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PRESYSTEMIC ELIMINATION OF TRICHLOROETHYLENE IN RATS FOLLOWING ENVIRONMENTALLY-RELEVANT ORAL EXPOSURES

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Running Title: Presystemic Elimination of Oral Trichloroethylene

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Nonstandard Abbreviations: 1,1,2-trichloroethylene (TCE), volatile organic contaminant/chemical (VOC); toxicokinetics (TK); National Health and Nutrition Examination Survey (NHANES); Headspace solid-phase microextraction (HS-SPME); selected ion monitoring (SIM)

Abstract

1,1,2-Trichloroethylene (TCE), a volatile organic contaminant (VOC) of drinking water in the U.S., is frequently present in trace amounts. TCE is currently classified by IARC and the U.S. EPA as a probable human carcinogen, as it produces tumors in some organs of certain strains of mice or rats in chronic, high-dose bioassays. Andersen (1981) and Bogen (1988) used physiological modeling principles to reason that the liver should remove virtually all of a well-metabolized VOC, such as TCE, so long as concentrations in the portal blood were not high enough to saturate metabolism. In order to test this hypothesis, groups of unanesthetized male Sprague-Dawley rats were injected i.v. with 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 bw. Serial micro blood samples were taken via an indwelling carotid artery cannula, in order 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 exhibited linear kinetics from 0.1 – 5.0 mg/kg. Bioavailability was consistent over this dosage range, ranging from 12.5 – 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.

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Introduction

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 U.S. TCE was often detected in the blood of 982 non-occupationally-exposed adults evaluated in the NHANES 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 U.S. range from parts per trillion (ppt) to parts per billion (ppb) (Moran et al., 2007).

Trace levels of TCE are primarily of concern to health, 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 (NRC, 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 chemical have developed lung tumors. A low incidence of kidney tumors has been reported in 3 of 7 strains of rats tested. Leydig cell tumors have been seen only in male Sprague-Dawley 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 (Caldwell and Keshava, 2006; Clewell and Andersen, 2004; Cohen et al., 2004; Lock and Reed, 2006). Some individuals 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 non-cancer effects in organs including the brain, liver, kidneys, testes and immune system.

Biotransformation plays a key role in modulating the toxicokinetics and the ensuing toxicity and carcinogenicity of TCE. The VOC is metabolized primarily via a cytochrome P450 (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, though metabolic activation of relatively small quantities of TCE reaching extrahepatic tissues, such as kidney (Lash et al., 2000b), testes (Forkert et al., 2002) and lungs (Forkert et al., 2006), can have toxicologically-significant impacts *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 by 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 L of water containing 20 or 40 µg TCE/L. Andersen (1981) proposed that the liver was capable of removing essentially all of orally administered VOCs with high extraction ratios from portal blood, if their concentrations were not high enough to saturate metabolism. If true, this could have profound implications for theoretical cancer risks in extrahepatic tissues. Our research group has recently developed analytical techniques (Liu et al., 2008a,b) 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 1,1,2-trichloroethylene (TCE) was purchased from Sigma-Aldrich (St. Louis, MO). Sulfuric acid was purchased from J.T. Baker (Phillipsburg, NJ). HPLC-grade acetonitrile was obtained from Fisher Scientific (Pittsburgh, PA). Deionized water was generated from a Siemens deionized water system (Warrendale, PA). Ultra-high purity helium and methane were purchased from National Welders (Charlotte, NC). Alkamuls®, formerly Emulphor®, a polyethoxylated vegetable oil, was obtained from Rhone-Poulenc (Cranbury, NJ) and used to prepare stable aqueous TCE emulsions the day of dosing.

Animals. Male Sprague-Dawley (S-D) rats (270 – 380 g) were obtained from Charles River Labs (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 AAALAC-approved animal care facility with a 12-h light cycle (light: 7:00 AM - 7:00 PM) at 22 ± 2° C and 55 ± 5% relative humidity for at least 7 days prior to 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 18 – 24 h prior to TCE dosing by i.m. injection of 0.1 ml/100 g bw of 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 i.v. 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.

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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 sec via the jugular vein. Doses of TCE injected i.v. were 0.1, 1.0 and 2.5 mg TCE/kg bw. 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 bw).

Blood Collection and TCE Analysis. A blood sample was collected from each rat and analyzed to insure there was no background level of TCE before dosing. No TCE was detected in any sample. Serial 300-µl blood samples were taken from the carotid artery cannula at 1- to 480-min intervals post dosing. Heparinized saline (300 µl) was reinjected after each sample to replace lost blood volume.

TCE concentrations in blood were analyzed by the headspace solid-phase microextraction (HS-SPME) gas chromatography mass spectrometry (GC-MS) method of 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).

Toxicokinetic (TK) Data Analyses. Means and standard error of the mean (SE) were calculated with Microsoft Excel 2003 (Microsoft Co., Redmond, WA). TK parameters, including area under the blood TCE concentration versus time curve (AUC_0^{∞}), volume of distribution (Vd), clearance (CL), and terminal elimination half-life ($t_{½}$) were calculated using WinoNonlin (vers. 4.1) noncompartmental model analysis by Pharsight, Inc. (Cary, NC). The maximum blood concentration (C_{max}) and time of maximum blood concentration after dosing (T_{max}) were observed values. Bioavailability (F) was calculated using the equation: $F = (AUC_{p.o.}/AUC_{i.v.})(Dose_{i.v.}/Dose_{p.o.})$. Differences among the TK parameters of different dosage groups were evaluated by One-Way ANOVA. A value of $p < 0.05$ was considered statistically significant.

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Results

Toxicokinetics of Intravenous TCE. Arterial blood TCE concentration versus time curves for groups of rats given three different i.v. 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 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 is reflected by its slightly longer t½ (Table 1). Clearance does not differ significantly in the 0.1 – 2.5 mg/kg groups, ranging from 49.7 to 68.8 ml/min/kg. AUC_0^{∞} 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 GI tract of the fasted animals. The first blood sample was taken 1 min post gavage. Observed T_{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_{max} and T_{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 i.v. experiment, the t½ 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. Increase in AUC_0^{∞} is also proportional to dose from 0.1 to 5.0 mg/kg. The 2-fold difference between 5 and 10 mg/kg, however, results in a 4-fold rise in AUC_0^{∞}.

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 doubles between 5 and 10 mg/kg.

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 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 ~ 60% of low oral bolus doses was removed. It was not possible then to measure uptake and pulmonary elimination of doses lower than ~ 1 mg/kg, due to lack of analytical sensitivity. TCE was not detectable with the current method (LOQ = 25 ppt) in rats given 0.1 µg/kg. A miniscule amount (AUC^{15}_0 = 0.9 ng·min/ml) reached the arterial circulation of rats ingesting 1 µg/kg. This would be the administered dose for a person who consumed 2 L 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 (CH), trichloroacetic acid (TCA), possibly dichloroacetic acid (DCA), and trichloroethanol (Lash et al., 2000). CH, TCA and DCA have been implicated in rodent liver carcinogenesis, though 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 of these metabolites enter the blood and bile, from which they may reenter the liver to be detoxified or be taken up by the kidneys 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, since their uptake is perfusion limited. By use of a physiologically-based pharmacokinetic (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 L 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_{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 suggests that the efficiency (i.e., ~ 85 – 88%) of presystemic elimination of trace levels of TCE observed in the current study may not exhibit significant interindividual or interspecies 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 employed 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 – 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

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exhibited linear kinetics at oral doses ≤ 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 – 5.0 mg/kg i.v. and 0.1 – 10.0 mg/kg p.o. dosage ranges. Values for $t_{½}$ were also quite consistent. The onset of metabolic saturation was manifest by a disproportionate increase in AUC between 5.0 and 10.0 mg/kg p.o. and by the ~ 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 – 64 mg/kg. These investigators estimated the K_m to be 2.68 µg TCE/ml. Blood TCE concentrations in animals given 0.1 – 5.0 mg/kg in the present study did not exceed this value.

A number of 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 VLDLs 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 hetergenous distribution of hepatic enzymes and transporters (Liu and Pang, 2006). Multizonal livers have been included in a number of PBPK models (Andersen et al., 1997; Frederick et al., 1992; 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 appears unlikely that the GI tract would contribute significantly to first-pass extraction of TCE, though 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). TCE and other VOCs, however, 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 (NRC, 1986). Lee et al. (1996) reported that lung first-pass elimination accounted for ~ 5 - 8% of oral doses of 0.7 – 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. It can thus 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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Footnotes

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Figure Legends

Figure 1A. Blood TCE concentration versus time profiles of S-D rats injected i.v. with 0.1, 1.0 and 2.5 mg TCE/kg in an aqueous emulsion. Each data point represents the mean ± S.E. of 5 – 7 animals.

Figure 1B. 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 ± S.E. of 5 – 7 animals.

Figure 2. Percent 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 – 7.

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TABLE 1

Toxicokinetic parameters in blood after i.v. injection of S-D rats with 0.1, 1.0 and 2.5 mg TCE/kg as an aqueous emulsion. Each value represents the mean ± S.E. for a group of 5 – 7 rats.

Toxicokinetic parameters	0.1 mg/kg	1.0 mg/kg	2.5mg/kg
Half-life (min)	201.4 ± 28[a]	157.5 ± 12[b]	131 ± 7[b]
Clearance (ml/min/kg)	68.8 ± 11[a]	49.7 ± 3[a]	58.4 ± 3.5[a]
Vd (ml/kg)	18.6 ± 2[a]	13.5 ± 2.4[b]	11.0 ± 0.9[b]
AUC (μg•min/ml)	1.6 ± 0.2[a]	20.6 ± 1.5[b]	43.7 ± 2.6[c]

Parameter values with different letters are significantly different from one another at $p \leq 0.05$.

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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 ± S.E. for a group of 5 – 7 rats.

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

Parameter values with different letters are significantly different from one another at $p \leq 0.05$.

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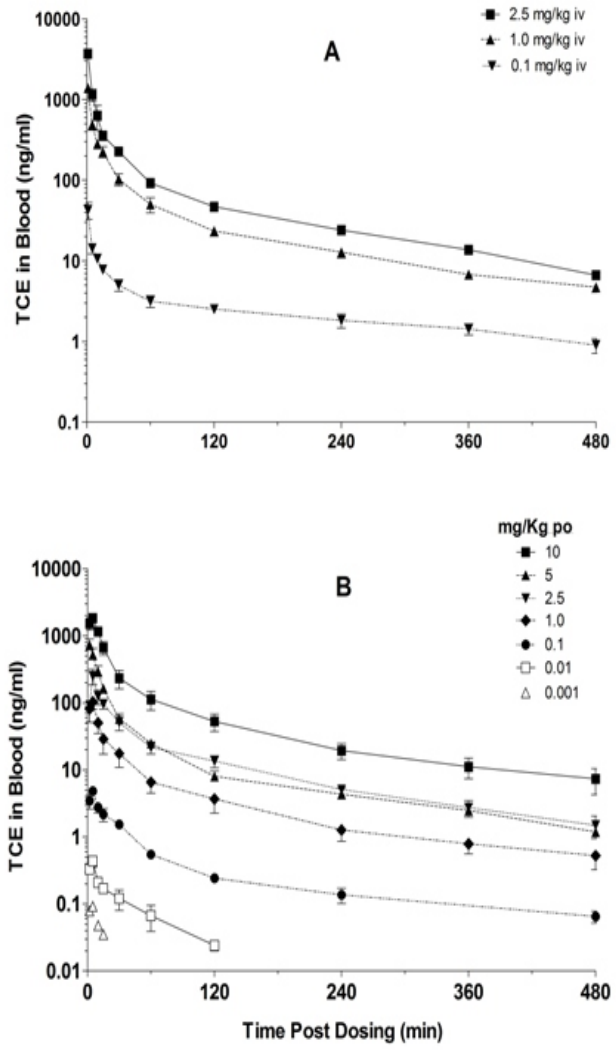

Figure 1

FIGURE 2