) and the rate of delivery due to blood flow and arterial concentration (the perfusion) as described by Thrall et al. (2000). In the PBPK model, this is written as:

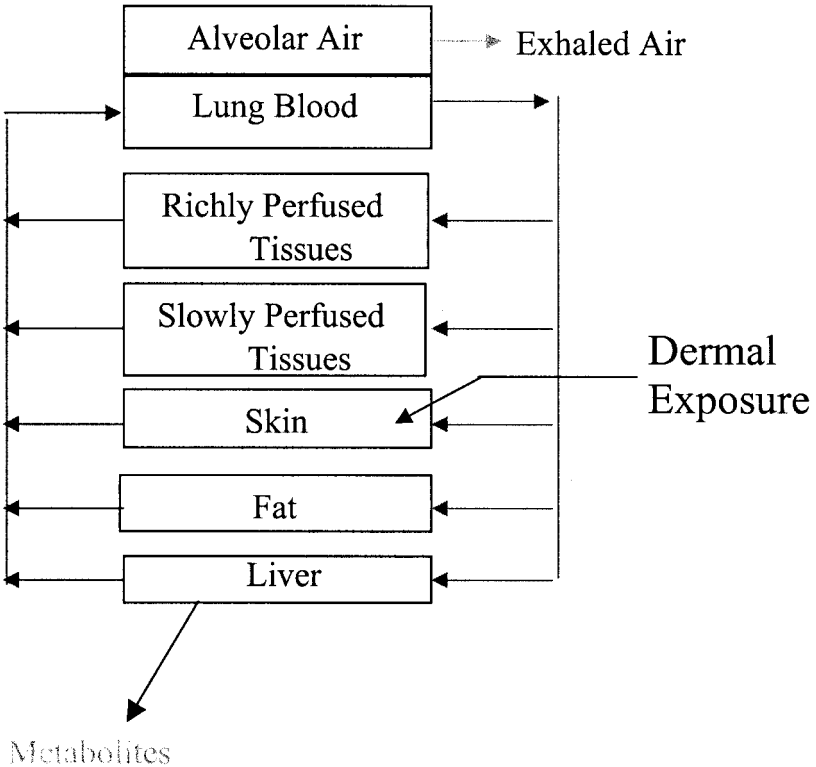

FIGURE 1. Schematic representation of the toluene PBPK model including the skin compartment.

$$V_{sk}\frac{dC_{sk}}{dt} = K_p A\left(C_{liq} - \frac{C_{sk}}{P_{sk/liq}}\right) + Q_{sk}\left(C_a - \frac{C_{sk}}{P_{sk/b}}\right)$$

where V_{sk} is the volume of the skin under the exposure cell (ml), K_p is the permeability coefficient (cm/h), A is the exposed surface area (cm^2), Q_{sk} is the blood flow rate to the skin (ml/h), C_a is the arterial concentration (μg/ml), C_{sk} is the skin concentration (μg/ml), C_{liq} is the liquid toluene concentration (μg/ml), $P_{sk/b}$ is the toluene skin to blood partition coefficient (unitless; calculated by dividing the toluene solubility ratio of skin:air by blood:air), and $P_{sk/liq}$ is the toluene skin to water partition coefficient (unitless; calculated by dividing the toluene solubility ratio of skin:air by water:air).

The skin permeability coefficient (K_p) for each individual data set was estimated from this equation based on the kinetics of absorption as described by the exhaled breath. A maximum likelihood search algorithm in SimuSolv (version 3.0; Dow Chemical Co., Midland, MI) was used to vary the K_p coefficient until an optimal fit was achieved that described the time-course data.

TABLE 1. Toluene PBPK Model Parameters

Parameter	Rat
Body weight (kg)	0.23-0.41
Cardiac output (L/h)	5.4
Alveolar ventilation (L/h)	5.4
Blood flow (% cardiac output)	
Liver	25
Fat	5
Rapidly perfused	51
Slowly perfused	15
Total skin	5
Tissue volume (% body weight)	
Liver	4
Fat	8
Rapidly perfused	5
Slowly perfused	64
Total skin	10
Metabolic constants	
V_{max} (mg/kg/h)	4.68
K_m (mg/L)	0.55
Partition coefficients	
Saline:air	1.2
Blood:air	18.0
Liver:air	83.5
Fat:air	1021
Muscle:air	27.7
Skin:air	43.0

Note. From Tardif et al. (1993).

Statistical Analysis

Estimates of the permeability coefficient (K_p) were based on the ability to describe each exhaled breath data set using the software optimization routines supplied with the commercial software package SimuSolv. The percent variability explained for all optimized values was always ≥80%. The use of these routines has been described previously (Agin & Blau, 1982). Optimized permeability coefficients were determined for each individual animal data set and averaged ($n = 3$) for each exposure dose level; data is expressed as the average ± standard deviation. An overall K_p for all animals, regardless of exposure level, was determined as an average ± standard deviation of $n = 6$ data sets.

RESULTS

Animals were exposed to aqueous toluene at a 0.5 or 0.2 mg/ml target concentration. The actual dosing concentrations were verified for each individual animal by GC analysis of the original dosing solution. Actual dosing

concentrations were found to range at the lower exposure from 0.19–0.20 mg/ml (1.75 ± 0.32 mg/kg) and from 0.42–0.51 mg/ml (4.14 ± 0.38 mg/kg) at the higher exposure (Table 2).

A representative exhaled breath profile from an animal treated dermally with a 2-ml volume of the 0.51 mg/ml aqueous toluene dose is given in Figure 2. The exhaled breath data, reflected as toluene chamber concentration, clearly shows an initial absorption phase, followed by a slower elimination phase. Exposures at this level resulted in a peak exhaled breath concentration (C_{max}) of approximately 500 ppb, which was achieved within 1 h after application of the dermal dose.

A similar exhaled breath profile was observed in animals treated with the lower toluene exposure dose level. For example, Figure 3 illustrates the exhaled breath data, reflected as toluene chamber concentration, from an animal treated dermally with a 2-ml volume of 0.19 mg/ml aqueous toluene. In comparison to the higher dose level, exposures at this level resulted in a peak exhaled breath concentration (C_{max}) of approximately 250 ppb, which was again reached within 1 h after application of the dermal dose. The reproducibility of the data is demonstrated in Figure 4, which contains the average (points) and standard deviation (shaded bars) of three individual data sets from rats exposed to toluene at the lower exposure dose concentration.

The toluene PBPK model, modified by addition of a skin compartment, was used to estimate the permeability coefficient (K_p) for dermal absorption of aqueous toluene for each individual rat. This was done by requiring the PBPK model to determine the rate constant (in cm/h) needed to match the achieved exhaled breath data for each animal through the use of the optimization routines in the modeling and optimization software package SimuSolv (Dow Chemical Co., Midland, MI). The estimated K_p values ranged from 0.076 ± 0.004 cm/h for the lower exposure dose level to 0.070 ± 0.004 cm/h for the higher exposure dose (Table 2). Regardless of exposure dose level, an overall average K_p of 0.074 ± 0.005 cm/h provided a good fit to all of the data sets ($n = 6$).

For each animal, the concentration of toluene remaining at the end of exposure was analyzed using GC headspace methodologies as described earlier. These analyses indicate that 45 ± 4% and 42 ± 18% of the toluene in the aqueous matrix was absorbed in the lower and higher exposure dose concentrations, respectively (Table 2). Regardless of exposure level, an overall average ($n = 6$) indicates that 43.8 ± 9.6% of the toluene was absorbed during the 5 h the animals were monitored. A comparison of the PBPK model

TABLE 2. Rat Dermal Exposures (Average ± Standard Deviation of $n = 3$ Individual Data Sets)

Exposure (mg/kg)	Percent dose absorbed	K_p (cm/h)
1.75 ± 0.32	45 ± 4	0.076 ± 0.004
4.14 ± 0.38	42 ± 18	0.070 ± 0.004

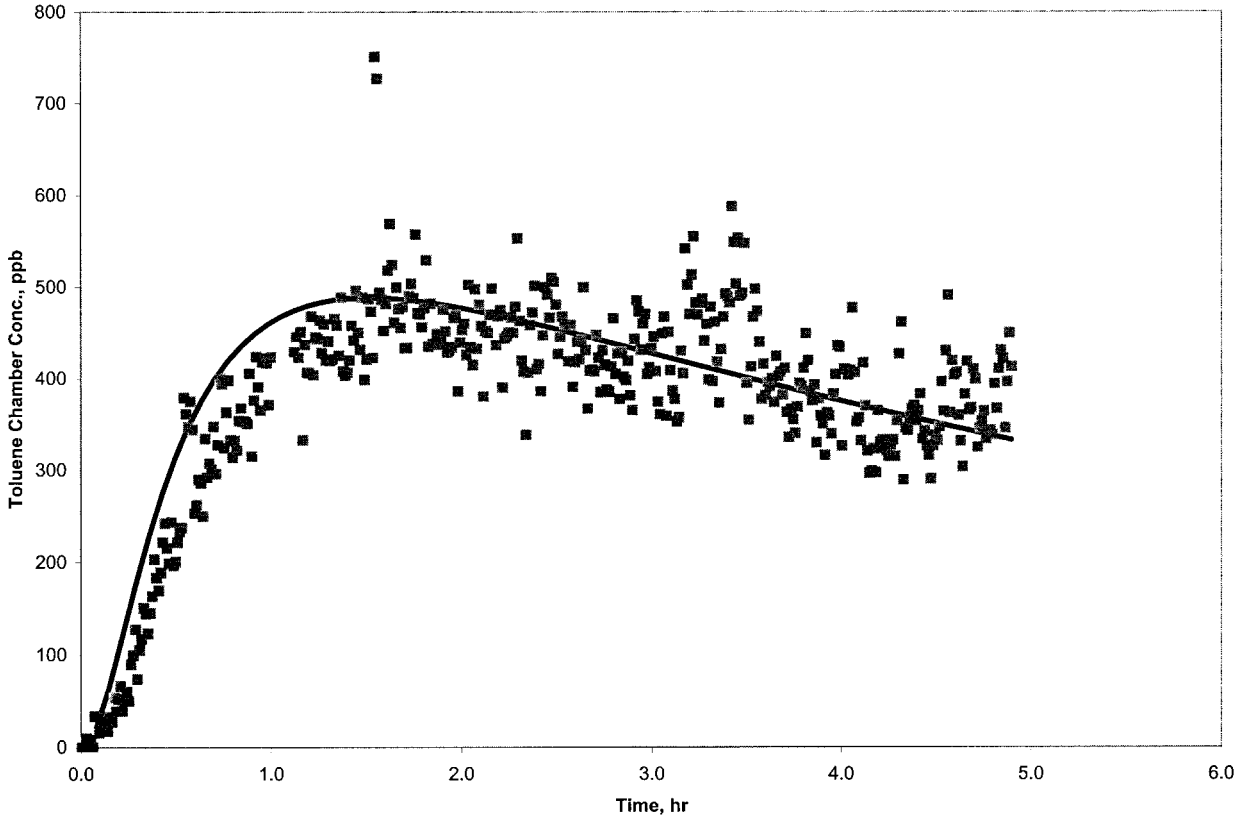

FIGURE 2. PBPK model prediction (line) and exhaled breath data, reflected as chamber concentration (points) for a rat exposed to a 2.08-ml volume of 0.51 mg/ml (4.41 mg/kg) aqueous toluene over a 4.91-cm^2 area of the back.

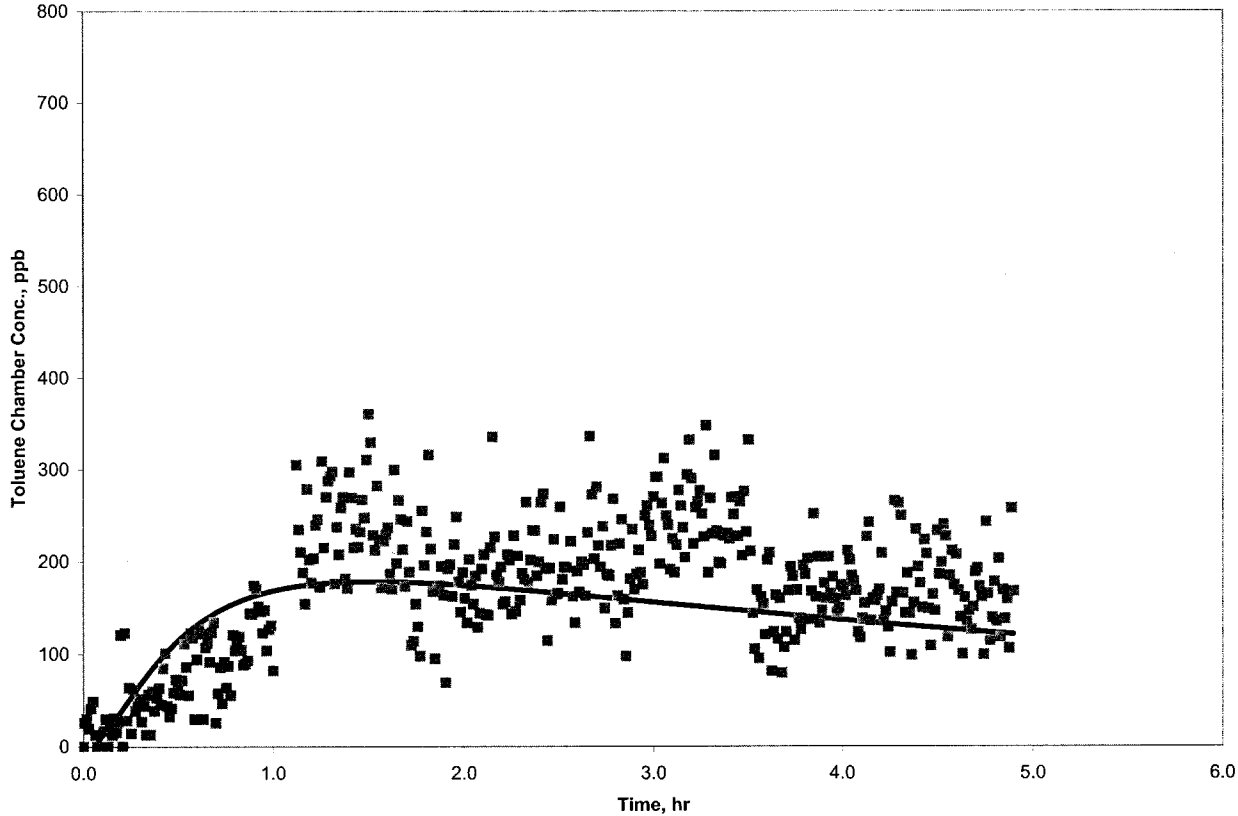

FIGURE 3. PBPK model prediction (line) and exhaled breath data, reflected as chamber concentration (points) for a rat exposed to a 2.05-ml volume of 0.19 mg/ml (1.51 mg/kg) aqueous toluene over a 4.91-cm^2 area of the back.

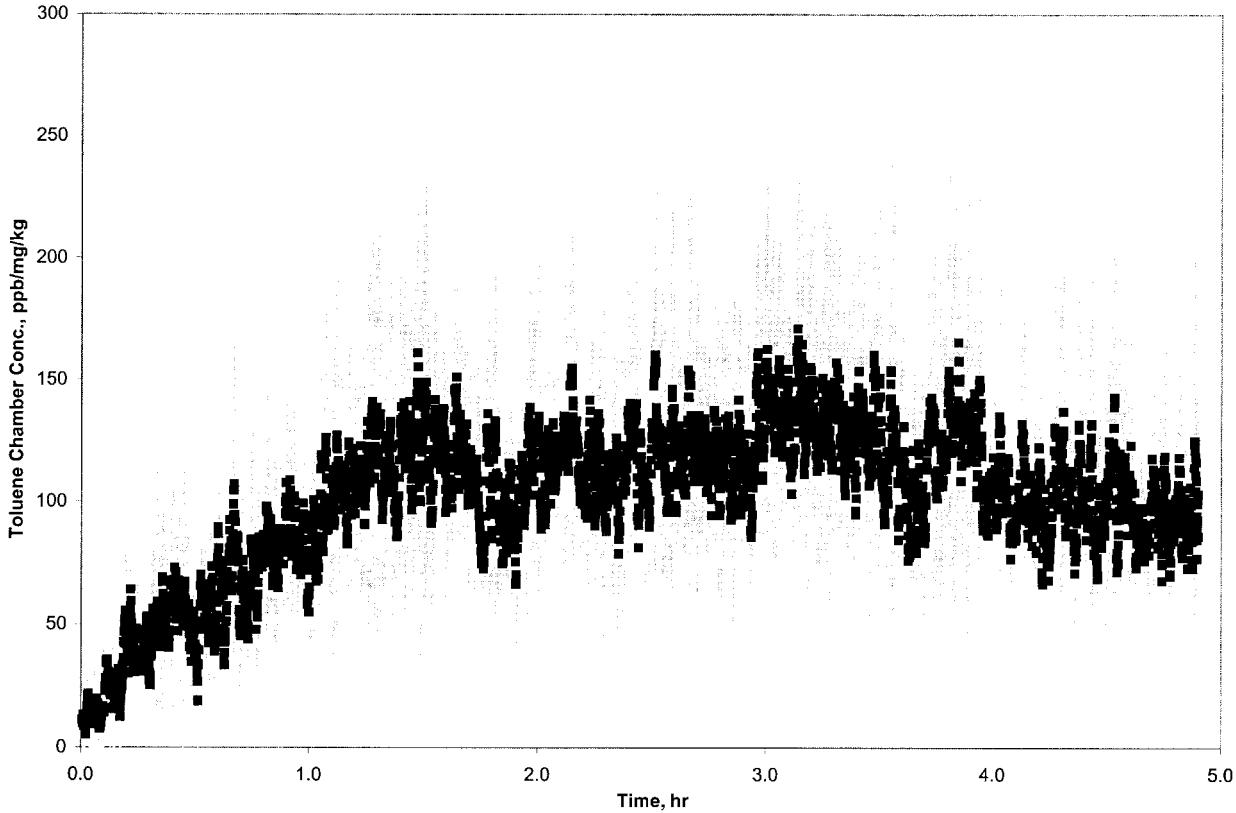

FIGURE 4. Average (points) and standard deviation (error bars) of exhaled toluene, reflected as toluene chamber concentration (ppb/mg/kg), for $n = 3$ individual rats exposed to toluene at the lower exposure dose (approximately 1.75 mg/kg) over a 4.91-cm^2 surface area.

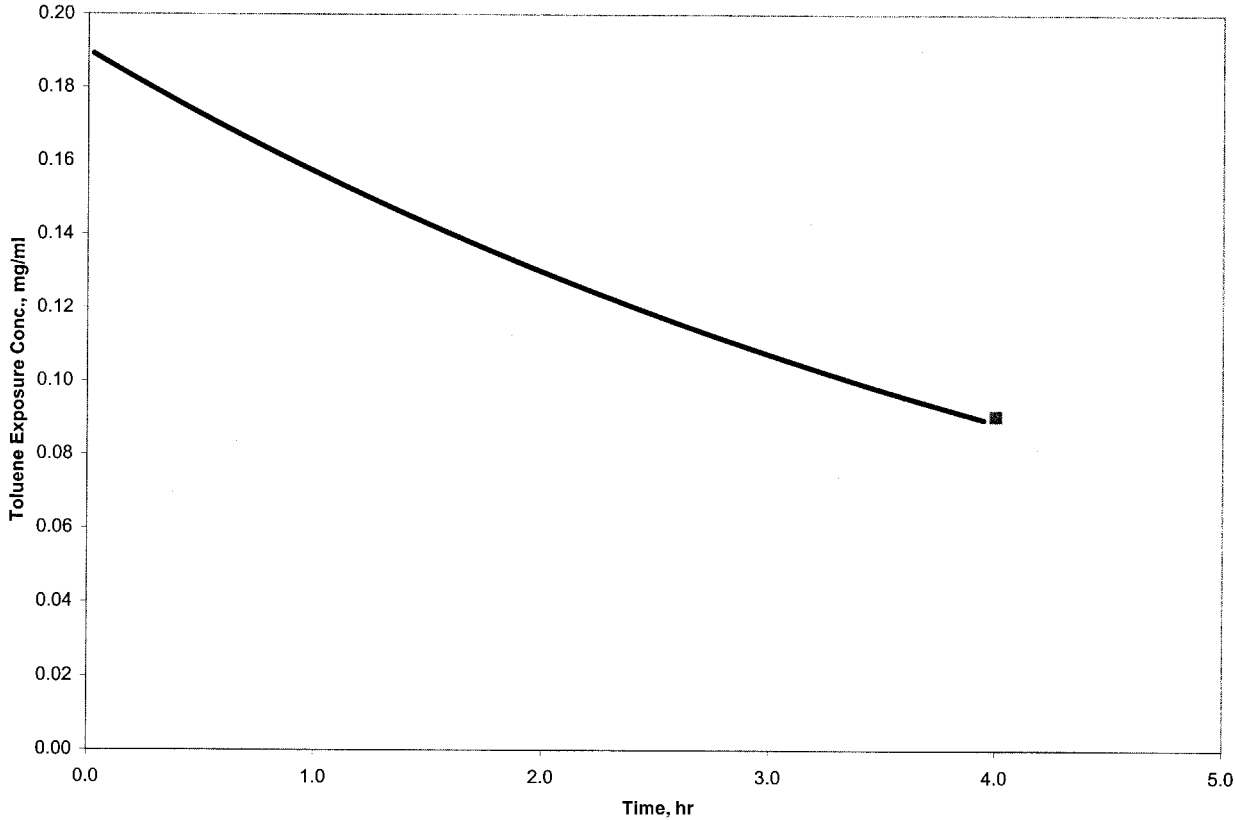

FIGURE 5. Comparison of the PBPK model prediction (line) of the toluene exposure concentration and the actual measured end of exposure concentration (point) for a rat exposed to 0.19 mg/ml (1.62 mg/kg) aqueous toluene over a 4.91-cm^2 area of the back.

estimates of the concentration of toluene remaining at the end of exposure and the measured values showed good agreement, as illustrated in Figure 5 for an animal exposed at 0.19 mg/ml. Chamber monitoring for toluene concentrations following sacrifice of the animals revealed that no leakage of the exposure system occurred.

DISCUSSION

The studies conducted here indicate that toluene, in an aqueous matrix, is rapidly absorbed through the skin of a rat. Although the current studies were conducted under occluded conditions, previous studies have shown that an occluded K_p value is identical to a nonoccluded K_p value once the PBPK model accounts for loss of the compound due to volatilization in the nonoccluded situation (Thrall et al., 2000). Unlike flux, the permeability coefficient is concentration independent and is therefore useful for expressing dermal absorption data (Jepson & McDougal, 1997). This was clearly illustrated for bromochloromethane and dibromomethane, where the permeability coefficients estimated from aqueous exposures ranging from 25 to 100% saturated solutions were not significantly different across concentrations (Jepson & McDougal, 1997). Thus, although the current studies were conducted at aqueous toluene concentrations well above the current drinking water standards, the calculated permeability coefficient should be applicable to lower concentrations.

In the PBPK model used in the current studies, a simple, well-stirred compartment was used to describe the dermal absorption. Previous investigators used two compartments, the stratum corneum and the viable epidermis, to represent the skin compartment (Shatkin & Szejnwald-Brown, 1991; Chinery & Gleason, 1993). However, McKone (1993), in developing a PBPK model for dermal exposure to chloroform, determined that for lipid-soluble compounds, the viable epidermis does not significantly limit either the speed or quantity of compound passing from the stratum corneum to blood. This is also consistent with the modeling of dermal exposure to organic vapors, as described by McDougal et al. (1986). In the current studies, the exhaled breath data was adequately represented using the PBPK with a simplistic skin compartment, thus no further refinement of skin structure was undertaken (Figures 2 and 3).

A single permeability constant for absorption of aqueous toluene in the rat, calculated from the exhaled breath data using the PBPK model, was found to be 0.074 ± 0.005 cm/h. Although a comparative rodent value for aqueous toluene was not located in the literature, the rat in vivo K_p for toluene vapor exposures was reported to be substantially higher at 0.72 cm/h (McDougal et al., 1990). Similar relationships are reported by Jepson and McDougal (1997) for aqueous versus vapor absorption of dibromomethane (K_p = 0.22 cm/h vs. 1.32 cm/h) and bromochloromethane (K_p = 0.12 cm/h vs. 0.79 cm/h).

Numerous investigators have shown that the dermal absorption of a variety of compounds is greater in rats than in humans (Bronaugh, 1998; Jepson & McDougal, 1997; McDougal et al., 1990; U.S. EPA, 1992). The U.S. EPA (1992) human K_p value for aqueous toluene was estimated to be 1 cm/h based on flux data from Dutkiewicz and Tyras (1968). In the human studies, the amount of toluene absorbed was quantified by measuring the loss of the compound from the donor solution, and steady-state conditions were not verified. However, as described by Jepson and McDougal (1997), permeability may be overestimated by assuming that the rate of chemical loss from the exposure solution represents the average flux into the skin. In addition, erroneous estimates of percutaneous absorption may be determined when standard Fick's law calculations of dermal flux are used without verifying that steady state was achieved (Jepson & McDougal, 1997). Given that the K_p value for aqueous toluene exposures in F344 rats determined here is lower than the human estimated value, a reevaluation of human dermal absorption may be warranted.

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