

## Short Communication

# Presystemic Elimination of Trichloroethylene in Rats Following Environmentally Relevant Oral Exposures

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### ABSTRACT:

1,1,2-Trichloroethylene (TCE), a volatile organic contaminant (VOC) of drinking water in the United States, is frequently present in trace amounts. TCE is currently classified by the International Agency for Research on Cancer and the U.S. Environmental Protection Agency as a probable human carcinogen, because it produces tumors in some organs of certain strains of mice or rats in chronic, high-dose bioassays. Previous studies (*Toxicol Appl Pharmacol* 60:509-526, 1981; *Regul Toxicol Pharmacol* 8:447-466, 1988) used physiological modeling principles to reason that the liver should remove virtually all of a well metabolized VOC, such as TCE, as long as concentrations in the portal blood were not high enough to saturate metabolism. To test this hypothesis, groups of unanesthetized male Sprague-Dawley rats received intravenous injections of 0.1, 1.0, or 2.5 mg TCE/kg as an aqueous emulsion. Other rats

were gavaged with 0.0001, 0.001, 0.01, 0.1, 1, 2.5, 5, or 10 mg TCE/kg b.wt. Serial microblood samples were taken via an indwelling carotid artery cannula, to generate blood TCE versus time profiles. Headspace solid-phase microextraction gas chromatography with negative chemical ionization mass spectrometry (limit of quantitation = 25 pg/ml) was used to quantify TCE. TCE was undetectable in rats given 0.0001 mg/kg, but it exhibited linear kinetics from 0.1 to 5.0 mg/kg. Bioavailability was consistent over this dosage range, ranging from 12.5 to 16.4%. The presence of these limited amounts of TCE in the arterial blood disprove the aforementioned hypothesis, yet demonstrate that first-pass hepatic and pulmonary elimination in the rat afford its extrahepatic organs protection from potential adverse effects by the majority of the low levels of TCE absorbed from drinking water.

Extensive use of volatile organic chemicals (VOCs), including 1,1,2-trichloroethylene (TCE), has resulted in their common occurrence in drinking water supplies. TCE is the most frequently found chemical contaminant in groundwater in the proximity of hazardous waste sites in the United States. TCE was often detected in the blood of 982 nonoccupationally exposed adults evaluated in the National Health and Nutrition Examination Survey (Churchill et al., 2001) and more recently in a subset of 951 persons (Blount et al., 2006). Concentrations typically found in finished drinking water in the United States range from parts per trillion (ppt) to parts per billion (ppb) (Moran et al., 2007).

Trace levels of TCE are primarily a health concern because the solvent is a potential human carcinogen. TCE-induced tumors seen in chronic, very high-dose rodent bioassays are organ-, species-, and strain-specific (National Research Council, 2006). Hepatocellular carcinoma, for example, is known to occur in only one strain of one species, the B6C3F1 mouse. Some strains of mice inhaling the chem-

ical have developed lung tumors. A low incidence of kidney tumors has been reported in three of seven strains of rats tested. Leydig cell tumors have only been seen in male Sprague-Dawley (S-D) rats. There is still controversy about the relevance of these high-dose rodent tumors to humans and about human risks posed by very low exposures (Clewell and Andersen, 2004; Cohen et al., 2004; Caldwell and Keshava, 2006; Lock and Reed, 2006). Some researchers and administrators have taken the position that scientific evidence is insufficient to rule out that certain TCE metabolites may be mutagenic and therefore have no dosage threshold. TCE, in sufficient amounts, can produce noncancer effects in organs including the brain, liver, kidneys, testes, and immune system.

Biotransformation plays a key role in modulating the toxicokinetics (TK) and the ensuing toxicity and carcinogenicity of TCE. The VOC is metabolized primarily via a CYP450-catalyzed oxidative pathway involving sequential formation of a series of products (Lash et al., 2000a). The initial step in oxidation of low TCE doses is catalyzed primarily in rodents and humans by CYP2E1, a constitutive CYP450 isoform (Lipscomb et al., 1997; Ramdhan et al., 2008). The second, relatively minor pathway involves conjugation of TCE with glutathione, followed by a series of subsequent metabolic activation and detoxification reactions (Lash et al., 2000a). This second pathway becomes important quantitatively only at quite high TCE doses. The majority of TCE biotransformation occurs in the liver, although metabolic activation of relatively small quantities of TCE reaching extrahepatic tissues, such as kidney (Lash et al., 2000b), testes (Forkert

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**ABBREVIATIONS:** VOC, volatile organic chemical; TCE, 1,1,2-trichloroethylene; ppt, parts per trillion; ppb, parts per billion; S-D, Sprague-Dawley; TK, toxicokinetics; MS, mass spectrometry;  $AUC_0^\infty$ , area under the blood TCE concentration versus time curve; Vd, volume of distribution; GI, gastrointestinal;  $t_{1/2}$ , terminal elimination half-life; PBPK, physiologically based pharmacokinetic.

et al., 2002), and lungs (Forkert et al., 2006), can have a toxicologically significant impact in situ.

There are a number of protection and repair systems that guard against cytotoxic, mutagenic, and carcinogenic actions of TCE and other chemicals. One of these processes is first-pass or presystemic elimination. Ingested chemicals that are absorbed into venous mesenteric blood vessels are conveyed via the portal vein through the liver before reaching the arterial circulation and extrahepatic organs. Lee et al. (1996) report that a substantial proportion of oral TCE, a well metabolized VOC, is eliminated its first pass through the liver and lungs of male rats. Weisel and Jo (1996) were essentially unable to detect TCE in the exhaled breath of persons who consumed 0.5 liter of water containing 20 or 40  $\mu\text{g}$  TCE/liter. Andersen (1981) proposed that the liver was capable of removing essentially all of the orally administered VOCs with high extraction ratios from portal blood, if their concentrations were not high enough to saturate metabolism. If true, this effect could have profound implications for theoretical cancer risks in extrahepatic tissues. Our research group has recently developed analytical techniques (Liu et al., 2008a,b) that are sensitive enough to evaluate the efficiency of first-pass elimination of trace levels of TCE in drinking water. The overall objective of the current project was to test the hypothesis of Andersen (1981), by directly characterizing the linearity of the kinetics and delineating the bioavailability of a series of very low oral doses of TCE in rats.

### Materials and Methods

**Chemicals.** Analytical-grade TCE was purchased from Sigma-Aldrich (St. Louis, MO). Sulfuric acid was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). High-performance liquid chromatography-grade acetonitrile was obtained from Thermo Fisher Scientific (Waltham, MA). Deionized water was generated from a Siemens deionized water system (Warrendale, PA). Ultrahigh purity helium and methane were purchased from National Welders (Charlotte, NC). Alkamuls, formerly Emulphor, a polyethoxylated vegetable oil, was obtained from sanofi-aventis (Bridgewater, NJ) and used to prepare stable aqueous TCE emulsions the day of dosing.

**Animals.** Male S-D rats (270–380 g) were obtained from Charles River Laboratories (Raleigh, NC). All protocols for this study were approved by the institution's Animal Care and Use Committee. The animals were housed in pairs in polycarbonate cages in an Association for Assessment and Accreditation of Laboratory Animals-approved animal care facility with a 12-h light cycle (light: 7:00 AM–7:00 PM) at  $22 \pm 2^\circ\text{C}$  and  $55 \pm 5\%$  relative humidity for at least 7 days before use. Food (5001 Rodent Diet; PMI Nutrition International, Brentwood, MO) and boiled tap water were provided ad libitum during this period.

Each rat was anesthetized at 18 to 24 h prior to TCE dosing by intramuscular injection of 0.1 ml/100 g b.wt. of ketamine HCl (100 mg/ml), acepromazine maleate (10 mg/ml), and xylazine HCl (20 mg/ml) (3:2:1, v/v/v). A cannula (PE-50) was surgically inserted into the right carotid artery and jugular vein of one group of animals. The jugular cannula was later used for intravenous injection. Serial blood samples were taken from the carotid artery. A second group of orally dosed rats had only a carotid artery cannulated for subsequent blood sampling. The cannulas were filled with heparinized saline, passed under the skin, and exteriorized at the nape of the neck to maintain patency and prevent the freely moving animals from disturbing them after they recovered. Boiled water was provided ad libitum, but food was withheld during the 24-h recovery period. Access to food was provided 3 h after TCE dosing.

**Dosing.** Five to seven unanesthetized, cannulated rats were assigned to each group. TCE was incorporated into a 5% aqueous Alkamuls emulsion in saline the day of study. Some groups were given a single injection of TCE in 0.2 ml over 30 s via the jugular vein. Doses of TCE injected intravenously were 0.1, 1.0, and 2.5 mg TCE/kg b.wt. Other groups were gavaged with one of the following doses of TCE in a total volume of 1 ml (0.0001, 0.001, 0.01, 0.1, 1.0, 2.5, 5.0, and 10.0 mg/kg b.wt.).

**Blood Collection and TCE Analysis.** A blood sample was collected from each rat and analyzed to ensure there was no background level of TCE before

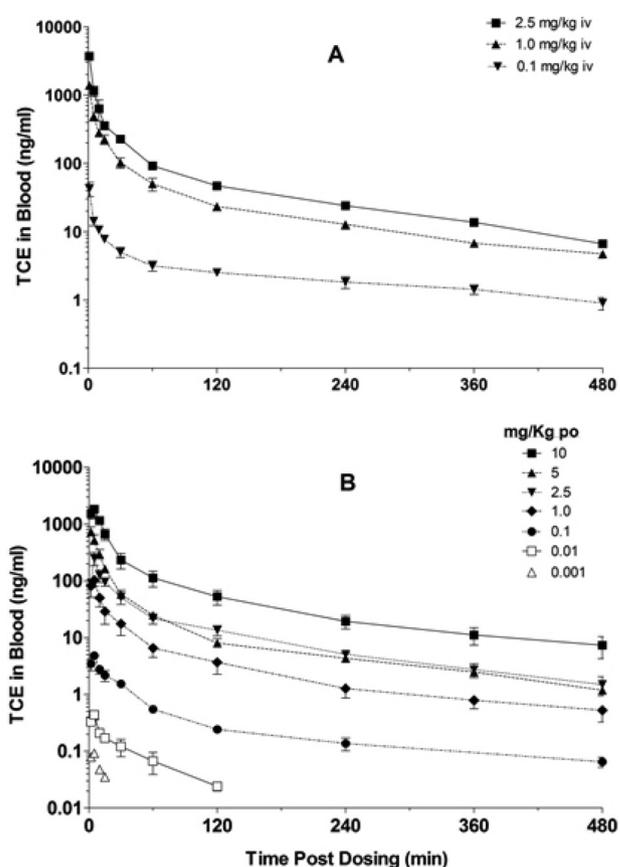


Fig. 1. A, blood TCE concentration versus time profiles of S-D rats given intravenous injections of 0.1, 1.0, and 2.5 mg TCE/kg in an aqueous emulsion. Each data point represents the mean  $\pm$  S.E. of 5 to 7 animals. B, blood TCE concentration versus time profiles of S-D rats dosed orally by gavage with 0.001, 0.01, 0.1, 1.0, 2.5, and 5.0 mg TCE/kg as an aqueous emulsion. Each data point represents the mean  $\pm$  S.E. of 5 to 7 animals.

dosing. No TCE was detected in any sample. Serial 300- $\mu\text{l}$  blood samples were taken from the carotid artery cannula at 1- to 480-min intervals postdosing. Heparinized saline (300  $\mu\text{l}$ ) was reinjected after each sample to replace lost blood volume.

TCE concentrations in blood were analyzed by the headspace solid-phase microextraction gas chromatography mass spectrometry (MS) method proposed by Liu et al. (2008a,b). For lower doses (0.0001, 0.001, 0.01, 0.1, 1.0 mg/kg), the MS was operated in negative chemical ionization mode (Liu et al., 2008a). For higher doses (2.5 mg/kg), the MS was operated in positive electron ionization mode (Liu et al., 2008b).

**TK Data Analyses.** Means and S.E.M. were calculated using Microsoft Excel 2003 (Microsoft, Redmond, WA). TK parameters, including area under the blood TCE concentration versus time curve ( $\text{AUC}_{0-\infty}$ ), volume of distribution ( $V_d$ ), clearance, and terminal elimination half-life ( $t_{1/2}$ ) were calculated using WinNonlin (version 4.1) noncompartmental model analysis by Pharsight (Mountain View, CA). The maximum blood concentration ( $C_{\text{max}}$ ) and time of maximum blood concentration after dosing ( $T_{\text{max}}$ ) were observed values. Bioavailability ( $F$ ) was calculated using the equation:  $F = (\text{AUC}_{\text{i.v.}} / \text{AUC}_{\text{p.o.}}) (\text{Dose}_{\text{i.v.}} / \text{Dose}_{\text{p.o.}})$ . Differences among the TK parameters of different dosage groups were evaluated by one-way analysis of variance. A value of  $p < 0.05$  was considered statistically significant.

### Results

**Toxicokinetics of Intravenous TCE.** Arterial blood TCE concentration versus time curves for groups of rats given three different intravenous doses are shown in Fig. 1A. TCE levels diminish very quickly following i.v. injection, indicative of rapid diffusion of the small lipophilic chemical from the bloodstream into body tissues. The

TABLE 1

Toxicokinetic parameters in blood after S-D rats received intravenous injections of 0.1, 1.0, and 2.5 mg TCE/kg as an aqueous emulsion

Each value represents the mean  $\pm$  S.E. for a group of 5 to 7 rats.

Toxicokinetic Parameters	0.1 mg/kg	1.0 mg/kg	2.5 mg/kg
Half-life (min)	201.4 $\pm$ 28 <sup>a</sup>	157.5 $\pm$ 12 <sup>b</sup>	131 $\pm$ 7 <sup>b</sup>
Clearance (ml/min/kg)	68.8 $\pm$ 11 <sup>a</sup>	49.7 $\pm$ 3 <sup>a</sup>	58.4 $\pm$ 3.5 <sup>a</sup>
Vd (ml/kg)	18.6 $\pm$ 2 <sup>a</sup>	13.5 $\pm$ 2.4 <sup>b</sup>	11.0 $\pm$ 0.9 <sup>b</sup>
AUC ( $\mu\text{g} \cdot \text{min/ml}$ )	1.6 $\pm$ 0.2 <sup>a</sup>	20.6 $\pm$ 1.5 <sup>b</sup>	43.7 $\pm$ 2.6 <sup>c</sup>

<sup>a,b,c</sup> Parameter values with different letters are significantly different from one another at  $P \leq 0.05$ .

curves in Fig. 1 parallel one another, although the rate of elimination of parent compound at the lowest dosage level (0.1 mg/kg) is somewhat slower. This effect is reflected by its slightly longer  $t_{1/2}$  (Table 1). Clearance does not differ significantly in the 0.1 to 2.5 mg/kg groups, ranging from 49.7 to 68.8 ml/min/kg.  $\text{AUC}_0^\infty$  values increase proportionally with dosage. These findings indicate that TCE exhibits linear kinetics in this dosage range.

**Toxicokinetics of Oral TCE.** Arterial blood TCE concentration versus time profiles for groups of rats given a series of oral doses of the VOC are pictured in Fig. 1B. It is obvious that TCE is very rapidly absorbed from the gastrointestinal (GI) tract of the fasted animals. The first blood sample was taken 1 min after gavage. Observed  $T_{\text{max}}$  values vary from 2.5 to 6.0 min. The blood TCE levels drop very rapidly during the initial distribution phase. The blood profiles generally parallel one another over the entire dosage range. TCE is not detectable in rats given 0.0001 mg/kg. TCE is measurable in the 0.001 and 0.01 mg/kg groups for the initial 15 and 120 min, respectively. These durations are not adequate to allow estimation of kinetic parameters other than  $C_{\text{max}}$  and  $T_{\text{max}}$ . The elimination curves of the higher dosage groups largely parallel one another. The 2.5 and 5.0 mg/kg curves are an exception, in that the two groups' TCE levels are comparable after the initial 30 min. As in the intravenous experiment, the  $t_{1/2}$  of the 0.1 mg/kg group is slightly longer than in the other groups (Table 2). Half-lives do not vary despite the 10-fold elevation from 1 to 10 mg/kg. Clearance and volume of distribution are constant over the 100-fold dosage range. An increase in  $\text{AUC}_0^\infty$  is also proportional to dose from 0.1 to 5.0 mg/kg. However, the 2-fold difference between 5 and 10 mg/kg results in a 4-fold rise in  $\text{AUC}_0^\infty$ .

**Bioavailability of TCE.** Oral bioavailability ( $F$ ) values for the 0.1, 1.0, 2.5, 5.0, and 10.0 mg/kg groups are 12.5, 12.6, 16.4, 12.9, and 26.5%. Thus, bioavailability is relatively consistent from 0.1 to 5.0 mg/kg, but it doubles between 5 and 10 mg/kg (Fig. 2).

## Discussion

The findings of the current investigation clearly demonstrate that presystemic elimination does not offer complete protection from potential adverse effects on extrahepatic tissues of rats upon ingestion of trace amounts of TCE. Almost 3 decades ago, Andersen (1981)

hypothesized that the liver was capable of removing virtually all of an orally administered VOC with a high extraction ratio, if the dose was not high enough to saturate metabolism. Bogen (1988) used physiologically based pharmacokinetic (PBPK) model-based algebraic formulas to calculate that 99.8% of low oral doses of TCE should be metabolized. Lee et al. (1996) demonstrated empirically that hepatic first-pass elimination of TCE by male S-D rats was inversely related to exposure level, and that  $\sim 60\%$  of low oral bolus doses was removed. It was not possible then to measure uptake and pulmonary elimination of doses lower than  $\sim 1$  mg/kg, due to lack of analytical sensitivity. TCE was not detectable with the current method (limit of quantification = 25 ppt) in rats given 0.1  $\mu\text{g/kg}$ . A minuscule amount ( $\text{AUC}_0^{15} = 0.9$  ng  $\cdot$  min/ml) reached the arterial circulation of rats ingesting 1  $\mu\text{g/kg}$ . This amount would be the administered dose for a person who consumed 2 liters of water containing 35 ppb TCE in one sitting. People normally consume water in divided doses, circumstances under which the liver is even more efficient in removing TCE.

Although the liver and lungs offer extrahepatic organs protection from ingested TCE, potentially toxic metabolites formed in the liver may be released into the systemic circulation. TCE is biotransformed primarily via a CYP450-catalyzed pathway involving sequential formation of a series of metabolites including chloral, chloral hydrate, trichloroacetic acid, possibly dichloroacetic acid, and trichloroethanol (Lash et al., 2000a). Chloral hydrate, trichloroacetic acid, and dichloroacetic acid have been implicated in rodent liver carcinogenesis, although apparently not in adverse effects in other organs (Bull, 2000). The second, relatively minor TCE biotransformation pathway involves glutathione conjugation and subsequent conversion to a series of products (Lash et al., 2000b). Certain metabolites enter the blood and bile, from which they may reenter the liver to be detoxified or be taken up by the kidney cells and undergo metabolic activation to cytotoxic, mutagenic moieties (Lash et al., 2006). Thus, the liver serves to both metabolically activate and inactivate TCE.

It would appear that presystemic elimination is an efficient but not a perfect means of preventing exposure of extrahepatic organs of humans upon ingestion of environmentally relevant levels of well metabolized VOCs. Saturation of first-pass metabolism will occur at moderate to high dosage levels, but not with the very low quantities at issue here, because their uptake is perfusion limited. By use of a PBPK model, Kedderis (1997) estimated that a 10-fold increase in CYP2E1 activity in humans inhaling 10 ppm TCE for 4 h would result in only a 2% increase in TCE metabolism. PBPK model simulations of an 8-h inhalation exposure to 50 ppm TCE and ingestion of 2 liters of water containing 5 ppb TCE predicted that the amount oxidized in the liver differed by only 2% in persons whose CYP2E1 content varied 10-fold (Lipscomb et al., 2003).  $V_{\text{max}}$  used in PBPK models for TCE in humans (Fisher et al., 1998) and rats (Keys et al., 2003) varied just 4- to 5-fold. This result suggests that the efficiency (i.e.,  $\sim 85$ – $88\%$ ) of presystemic elimination of trace levels of TCE observed in the current study may not exhibit significant interindividual or inter-

TABLE 2

Toxicokinetic parameters in blood after gavage of S-D rats with 0.1, 1.0, 2.5, 5.0, and 10 mg TCE/kg as an aqueous emulsion

Each value represents the mean  $\pm$  S.E. for a group of 5 to 7 rats.

Toxicokinetic Parameters	0.1 mg/kg	1 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
$C_{\text{max}}$ (ng/ml)	5 $\pm$ 0.5	107 $\pm$ 27	250 $\pm$ 65	790 $\pm$ 201	1916 $\pm$ 272
$T_{\text{max}}$ (min)	4.4 $\pm$ 0.6	4.4 $\pm$ 0.6	6 $\pm$ 1.0	2.5 $\pm$ 0.6	3.8 $\pm$ 0.7
Half-life (min)	209 $\pm$ 25 <sup>a</sup>	134 $\pm$ 9 <sup>b</sup>	107 $\pm$ 14 <sup>b</sup>	132 $\pm$ 12 <sup>b</sup>	126 $\pm$ 17 <sup>b</sup>
Clearance (ml/min/kg)	64 $\pm$ 5	70 $\pm$ 18	68 $\pm$ 14	68 $\pm$ 14	70 $\pm$ 16
Vd (ml/kg)	19.5 $\pm$ 3.3	13.7 $\pm$ 4.0	9.6 $\pm$ 1.0	12.4 $\pm$ 3	12.5 $\pm$ 3.5
AUC ( $\mu\text{g} \cdot \text{min/ml}$ )	0.20 $\pm$ 0.015	2.6 $\pm$ 0.8	7.1 $\pm$ 1.3	11.2 $\pm$ 2.1	44.4 $\pm$ 9.5

<sup>a,b</sup> Parameter values with different letters are significantly different from one another at  $P \leq 0.05$ .

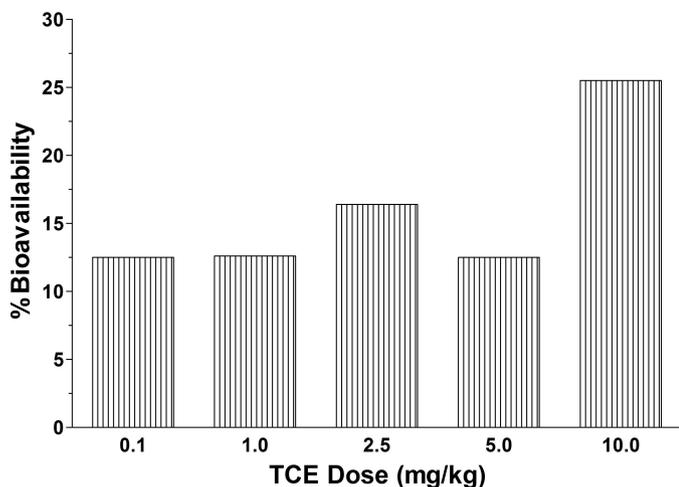


FIG. 2. Percentage of bioavailability of TCE in S-D rats dosed orally by gavage with 0.1, 1.0, 5.0, and 10.0 mg TCE/kg as an aqueous emulsion.  $n = 5$  to 7.

species differences. A study is underway in our laboratory to determine whether pronounced CYP2E1 induction significantly enhances oxidation of very low TCE doses in rats. Persons with compromised liver function would be anticipated to exhibit less efficient first-pass elimination.

First-pass hepatic elimination was linear over most of the range of doses used in the current study. GI absorption of TCE from the aqueous emulsion in the fasted rats was very rapid, with  $T_{max}$  values ranging from just 2.5 to 6.0 min. TCE was promptly distributed to tissues, as reflected by the quick decline in blood levels during the distribution phase in the p.o.- and i.v.-exposed animals. TCE exhibited linear kinetics at oral doses  $\leq 5$  mg/kg, although the blood elimination profiles for the 0.001 and 0.01 mg/kg groups were incomplete. Clearance was constant over the 0.1 to 5.0 mg/kg i.v. and 0.1 to 10.0 mg/kg p.o. dosage ranges. Values for  $t_{1/2}$  were also quite consistent. The onset of metabolic saturation was manifest by a disproportionate increase in area under the curve between 5.0 and 10.0 mg/kg p.o. and by the  $\sim 2$ -fold increase in bioavailability. Lee et al. (1996) reported a progressive increase in metabolic saturation in male S-D rats given oral doses  $> 8$  mg/kg. The doses they evaluated ranged from 0.17 to 64 mg/kg. These investigators estimated the  $K_m$  to be 2.68  $\mu\text{g}$  TCE/ml. Blood TCE concentrations in animals given 0.1 to 5.0 mg/kg in the present study did not exceed this value.

Several factors may have contributed to the incomplete hepatic elimination of TCE in its linear kinetics range. Blood-borne TCE may simply pass through the sinusoids into the central vein before there is time for all the TCE to be removed. The rat's liver homogenate/blood partition coefficient is 1.24, which would contribute modestly to hepatic uptake (Gargas et al., 1989). Periportal hepatocytes initially receive the highest concentrations of blood-borne xenobiotics, but typically have the lowest activities of CYP2E1, CYP2B1/2, and most other enzymes that metabolize TCE (Oinonen and Lindros, 1998). Dietary fatty acid uptake and incorporation into very low-density lipoproteins is relatively high in periportal hepatocytes. Cholesterol synthesis and ketogenesis are also known to occur primarily in this region (Gebhardt, 1989). Preferential delivery and retention of TCE in these areas likely contribute to incomplete clearance. Relatively simple "well stirred" and "parallel tube" hepatic clearance models have been upgraded to "zonal models," to accommodate the heterogeneous distribution of hepatic enzymes and transporters (Liu and Pang, 2006). Multizonal livers have been included in several PBPK models (Frederick et al., 1992; Andersen et al., 1997; Keys et al., 2003). The latter

researchers incorporated a deep liver compartment into their PBPK model to account for slower than anticipated systemic TCE clearance in male S-D rats.

It seems unlikely that the GI tract would contribute significantly to first-pass extraction of TCE, although the VOC is exhaled to some extent during each pass through the pulmonary circulation. The bioavailability of some drugs is limited by the presence of transporters and CYP450s in both enterocytes and hepatocytes (Hall et al., 1999). However, TCE and other VOCs are small, uncharged, lipophilic molecules that do not depend on transporters but readily diffuse across membranes. CYP2E1, the isoform primarily responsible for oxidation of low doses of TCE in rats and humans, has not been found in the small intestines of either species (Kaminsky and Zhang, 2003; Paine et al., 2006). Pulmonary first-pass effect, determined primarily by a VOC's blood/air partition coefficient, is considered to be blood level-independent (National Research Council, 1986). Lee et al. (1996) reported that lung first-pass elimination accounted for  $\sim 5$  to 8% of oral doses of 0.7 to 16.0 mg TCE/kg in rats. Forkert et al. (2005, 2006) measured efficient oxidation of TCE to chloral by CYP2E1, CYP2F2, and CYP2B1 in Clara cells in mouse airways. Clara cell numbers and TCE metabolic activation are much lower in rats and largely undetectable in human lung microsomes. TCE storage in the lungs should be negligible. Thus, it can be concluded that exhalation contributes modestly to presystemic elimination of TCE, but that saturable first-pass hepatic uptake is the predominant process.

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