ALTERATIONS IN TOXICOKINETICS OF TRICHLOROETHYLENE (TCE) AND TRICHLOROACETIC ACID (TCA) DUE TO CYTOCHROME P450 2E1 (CYP2E1) INDUCTION

by

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ABSTRACT

Trichloroethylene (TCE) is a chlorinated solvent used primarily as a degreaser. It has been reported that TCE produced elevated incidences of tumors in rodents by both the oral and inhalation routes of exposure. There is limited evidence to support TCE as a cause of cancer in humans. Trichloroacetatic acid (TCA) is of considerable interest to the scientific and regulatory communities, since it is a toxicologically important metabolite of TCE and perchloroethylene (PERC), as well as one of the byproducts of drinking water chlorination. TCA is generally believed to be the proximate hepatocarcinogenic metabolite of TCE in mice. CYP2E1, which catalyzes the oxidation of many small volatile organic chemicals, is responsible for the first step of TCE oxidation. CYP2E1 is induced by a variety of xenobiotics (i.e., ethanol, acetone and aspirin), as well as by certain conditions and diseases (i.e., obesity, alcoholism and diabetes). Thus, induction of CYP2E1 is generally expected to cause a significant increase in the biotransformation of

highly metabolized compounds, such as TCE, possibly leading to an increase in cancer risks. Low-level TCE exposure scenarios have not received much attention. Information on the carcinogenic responses to TCE and its metabolites has been obtained at very high doses, which have been used to predict cancer risks of low-level TCE exposure by linear extrapolation. Thus, the main objective was to investigate changes in the metabolism of low doses of TCE and on the pharmacokinetics of downstream metabolites (especially TCA), due to CYP2E1 induction by pyridazine (PZ) as a inducer. The most prominent effects of CYP2E1 induction were on the toxicokinetic profiles of TCA. The data suggest that CYP2E1 induction enhances systemic and renal clearance of TCA, possibly by affecting organic anion transporters/multidrug resistance-related protein (OATs/MRPs) in the kidneys. So, future investigation of OATs/MRPs should provide a better understanding of the urinary elimination mechanism of small organic acids such as TCA. Rapid clearance of the TCA may, in fact, be beneficial in that liver cancer risk from TCE would be reduced.

INDEX WORDS: Trichloroethylene, TCE, Trichloroacetic acid, TCA, Toxicokinetics, Enzyme Induction, Cytochrome P450 2E1, Head-Space Gas Chromatography, GC, Volatile Organic Compounds, VOC

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in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILASOPHY

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2007

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May 2007

ACKNOWLEDGEMENTS

I would like to thank my co-major professors, Prof. James V. Bruckner for introducing me to the world of toxicology with his sagacious insights and encouragement and Dr. Catherine A. White for her insightful guidance of research direction as well as for her expertise of pharmacokinetic data analysis. Without their support and mentoring over the past years, nothing would have been possible. I would also like to express my sincere appreciation to the rest of my committee, thank you Dr. Jeffrey W. Fisher for your invaluable teachings on TCE and PBPK modeling, as well as Dr. Michael Bartlett and Dr. Brian S. Cummings for their time and advices during my time at the University of Georgia. I thank Mr. Srinivasa Muralidhara for assisting me from the beginning till the end whether helping the animal studies or performing animal surgeries in this work, in addition to the all the vigorous discussion covering various topics. I really enjoyed your company. I also would like to extend my appreciation to my labmates including Dr. Satheesh Anand, Kyu-Bong Kim and Brooks McPhail as well as the departmental secretaries for their excellent help and kindness.

Last, but the foremost, my indescribable thanks go to my family, especially wife YJ and baby Hanvit of my joy and pride, without their constant support I would never been able to survive these so many years as a graduate student. I am deeply obliged to my parents for their immeasurable love and everlasting encouragement throughout my life.
Thank you to you all—I could not have done it without your help.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Trichloroethylene (TCE) is a volatile, lipophilic chlorinated alkenyl halide. Since its first commercial production in the 1920s, by chlorination of ethylene, TCE has been used as a general purpose solvent for degreasing and was introduced in use for dry cleaning in the 1930s. TCE was much less toxic than other similar volatile organic chemicals (VOCs), such as carbon tetrachloride (CCl_4) and chloroform ($CHCl_3$). Currently, about 80 ~ 90 % of TCE usage worldwide is for degreasing in metal cleaning operations, but it also has been used as paint stripper, adhesive solvent, ingredient in paints, precursor for solvents or polymers and for plutonium disposition in nuclear production facilities.

TCE is mainly released to air as vapor from degreasing operation sites, as well as lesser amounts from waste disposal and treatment facilities (U.S. EPA, 1985). Poor handling and improper disposal of TCE in landfills have been the main causes of groundwater contamination and release to surface waters from industrial discharges (IPCS, 1985). Thus TCE is the most abundant contaminant of groundwater at Superfund sites (identified at 47 % of > 1,000 NPL sites) in US. Up to 34 % of municipal drinking water supplies tested had TCE contamination (ATSDR, 1993).

TCE is prevalent in urban air, water, soil and even in food, but the U.S. EPA concluded that exposure of the general population to TCE from food was probably low (U.S. EPA, 2001). In the atmosphere, TCE is highly reactive and does not persist for a significant length of time. In surface water, TCE is mainly removed via volatilization

with minor contributions from photo-degradation and hydrolysis. TCE is degraded slowly by microorganisms in groundwater (ATSDR, 1993). TCE has been found in animal and human biological specimens, such as blood, breast milk, sweat, saliva, seminal fluids and particularly in adipose tissues. Certain TCE metabolites (i.e., trichloroacetic and dichloroacetic acids (TCA and DCA)) are also produced during disinfection of drinking water by chlorination or chloramination (U.S. EPA, 1985). In the U.S., TCE is listed as a priority pollutant under the Clean Water Act (CWA) and Safe Drinking Water Act (SDWA), with maximum contaminant limit set at 5 μg/L (ppb). TCE is also regulated under the Resource Conservation and Recovery Act (RCRA) as a spent solvent process waste and as a characteristically toxic waste (any material leaching at more than 0.5 mg/L). The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) requires reporting of releases of TCE above 100 pounds (about 8 gallons), while the Superfund Amendments and Reauthorization Act (SARA) lists TCE as a chemical requiring reporting under its community right-to-know provisions.

TCA, a major end metabolite of TCE, has been used as a laboratory reagent in the synthesis of various medicinal and organic chemicals, as a soil sterilizer and a selective herbicide for control of many annual and perennial grasses in crop and non-crop fields (Hoekstra, 2003). Medical uses of TCA include application as an antiseptic and a peeling agent used for the topical treatment of warts and other dermatological conditions. TCA also is used as an etching agent for the metal surfaces, and as a solvent in the plastics and textile industries (HSDB, 2002).

In the atmosphere, TCA can be formed as a combustion by-product of organic compounds in the presence of chlorine and as a photo-oxidation product of tetrachloroethylene and TCE (Juuti and Hoekstra, 1998). However, Sidebottom and Franklin suggested that atmospheric degradation of chlorinated solvents contributes only a minor amount of TCA to the atmosphere (Sidebottom & Franklin, 1996). Also, the U.S. EPA data for U.S. drinking water supplies indicate that TCA is detected in groundwater and surface water at mean concentrations of 5.3 and 16 μg/L, respectively. It has been measured in concentrations ranging up to 80 μg/L in groundwater and up to 174 μg/L in surface water distribution systems. TCA is also likely to be found as a disinfection by-product in meat and other food products, as chlorine is used in food production processes including disinfection of chicken in poultry plants (U.S. EPA, 2002).

Toxicokinetic aspects of TCE and TCA

TCE is readily absorbed across biological membranes, as a result of its volatile and lipophilic properties. At high vapor levels, TCE is an eye and skin irritant. There are essentially three routes of exposures to consider for humans or laboratory animals: inhalation (vapor), dermal (vapor or liquid), and oral (liquid). The most common route of TCE in occupational settings is inhalation. Ingestion is another major route of exposure to TCE, particularly in environmental settings. Since TCE is uncharged and highly lipophilic, uptake can readily occur by passive diffusion via the gastrointestinal (GI) tract, skin and mucous membranes, and alveoli.

With inhalation and oral administration, TCE is rapidly and extensively absorbed into the systemic circulation, and subsequently distributed to different target organs (e.g.,

lungs, liver, kidneys, and nervous system, etc.) according to their blood supply and lipid content (Lee et al., 1996). Clearance occurs by two major processes: exhalation of the parent compound; and by metabolism, mainly in liver with subsequent urinary and biliary elimination of metabolites. Most of the TCE absorbed from the GI tract goes to the liver, where much of low doses is metabolized. The lipophilic chemical primarily accumulates in adipose tissue, regardless of the route of administration. Estimated TCE half-lives in richly and poorly perfused compartments (e.g., adipose tissue) are 2 ~ 4 min vs. 3.5 ~ 5 hr, respectively (Davidson and Beliles, 1991).

Many studies have showed marked differences in TCE pharmacokinetics in rodents and humans. Prout *et al.,* (1985) demonstrated the different elimination patterns in rats and mice given a single *po* dose (10 to 2,000 mg/kg) of isotope-labeled TCE. Linear kinetics were observed in the mouse at dose of 1,000 mg/kg and above (Prout *et al.,* 1985). They showed mice and rats metabolized TCE almost completely at a 10 mg/kg dosage. Sixty % of this dose was excreted as metabolites in urine with only ~ 4 % eliminated unchanged in expired air during the first 24-hr period. However, almost 78 % of the dose was eliminated unchanged in the rat with 2,000 mg/kg TCE dose, compared to only 14 % in the mouse. These findings reveal at high dosages, the mouse is exposed to significantly higher concentrations of potentially toxic and/or carcinogenic TCE metabolites than the rat. The researchers also examined differences in pharmacokinetics of TCE and its metabolites in the blood, with mice exhibiting higher rates of metabolism, with the mouse exhibiting significantly higher blood concentrations of both trichloroethanol (TCOH) and TCA than the rat (4-fold and 7-fold differences,

respectively). They reported the time for reaching maximum metabolite concentration to be 2 hr for mice and more than 10 hr for rats.

Human TCE pharmacokinetic data come mostly from the case studies after accidental or intentional exposure. Yoshida *et al.* (1996) reported the pharmacokinetic profiles of TCE and its metabolites in blood and urine following accidental TCE ingestion. The authors described two different phases of TCE elimination from the serum and urine with excretion persisting for 2 days. The half-life of urinary TCA excretion was 26 hr for the initial phase versus 52 hr for the terminal elimination phase (Yoshida et al., 1996).

Differences in partition coefficients for TCE (including blood/air, P_B; fat/blood, P_{FAT}; liver/blood, P_{LIVER}; and rapidly perfused tissue, P_{RAP}) across the species have been reported. For example, Allen and Fisher (1993) and Fisher *et al.* (1991) reported that TCE's partition coefficient for blood/air (P_B) is higher in mice (14.0) and rats (18.5) than that in humans (9.2). This is another important factor to be considered when extrapolating animal data to humans (Allen and Fisher, 1993; Fisher *et al.*, 1991).

Due to its high water solubility (ca. 13 g/L), TCA is rapidly absorbed from the GI tract of rats and humans (Kim and Weisel, 1998). It is then distributed primarily into the plasma and richly perfused organs, leading to lower concentrations in fat. The majority of TCA is excreted in urine unchanged in humans and rodents (Larson and Bull, 1992b). Thus, TCA's toxicokinetic profiles are much different from those of TCE. The half-life

of TCA is much longer than that of TCE, whether given orally or formed as a metabolite after the administration of TCE or TCOH.

The toxicokinetics of TCA also show clear differences across species. Fisher *et al.* and Schultz *et al.* showed that the plasma half-lives for TCA were much shorter in rodents than in humans. For example, a TCA plasma half-life of 12 hr was found after the iv injection of 5 mg/kg TCA in male rats. Male mice given intraperitoneal doses of 5 mg TCA/kg exhibited a plasma half-life of 7 hr. In humans, administration of 3 mg TCA/kg resulted in a plasma half-life of 51 hr (Fisher et al. 1991; Fisher et al. 1998; Allen and Fisher 1993; Schultz et al. 1999). Volkel et al. (1998) reported the mean elimination half-lives for TCA in urine (46 hr in humans and 11 hr in rats) from a study of the inhalation of perchloroethylene, which is also metabolized to TCA. Fisher *et al.* (1991) estimated the half-lives of TCA formed after TCE exposure. The plasma TCA half-life in male mice exposed for 4 hr to TCE vapors (42 ~ 889 ppm) was estimated to be 16 hr, which is comparable to 15 hr for rats exposed to TCE vapors (500 ~ 600 ppm). In contrast, the plasma half-life of TCA in humans after TCE inhalation of either 50 or 100 ppm was significantly longer (86 to 99 hr) (Fisher *et al.*, 1998).

Another important property of TCA is its plasma protein binding (or sequestration) capacity. This plays a major role in distribution and elimination of TCA, and leads to differences in TCA dosimetry in different species. Many studies have demonstrated that TCA binds in significant amounts to plasma proteins. For example, Muller *et al.* (1972) stated that approximately 90 % of TCA in human blood was bound to plasma proteins, but provided no binding data.

Protein binding capacity (B_{max}) of TCA shows marked species dependence. Templin *et al.* (1995) investigated the binding of TCA to plasma proteins in the rat, dog, mouse and human. The authors reported that rat plasma had approximately one-half the TCA binding capacity of human plasma (Templin et al., 1995). Lumpkin *et al.* (2003) reported that the fraction of TCA bound to plasma proteins was both species- and TCA concentration-dependent. They reported the binding capacities (709, 283 and 29 μM, respectively) and mean percentage bound values (82 %, 39 % and 19 %, respectively) for humans, rats and mice. This suggests the relatively low plasma binding of TCA in rodents would result in higher TCA exposures of their liver (Lumpkin et al., 2003).

TCE is a modest toxic substance, as revealed by many studies of a wide range of toxic end-points (NTP, 1990; Barton *et al.,* 1996; Kaneko *et al*., 1997). Due to its relatively poor solubility in water (1.1 ~ 1.4 g/L), few researchers used water as a vehicle in their toxicity or carcinogenicity studies. Many such study results are therefore confounded by the use of a vegetable oil diluent, which has been found to alter TCE pharmacokinetics and to affect lipid metabolism and other pharmacodynamic processes (ATSDR, 1997; Tucker et al., 1982).

Acute exposures of rats and mice have shown TCE to have low toxicity following inhalation and oral exposure. Oral LD_{50} values were determined to be 2,400 mg/kg in mice (Tucker *et al.,* 1982) and 4,920 mg/kg in rats in a 14-day acute toxicity study (IPCS, 1985; ATSDR, 1997). Long-term gavage studies in rats and mice with very high doses TCE have been revealed nephropathy (with its characteristic degenerative changes in the renal tubular epithelium) (NCI, 1976), along with toxic nephrosis in other cancer

bioassays in mice and rats (characterized by cytomegaly of the renal tubular epithelium) (NTP, 1988 and NTP, 1990). When TCE toxicity was investigated using F344 rats and B6C3F1 mice given 500 or 1,000 mg/kg in corn oil 5 days per week, for 103 weeks, the rate of survival was reduced in male rats and mice (NTP, 1983). The Lowest Observed Adverse Effect Level (LOAEL) of this chronic study was 500 mg/kg/day for rats and 1,000 mg/kg/day for mice (NTP, 1990).

TCE has not caused biologically-significant embryotoxic or teratogenic effects in animal studies. Evidence for mutagenic effects was inconclusive. There is clear evidence that TCE is carcinogenic in B6C3F1 mice after lifetime (2-year) inhalation exposures to 1,620 $mg/m^3/kg/day$ (or 300 ppm/day) or oral administration of 700 – 1,200 mg/kg/day. There is also evidence that TCE caused a low incidence of renal tumors in some strains of rats exposed for 2 years to levels of 3,240 $mg/m^3/day$ (or 600 ppm/day) by inhalation or to 500 - 1,000 mg/kg/day orally (NCI, 1976; NTP, 1988; NTP, 1990). Liver tumor induction in mice by TCE is one of the most critical effects from the standpoint of environmental regulations.

In humans, exposure to high enough dose of TCE causes a variety of disorders, including central nervous system (CNS) depression, hepatotoxicity and nephrotoxicity (IPCS, 1985). The LOAEL for CNS depression from acute TCE exposure is $\geq$ 200 ppm, with symptoms including dizziness, headache, nausea and blurred vision, etc. Anesthesia occurs upon inhalation of > 2,000 ppm. Coma and even death, associated with cardiac arrhythmias and respiratory failure, have been reported at 10,000 ppm or higher

concentrations. For example, a case of accidental TCE exposure was reported by Yoshida *et al.* (1996). A worker fell into a TCE reservoir bath, resulting in deep coma. Unlike the positive findings in the studies with rodents, carcinogenic effects of TCE and its metabolites in human exposure are not clear. The official classification of TCE by the International Agency for Research on Cancer (IARC) is "probable carcinogen to humans' (Group 2A). Nonetheless this designation was based on limited evidence from several human epidemiological studies (IARC, 1995).

TCA exposure

Effects of acute TCA exposure were reported by Davis (1998) in a study of the oral administration of TCA (30 or 300 mg/kg/day for 7 days) in the drinking water of rats. At high dose of TCA (or DCA), weight loss along with decreased food consumption was observed. These were attributed to decreased water consumption (Davis, 1986).

A study of the effects of subchronic administration of TCA (or DCA), at doses as low as 350 mg/kg/day for 90 days, showed decreased body weight and substantial toxicity to the liver and kidney, along with histopathologic changes in male S-D rats. These doses, of course, are far greater than those expected to occur in the environment (Mather et al., 1990). In another 90-day subchronic study, the toxic effects of monochloroacetic acid (MCA), DCA and TCA were compared after oral exposure of rats via their drinking water. Morphological changes were predominantly localized to the portal triads in the liver, which were mildly to moderately enlarged. Minimal alterations were observed in the lungs. This study also indicated that DCA was more toxic than TCA (Bhat et al., 1991).

In a long-term exposure study by DeAngelo *et al.* (1997), groups of male F344 rats were given TCA in drinking-water at 0.05, 0.5 or 5.0 g/L (3.6, 32.5 or 364 mg/kg per day) for 2 years. Some effects were observed including increased serum alanine aminotransferase (ALT) activity and limited hepatic necrosis only at the highest dosage level. No changes in kidney, spleen or testis weights were observed at the same dose, nor was there evidence of hepatocellular proliferation, as measured by radiolabelled thymidine incorporation rates. The investigators reported the NOAEL for this study to be 32.5 mg/kg of body weight per day, based on non neoplastic effects (DeAngelo et al., 1997).

As for the possible carcinogenicity, TCA has given mixed results in *in vitro* assays for mutations and chromosomal aberrations and has been reported to cause chromosomal aberrations in *in vivo* studies. IARC concluded that TCA is not classifiable as to its human carcinogenicity (Group 3), due to inadequate evidence for the toxicity and carcinogenicity (IARC, 2002; IARC, 2004). There are major health concerns about TCA (and TCE) mainly because TCA induces peroxisome proliferation in mouse liver (but does not induce the same response in rats) in the same range of doses that induces hepatic tumors (Prout *et al.,* 1985). U.S. EPA also classified TCA as C, possible human carcinogen in 1994, in accordance with the 1986 EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1994). However, under the 1999 EPA Draft Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), there is suggestive evidence of TCA carcinogenicity, but the data are not sufficient to assess human carcinogenicity (U.S. EPA, 2002).

TCE risk assessment

Assessment of the human health risks due to TCE exposure remains challenging, because TCE is a chemical with inherently complex metabolism, effects, and mode of action (MOA). Since U.S. EPA began to publish TCE health risk assessments in the late 1980s (U.S. EPA, 1985; U.S. EPA 1987), a substantial amount of scientific research assessing TCE health risks has been reported. Yet, there is a wide spectrum of perspectives on a number of critical and controversial scientific issues related to TCE health risks. These are currently the subject of a scientific review by the National Academy of Sciences (NAS) co-sponsored by a number of federal agencies, including the U.S. EPA, the U.S. Air Force, and the U.S. Department of Energy, etc. State-of-the-science (SOS) papers were published as a monograph in *Environmental Health Perspectives Supplement* (Scott and Cogliano, 2000), which reviewed a range of scientific subjects relevant to TCE health risk assessment, including pharmacokinetics, MOA, epidemiology and dose–response analysis. Since then, a substantial amount of new literature relevant to characterization of the human health risks from TCE have been published and reviewed extensively (Chiu, et al., 2006; NRC, 2006).

As stated, many aspects of human health risks from TCE have to be considered to attempt to correlate between the exposure to a chemical, its toxicity and subsequent cancer risks, such as dose, duration and route of exposure, different susceptibilities, etc. By definition, U.S. EPA dose-response-based cancer risk assessments as well as the reference dose-reference concentration (RfD/RfC) approach for non-cancer risk assessments are assumed to protect vulnerable subpopulations (Renwick and Lazarus,

1998). However, many of these applications have been based on default assumptions, without considering specific biological data. The EPA's default cancer risk assessment policy is applied to most chemicals, including TCE. It is based on a linear extrapolation from effects of high doses in rodents to risks for humans at low doses. However, data from many metabolism, toxicology and epidemiology studies on TCE and its metabolites casted doubts on this traditional approach. Many studies, including one by Steinberg and DeSesso (1993) suggested that it is possible to increase substantially the allowable TCE level in drinking water without increasing health hazards using a more appropriate threshold model, rather than a straight-line extrapolation model. Chlorination of drinking water can produce much higher levels of haloacetic acids (HAAs) than originate from metabolism of TCE under current regulations (Steinberg and DeSesso, 1993). The U.S. EPA Guidelines for Risk Characterization of 2005 have since emphasized the need to identify and include susceptible populations in risk assessment processes (U.S. EPA, 2005). Predisposing factors (susceptibilities) in subpopulations include genetic factors (e.g., specific polymorphisms), acquired factors (prior and/or concurrent exposure to other substances), behavioral patterns (smoking, drinking), altered health status (diabetes, acute renal failure) as well as fasting, obesity and age differences.

In this project, the aim has been to develop a specific P450-induced animal model with S-D rats, which mimics some altered physiological conditions/disease states (e.g., obesity, fasting, P450-inducing xenobiotics, diabetes) in which CYP2E1 is elevated, possibly resulting in increased formation of carcinogenic metabolites and resulting increased cancer risks. For the convenience of this discussion, background information

on the metabolism of TCE and its major metabolites is described before the elucidation of experimental aspects of this work. The following discussion summarizes the experimental evidences accumulated to date on the metabolism and pharmacokinetics of TCE and its major oxidative metabolites, including TCA, TCOH, chloral hydrate (CH) and DCA, some of which are believed to play a role in cancer risks posed by TCE.

TCE and TCA Metabolism

A schematic diagram of TCE metabolism pathways is shown in Figure 1-1. Based on both *in vivo* and *in vitro* data, TCE is known to be metabolized by: 1) a P450-dependent oxidative pathway; catalyzed by CYP2E1 and certain other P450s and 2) glutathione (GSH)-dependent pathway, mediated by glutathione-S-transferases (GSTs). The two key enzymes, CYP2E1 and GST, exhibit different kinetics (i.e., affinity and capacity) for each pathway. Shown on the right side of the diagram is the major oxidative pathway, which consists of TCE oxidation to CH by CYP450, followed by either oxidation of CH to TCA by aldehyde dehydrogenase (ALDH), or reduction of CH to TCOH by alcohol dehydrogenase (ADH). The oxidative metabolism of TCE takes place primarily (but not exclusively) in the liver, which has the highest quantities and activities of the CYP2E1 and other P450s. The minor pathway is far less important quantitatively and is present mainly in the liver and kidney. It involves conjugation of TCE with GSH and is shown to the left. TCE metabolism may also occur in lungs, spleen, small intestines and brain. CYP2E1 is believed to be present in rat kidney proximal tubules. The GSH-dependent conjugation pathway is believed to be responsible for the metabolites that are detoxified or activated in the kidney. Certain of these are

thought to be responsible for nephrotoxicity and potential nephrocarcinogenicity (Cummings et al., 2001; Lash et al., 1995). But, in accord to the purpose of the current project, the focus will be on the oxidative pathway.

The first oxidation step catalyzed by CYP2E1 involves formation of a TCE-oxygen-P450 (or epoxide) intermediate. It rearranges to form the oxidative metabolites chloral and CH. Relatively small amounts of CH can be recovered *in vivo*, as it is rapidly converted to other compounds in the liver. Thus the circulating concentration of CH in the blood is relatively low compared to levels of TCA and TCOH, as shown by experiments demonstrated in Chapter 2.

CH is not likely to be a major hepatotoxic or hepatocarcinogenic metabolite, due to its lack of longevity *in vivo*. Mayers *et al.* found that CH was detectable for several hours in children given 50 mg/kg, which is contrary to what has been observed in the adult, whose clearance profile is characterized by rapid and almost complete clearance of CH. The authors suggested a continuing production of CH from TCOH, since blood concentrations of CH resembled the time-course profile of TCOH, but were approximately an order of magnitude lower (Mayers et al., 1991; Mayers et al., 1992). CH is commonly-used sedative for dental, diagnostic and minor surgical procedures in children.

One of the distinctive examples of CH toxicity, however, is in the lungs of male CD-1 mice. Forkert and Birch showed Clara cell injury after TCE exposure was attributed to the accumulation of CH (Forkert and Birch, 1989; Forkert and Birch, 1993). The metabolism of CH is much slower than the conversion from TCE to CH in Clara

cells, leading to the buildup of CH (Odum et al., 1992). Furthermore, the rate of formation of CH in mouse lung was found to be markedly higher than that in either rat or human lungs, in addition to the slower rate of CH metabolism (Green et al., 1997).

Metabolism of CH involves several steps and other oxidative and reductive enzymes, besides P450. CH is reduced to TCOH in the cytosol or oxidized to TCA in either the cytosol or mitochondria, with marked species differences (Ikeda *et al.,* 1980). CH can be either reduced, which requires NADH as a cofactor, by alcohol dehydrogenase (ADH) to TCOH, or oxidized by aldehyde dehydrogenase (ALDH) to TCA in the presence of NAD^+ (Larson and Bull, 1989). Metabolism studies of CH by Ni *et al.* (1996) and by Lipscomb *et al.* (1996) with the male B6C3F1 mouse have suggested the involvement of CYP2E1 in TCOH formation in mouse liver. The precise role of each enzyme in conversion of CH to TCOH, however, remains to yet be determined.

A relatively small proportion of TCA may be metabolized (reduced) in the liver to DCA, which is considered hepatotoxic and hepatocarcinogenic along with TCA, although there have been considerable controversies about the formation of DCA especially in rats and humans (Lipscomb et al., 1996; Templin et al., 1993). In some studies, DCA has been identified as a metabolic product of both TCOH and TCA in rodents and humans. For example, after TCE administration to mice via gavage, low DCA concentrations were found in blood and tissue samples (Abbas and Fisher, 1997). Bruning *et al.* (1998) identified DCA and MCA for the first time in human urine as metabolites of TCE in a 17-year-old male who ingested approximately 70 ml of TCE in a suicide attempt. In a study

by Larson and Bull, the formation of DCA along with carbon dioxide, glyoxylic acid, oxalic acid, glycolic acid was observed in rats and mice following oral administration of 20 or 200 mg/kg of isotope-labeled ^{14}C-TCA. The authors suggested that TCA was metabolized by reductive dehalogenation to DCA, while others suggested that DCA could be formed from dichloroacetyl chloride via TCE oxide (Hathway, 1980; Larson and Bull, 1992a).

Other investigators have argued that metabolism of TCA to DCA may have been over-reported in some of the earlier studies due to analytical artifacts (Lash *et al.,* 2000). A study by Yu *et al.,* (2000) reported that in Fischer 344 rats given intravenous injections of isotope-labeled ^{14}C-TCA at doses of 6.1, 61 or 300 μmol/kg (approximately 1, 10 or 50 mg/kg), as much as 84 % of the administered radioactivity was excreted in the urine within 24 h of dosing. Furthermore, HPLC assay of plasma, urine and liver homogenate failed to detect any oxalate, glyoxalate, glycolate or DCA, suggesting that TCA was poorly metabolized by the rats (Yu et al., 2000). Clear conclusions and accurate quantitative analyses of DCA have been hindered by post sampling conversion of TCA to DCA (Brown et al., 2003; Dixon et al., 2005).

Another important question about the TCE oxidative pathway is the identity of the enzyme(s) responsible for the conversion of TCOH to TCA. Direct conversion of TCA to TCOH is highly unlikely. CYP2E1 has been postulated to be the predominant isoform to catalyze the oxidation of TCOH to TCA (Larson and Bull, 1989), and this issue will be addressed in TCOH or TCA intravenous administration experiments in Chapters 3.

A substantial percentage of TCOH, recovered in the urine and bile of animals and humans exposed to TCE, has undergone glucuronidation by UDP-glucuronosyltransferase (UGT) to TCOH-glucuronide (TCOG) in the liver. TCOG in bile may undergo bacterial cleavage of the glucuronide and enterohepatic recirculation of the TCOH rather than fecal excretion. Once TCOG returns to the liver, it may be hydrolyzed back to TCOH and be metabolized further to TCA or DCA. The involvement of UGT in TCE metabolism raises the question of whether CYP2E1 inducers would also cause induction of UGT.

CYP450s and CYP2E1

The key mechanistic aspects of the isozymes responsible for the metabolism of TCE, their induction mechanism and its significance are described below. Cytochrome P450s (CYP450s) are a superfamily of Phase I drug-metabolizing enzymes with a heme-containing moiety. CYP450s are the major catalysts involved in the bio-activation and bio-transformation of many xenobiotics, including drugs, toxicants and potential chemical carcinogens. CYP450s frequently convert chemicals to potentially reactive products, which can cause cell toxicity and even cancer. Other groups of compounds are detoxified by P450s. A limited number of other CYP450s is also responsible for the metabolic conversion of endogenous compounds such as steroid hormones and bile acids, as well as the metabolism of retinoic acid and fatty acids, including prostaglandins and eicosanoids. Thus, CYP450s have become a significant focus of interest, especially in the areas of drug metabolism, pharmacology and toxicology. Although most of the reactions mediated by CYP450s are oxidation processes, they also catalyze a variety of other

reactions, including reduction, desaturation, ring formation and expansion, dehydration, one-electron oxidation, coupling reactions, etc. Since a single CYP450 can metabolize a large number of structurally-diverse compounds, these isozymes can collectively metabolize a wide array of drugs and other chemicals in the diet, environment and workplace (Guengerich, 2001; Guengerich, 2004; Guengerich, 2006).

Among the various member of the CYP450 superfamily (xenobiotic-metabolizing CYP450s are found in families 1 through 4), four different isoforms (CYP1A1/2, CYP2B1/2, CYP2C11/6 and CYP2E1) have been identified in rodents as playing a role in TCE metabolism. CYP2E1 (EC 1.14.14.1) is the major isoform for metabolism of low dose TCE in rodents and humans, as it is a high affinity and low capacity isoform (Guengerich et al., 1991; Nakajima et al., 1990). In rats, CYP2E1 was found to account for more than 60 % of TCE metabolism, with smaller contributions from CYP1A1, CYP1A2 and CYP3A4. The identity of other isoforms that participate in metabolism of high TCE doses in humans is not still clear (Nakajima et al., 1992a). CYP2E1, which is the only constitutive isozyme of the 2E subfamily in humans and in rats, is responsible for the oxidative xenobiotic biotransformation of various endogenous and exogenous compounds, including ethanol, isoniazid and acetaminophen, as well as volatile hydrocarbons of low molecular weight (Nakajima, 1997; Guengerich et al., 1991). CYP2E1 is found mainly in the liver, but also exists in the extra-hepatic tissues including lungs, GI tract, testes, brain, etc. CYP2E1 is expressed in different levels in these tissues, in different species and among different humans. Previous structural and immunoassay studies have shown that CYP2E1 has well conserved gene and protein among P450s with

high similarities between the human and rat (Snawder and Lipscomb, 2000). Thus, the rat appears to be an excellent animal model to generate data relevant to CYP2E1 metabolism, which can be extrapolated to humans.

CYP2E1 induction and inducers

While baseline CYP2E1 activity in human liver showed interindividual variation of ~ 7-fold, control levels of CYP2E1 fluctuated only ± 20 % in rats (Lipscomb et al., 1997). Nakajima *et al.,* (1992b) showed sex-, pregnancy-, and age-related differences in metabolism of VOCs can result from variations in CYP2E1 content. Many structurally-diverse chemicals participate in microsomal enzyme induction, leading to up-regulation of a wide array of hepatic multifunction oxidases (MFOs). These microsomal enzyme inducers can affect other drug-metabolizing enzymes as well, including UGTs and GSTs. These, of course, are Phase II drug-metabolizing enzymes that are necessary for adding conjugates or co-substrates to xenobiotics to further enhance the chemicals' hydrophilicity, and thus facilitate elimination.

Induction of most CYP450s and other drug-metabolizing enzymes by microsomal enzyme inducers generally occurs at the transcription level, resulting in subsequent increases in CYP450 proteins and their functional activities. These inducers also can be classified as ligands for different nuclear receptors and DNA enhancer elements that influence the genes and their transcriptional activation. For example, Wilson and Safe showed that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other CYP1A inducers bind to the aryl hydrocarbon receptor (AhR). The subsequent protein complex, in turn, undergoes nuclear translocation and dimerization with AhR cofactor. This nuclear

heterodimer interacts with the xenobiotic response element (XRE) and activates CYP1A transcription (Wilson and Safe, 1998). However, the regulation involving CYP2E1 expression seems to be more complicated. It generally appears to be under tight control, since mRNA and protein levels are typically elevated. Several mechanisms of CYP2E1 induction have been proposed. These other mechanisms include: 1) increases of CYP2E1 mRNA due to transcriptional activation or post-transcriptional change (e.g., mRNA stabilization); 2) an increase in mRNA translation, and 3) decrease in CYP2E1 degradation due to protein stabilization (Koop et al., 1991).

CYP2E1 levels are altered in response to endogenous pathophysiological conditions caused by hormones such as insulin and by growth factors (including epidermal growth factor). Xenobiotic inducers also elevate CYP2E1 protein levels through both increased translation and stabilization of the protein from degradation, which appear to occur primarily through ubiquitination and proteasomal degradation (Novak and Woodcroft, 2000). The induction of CYP2E1 expression in liver can also be affected by other factors, including prior- or co-exposure to xenobiotics, fasting (Soh et al., 1996) and obesity (Murray, 2006), smoking (Czekaj et al., 2005) and by acute renal failure (via increases in plasma urea in conjunction with L-arginine metabolism) (Chung et al., 2002). Parkinson *et al.* (2004) showed that CYP2E1 (along with CYP1A2, CYP2B6, CYP2C19, and CYP2D6) activity in human liver microsomes appeared to decrease (at least 25 %) with age when subjects were grouped as follows < 20 years, 20 to 60 years, and 60 + years. However, it seemed doubtful that whether these decreases

would be biologically significant. The authors pointed out that the clearance (and volume of distribution of many drugs) is generally diminished in elderly people.

Although it is quite unrealistic to expect that CYP2E1 inducers will influence only CYP2E1, a list of CYP2E1 inducers presented in the following discussion encompasses 'relatively' specific ones. An original aim of this dissertation work was to establish a robust CYP2E1 induction model with ethanol, which then could be employed to test assumptions about the inducer's impact on TCE metabolism.

Hu *et al.* (1995) showed that the chronic ethanol exposure caused the marked induction of CYP2E1 in the centrilobular liver region, where alcoholic damage commonly is initiated, using male Wistar rats. Although the level of induction was very high (20-fold increase of liver CYP2E1 protein and 16-fold increase of catalytic activity, respectively), a very complicated and impractical induction protocol was provided. To maintain high levels (20 ~ 70 mM) of ethanol in blood, the authors initiated ethanol tolerance in animals by addition of ethanol to the drinking water by stepwise increase from 3 to 7 %, starting at 6 days prior to the forced ethanol administration regimen. Ethanol was then dosed by gavage as a 20 % (v/v) solution, increasing the total daily dose from 8.5 to 12 g/kg. In addition, all animals had to be subsequently maintained on water containing 7 % ethanol (Hu et al., 1995). Bardag-Gorce *et al.* (2005) utilized a simpler induction protocol that produced a 3.5-fold induction of CYP2E1 in male Wistar rats fed intragastrically with a liquid diet containing ethanol (13 g/kg/day) feeding for 15 days (Bardag-Gorce et al., 2005).

Some of the published induction regimens, like that of Hu et al. (1995) were too complex and time consuming. Several reported regiments for CYP2E1 induction models with ethanol in rats were tried, but our results from the set of pilot experiments were inconsistent and the extent of induction less impressive than anticipated. The decision was made to select a CYP2E1 inducer other than ethanol for the initial phase of the current project. It was also recognized that ethanol also competes for enzymes responsible for later steps in the oxidative pathway.

There were several examples of CYP2E1 induction by acetone. For example, a study by Buhler *et al.* (1992) showed that CYP2E1 induction by acetone (along with CYP2C11 and CYP3A1 induction) were occurred in the centrilobular area of the rat's liver, where they are constitutively expressed. This was the case irrespective of inducers (Buhler et al., 1992). Forkert *et al*. (1994) observed that both an acute treatment (5 ml/kg, single dose, intragastric) and a subacute regimen (1 % acetone in drinking water for 8 days) produced significant increase in the level of CYP2E1 protein (by 4.4- and 5.3-fold, respectively) in mice without significant alterations in the levels of CYP2E1 mRNA. *P-Nitrophenol* (PNP) hydroxylation was also increased in liver microsomes of acutely and chronically exposed animals (by 2.3- and 3.7-fold, respectively), when compared with controls (Forkert et al., 1994).

Longo and Ingelman-Sundberg (1993) examined the inducibility and molecular regulation of CYP2E1 in nasal mucosa of rats after acetone (5 mL/kg) treatment for 2 days. They showed that the amount of CYP2E1, as well as the rate of microsomal PNP hydroxylase activity, had increased by 2- to 3-fold in microsomes isolated from nasal

mucosa 24 hours following treatment with acetone. Interestingly they reported the CYP2E1 increase was accompanied by a corresponding increase of CYP2E1 mRNA, which was contrary to conclusions of others.

Pankow *et al.* (1994) showed increased PNP hydroxylase activity in liver microsomes from rats pretreated with acetylsalicylic acid (ASA), suggesting CYP2E1 involvement in the metabolism of salicylic acid (SA), and SA as an inducer of CYP2E1. Studies by Damme *et al*. (1996) of the mechanism of CYP2E1 induction by ASA or its metabolite salicylate (SAL) showed significantly elevated CYP2E1 mRNA levels in livers of ASA-treated rats compared with the control group. Pretreatment of ASA-treated rats with a blocker of mRNA transcription, actinomycin D, or a blocker of protein synthesis, cycloheximide, markedly suppressed PNP hydroxylase activity. This mechanism of CYP2E1 is different from that of certain other inducers of CYP2E1, which achieve induction via post-transcriptional activation without elevation of the mRNA level (Damme *et al*., 1996).

Kim *et al.* (2001) showed that pyridine induced CYP2E1 protein in the absence of an increase in CYP2E1 mRNA levels. CYP2B1/2, CYP3A1 and CYP3A2 protein levels and their mRNA levels, however, were increased. Hotchkiss *et al.* (1995) demonstrated a selective induction of CYP2E1 in kidney tubular epithelial cells, providing a basis for organ-specific nephrotoxicity, when certain xenobiotics are bioactivated to toxic metabolites by renal CYP2E1 in situ.

Schoedel and Tyndale (2003) found that nicotine also induced CYP2E1 protein levels and activity, without producing changes in levels of CYP2E1 mRNA. The authors also demonstrated that ethanol treatments increased CYP2B1 protein, mRNA and

CYP2B1-mediated nicotine metabolism, suggesting that metabolic cross-tolerance may occur between nicotine and ethanol.

Other xenobiotic compounds identified as CYP2E1 inducers include skatole (a tryptophan derivative produced in the hind-gut of pigs and metabolized via hepatic CYP2E1), which showed post-translational induction (Doran et al., 2002); dimethyl sulfoxide (CYP2E1 mRNA level was induced, while the UGT1A9 mRNA level was decreased by 2.5 % DMSO) (Nishimura et al., 2003); 4-methylpyrazole (which also induced several other CYP450s (Wu and Cederbaum, 1993; Wu and Cederbaum, 1994); isoniazid (CYP2E1 induction by isoniazid is due to activation of CYP2E1 mRNA translation) (Park et al., 1993; Poloyac et al., 2004); and GYKI-47261, a new AMPA [2-amino-3-(3-hydroxymethylisoxazole-4-yl)propionic acid] antagonist (Tamasi et al., 2003), etc.

Morel *et al.* (1999) studied the effects of the sex of animals on rat CYP2E1 activity by estimating the responses of 5-, 7- and 9-week-old male and female rats to different induction conditions. The results showed that hepatic PNP hydroxylase activity decreased significantly in control male rats in as animals matured. CYP2E1 induction by ethanol also decreased during this period. The effects of ethanol, acetone and pyridine on PNP hydroxylase activity were evaluated in 7-week-old male and female rats. The male rats exhibited significantly higher PNP hydroxylation than females. Seven-week-old male controls had higher PNP hydroxylase activity than male controls in age-groups, as well as larger increases in the enzyme's activity in response to the inducers, suggesting this is the most suitable age for CYP2E1 induction experiments. Morel et al.'s results strongly suggested that 7-week-old male S-D rats would be appropriate animal models for

studying the role of CYP2E1 in the metabolism of TCE and the toxicokinetics of it and its metabolites in the current research project.

Some of the most useful information for the present experimental design came from the report by Kim and Novak (1993). They studied several structurally-related sulfur- and nitrogen-containing heterocycles including thiazole, pyrazine, pyridazine, pyrimidine, thiophene and triazole, which are present in tobacco, tobacco smoke and certain foods. These compounds have been employed to obtain not only profiles of the inhibition and expression of CYP2E1 in hepatic tissue, but the molecular basis for the regulatory events governing induction. The results of Kim and Novak's study showed pyrazine and pyridazine (PZ) to increase CYP2E1 levels ~ 4- and 5-fold, respectively. They also showed that CYP2E1 induction by these compounds resulted in a substantial decrease in CYP2E1 poly (A) + RNA levels in treated animals relative to untreated animals, thus differentially affecting its protein expression.

Induction of CYP2E1 by some exogenous chemicals (including long-term exposure of ethanol at highly intoxicating levels) appear to primarily reflect a post-transcriptional mechanism, associated with a decrease in the rate of protein degradation due to inhibition of oxidative uncoupling by substrate ligands (Wu and Cederbaum, 1993). On the other hand, induction of CYP2E1 activity by fasting, diabetes and obesity, etc has been attributed to CYP2E1 transcriptional and post-transcriptional changes (Hu *et al.*, 1995). It is very important to mention that many of these compounds and inducers are suspected of inducing classes of CYP450s, in addition to CYP2E1. It should be also

noted that there are several types of compounds that suppress constitutive and inducible expression of CYP2E1, including organosulfur compounds (for example, allylsulfide, allylmercaptan and allylmethylsulfide) (Kwak et al., 1994). There are also studies that which showed metabolic interactions between VOCs metabolized by CYP2E1 (Kedderis, 1997; Gonzalez, 2005). Concurrent exposures to sufficiently high doses of such chemicals can result in competitive metabolic inhibition.

Muller *et al.* (1975) examined metabolism of TCE when it was co-administered with ethanol in humans. Such an investigation provides information pertinent to the influences of alcohol on TCE hepatotoxicity and carcinogenicity. Volunteers inhaled 50 ppm TCE for 6 hr per day on 5 consecutive days and were subjected to simultaneous ethanol ingestion (blood level of 0.6 %). The authors reported that the simultaneous exposure to TCE and ethanol caused inhibition of the metabolism of TCE to TCOH and TCA by 40 % on the average. They also reported the increases of TCE concentration in the blood (2.5-fold) and in the expired air (4-fold), as compared to TCE inhalation without ethanol. They reported that no change was observed in the glucuronidation of TCOH.

A study by Larson and Bull (1989) also investigated co-administration of ethanol and TCE in male S-D rats. The animals were administered oral doses of 0.2, 0.6 or 3 g TCE/kg, while the ethanol-treatment group was given an additional 0.07, 0.2, or 2 g/kg ethanol, respectively. The researchers reported that the peak-concentration time (T_{max}) of metabolites was delayed with increasing doses of ethanol. TCE and its metabolites' elimination was prolonged in the ethanol-treatment groups. Authors also reported that

decreased net metabolic conversion of TCE, even at the high dose of TCE where metabolism was saturated. They found that ethanol decreased blood levels of TCA, but only at early times at the highest TCE dose. The urinary TCOH/TCA ratio was increased at all dose-levels, suggesting the metabolism of TCE was shifted toward reduction to TCOH, away from oxidation to TCA (Larson and Bull, 1989).

Watanabe *et al.* (1998) investigated the effect of 2 mM (common concentration of consumed) ethanol on TCE metabolism in perfused Wistar rat liver. They showed that ethanol infusion significantly increased the rate of TCOH production (and TCOH/TCA ratio), while producing a comparable decrease in the TCA production rate. These observed shifts in TCE metabolism in the presence of ethanol suggested that alcohol altered the NAD+/NADH ratio (intracellular oxidation-reduction state) in the hepatocytes (Watanabe et al., 1998).

An *in vitro* metabolism study by Nakashima et al. (1990) of TCE and TCOH using liver microsomes from control and ethanol-treated rats showed that ethanol pretreatment enhanced TCE metabolism, predominantly at low TCE concentrations. A microsomal TCOH-metabolizing enzyme was induced. They observed TCE metabolism by enzymes from ethanol-treated rats was inhibited by the substrate (TCE) itself at high concentrations (suicide inhibition). They argued that ethanol pretreatment enhanced the microsomal conversion of TCOH to CH *in vitro* (Nakajima et al., 1990). This conversion was not observed *in vivo* in the current project when pyridazine (PZ) was used as a inducer.

Dekant *et al.* (1986) used radioisotope-labeled ^{14}C-TCE to demonstrate changes in TCE metabolism after P450 induction by phenobarbital (PB) in Wister rats. They

showed an increase in radioactivity covalently bound to liver and kidney macromolecules in induced rats, suggesting a dose-dependent increase in TCE metabolic capacity. TCA, TCOH and TCOG comprised 89 to 94 % of the radioactivity excreted in the urine, according to HPLC analysis (which is consistent with data presented in Chapter 2 and 3). Other minor metabolites including N-(hydroxyacetyl)aminoethanol (< 7 %), DCA (< 2 %) and oxalic acid (< 2 %) were found in urine by Dekant et al. (1986).

Pankow *et al.* (1994) showed that pretreatment of rats with ASA or sodium salicylate stimulated the metabolism of dichloromethane to carbon monoxide, as measured by the carboxyhemoglobin level in blood. They also showed simultaneous administration of dichloromethane and ASA or sodium salicylate was accompanied by reduced carboxyhemoglobin formation.

Raucy *et al.* (1993) also found that CYP2E1 induction by prior exposure to ethanol played a pivotal role in potentiating the toxicity of halogenated hydrocarbons including TCE. Kraner *et al.* (1993) demonstrated that acetone increased CYP2E1 protein levels in cultured rabbit hepatocytes. Furthermore, CYP2E1 was also shown to be induced by acute renal failure and by certain drugs (including aspirin) (Peng et al., 1983). These diverse factors have the potential to alter TCE metabolism by induction of CYP2E1, thereby affecting the susceptibility of individuals to TCE.

ANAYLTICAL PERSPECTIVE

Overview

It is necessary to search for ideal instrumental conditions to apply to separation, identification and quantitation of TCE and its metabolites in different matrices (e.g., blood, urine, different types of tissues). An extensive review of this subject was provided by Delinsky *et al.* (2005). Utilization of GC with electron capture detection (ECD) is prevalent for the separation and detection of chlorinated volatiles (TCE and its metabolites). A number of scientists have used GC with mass spectrometry (MS) in tandem in order to improve sensitivity. Since, the real-time and rapid quantitation of TCE and its metabolites in large number of blood and tissue samples was more important than the detection limit in this work, GC-ECD was chosen as the analytical tool. GC-MS was not needed to identify the compounds responsible for the peaks obtained by GC-ECD analysis. A general description of GC, ECD and headspace GC, along with some of the drawbacks, are described below.

Headspace Gas Chromatography

Many types of chromatography (including ion-pair; reverse phase; ion-exchange; hydrophilic interaction) can be used in the analysis of TCE and its metabolites (particularly, DCA and TCA) in combination with HPLC. Still, GC is by far the most commonly used procedure for separation and quantification for the analysis of TCE and

related compounds. Headspace analysis is generally defined as a vapor-phase extraction, involving the partitioning of analytes between liquid and vapor phases. There were essentially two types of headspace-sampling techniques available as headspace-GC: dynamic (trap-and-purge analysis) and static (vapor-phase extraction). These have been repeatedly renovated and have become automated. These techniques have been reviewed extensively in the literature (Hachenberg and Schmidt, 1977; Kolb and Ettre, 1997). With dynamic headspace analysis, a continuous flow of gas is swept over the surface of the sample matrix. Volatiles from the sample matrix are conveyed into a trap where the volatile analytes are accumulated prior to analysis. This trap usually consists of a column containing a sorbent such as Tenex®, Chromosorb®, Porapak® or Amberlite® XAD resins (B'Hymer, 2003). Because the "total" amount of a volatile substance is extracted, trapped and analyzed at one time, dynamic headspace analysis is particularly suited for the determination of VOCs at very low concentrations (detection limits up to pg/mL levels) (Camarasu et al., 1998). The classical static headspace technique is the simplest method. A liquid sample is placed into a sealed vial that is heated (and also pressurized which allows more rapid analyte transfer and equilibration) until a thermodynamic equilibrium between the sample and the gas phase is reached. An aliquot of the headspace gas is transferred via a heated transfer line and injected automatically into the GC for analysis. The main advantages of static headspace analysis are the ease of use and automation as a result of available commercial systems from major manufacturers. Many of these have detection limits as low as ng/mL (Camarasu et al., 1998). Among other techniques, sorbent-based solid-phase microextraction (SPME) and its combination with

headspace analysis is used much more extensively in recent years, as reviewed by Pawliszyn (2001) and by Mills and Walker (2000).

Electron Capture Detector

The requirements for a GC detector include a fast and linear response, high sensitivity, good stability and uniform response to various chemical species. Flame ionization detection (FID) and electron capture detection (ECD) can be used along with mass selective detection (MS). The latter is frequently employed for the quantitation of TCE (Brown et al., 2003; Dixon et al., 2005).

ECD, invented by Lovelock in late 1950s, uses a radioactive β-emitter (such as ^{63}Ni or tritium absorbed on platinum foil). An electron from the emitter causes ionization of the carrier gas (often N_2 or Ar/CH_4) and the production of a burst of electrons. In the absence of analyte, a constant standing current is generated from the ionization process. This current decreases, however, in the presence of those organic molecules that tend to capture electrons (Lovelock, 2001). ECD is selective and sensitive to molecules containing highly electronegative functional groups, while it is insensitive toward functional groups such as amines, alcohols, and hydrocarbons. Therefore, ECD remains one of the most widely used GC detectors for determination of halogenated solvents and pesticides.

Some of the U.S. EPA-approved methods employ GC-ECD. EPA Method 551.1, demonstrated by Munch and Hautman (1995) for measuring TCE in drinking-water, involves a liquid–liquid extraction procedure, followed by GC-ECD. GC-ECD is used to

monitor the levels of HAAs in drinking water in the U.S. and other countries (Krasner et al., 1989; Williams et al., 1997), as demonstrated by EPA Method 552.1 and EPA Method 552.2 (APHA, 1998).

GC-ECD is also used for the simultaneous analysis of TCE and its metabolites in several biological matrices, including lung, liver, kidney, and blood. Merdink *et al.* (1998) studied DCA as a possible metabolite after dosing of male B6C3F1 mice with TCE, CH, TCOH, or TCA. TCE, CH along with methyl esters of DCA and TCA (after derivatization) were analyzed by headspace GC-ECD. The investigators could not detect DCA in the blood of mice dosed with any of the above compounds, possibly due to inadequate sensitivity. Muralidhara and Bruckner (1999) reported a rather simple method for the determination of TCE, TCA, TCOH and DCA in rat lung, liver, kidney and blood by headspace GC-ECD. A mixture of water: sulfuric acid: methanol (6:5:1) was used to derivatize DCA and TCA to their methyl esters. The authors reported a LOD of 5 ~ 10 ng/mL for each compound and percent recovery values for TCE metabolites, including TCA and TCOH (68 ~ 100 % in blood, 57 ~ 87 % in liver, 63 ~ 86 % in kidney and 64 ~ 98 % in lung) at different concentrations.

GC-ECD was utilized in the analysis of TCE and its metabolites in seminal fluids of workers exposed to TCE occupationally. Forkert *et al.* (2003) measured levels of TCE, TCOH, DCA and TCA with headspace GC-ECD analysis in the seminal fluid of eight infertile mechanics. TCE and TCOH were found in all of the workers, while TCA was found in one individual, and DCA was found in two people. When urine of the same eight workers was analyzed for TCA and TCOH, all workers had observable levels of each metabolite (Forkert et al., 2003). Other examples of GC-ECD uses include

determination of other disinfection by-products in drinking water (Weisel et al., 1999) and the analysis of polychlorinated biphenyls (PCBs) in human serum (DeCaprio *et al.,* 2000).

Derivatization for GC analysis of TCE and metabolites

It is important to take appropriate steps to minimize errors associated with sample preparation and handling to ensure reproducibility in any assay dealing with volatile or acid-labile compounds. Should any reaction occur with the analytes, the entire reaction products should be identified along with the extent of this change. Thus, more studies may be needed to evaluate the changes of analytes which occur after sample collection. It is necessary to find out 1) if there is a loss, whether it is accountable and consistent; 2) whether any other change is happening to the analytes during the process of analysis and how it happens. Thus, in the course of analysis for TCE and its metabolites, it is essential to ensure the reliability of the assay for measuring the concentration and/or amount of TCE and its metabolites in biological samples. Thus, for reliable measurement and analysis of TCE (since it is volatile) and its metabolites (since they are intrinsically labile and unstable, especially in the acidic media) in the blood, urine and target tissues, several potential sources of species conversion and possible loss of analytes during the analysis should be addressed. It is also possible to assume enzymatic involvement in converting one chemical entity to another after the sample collection. For example, it is possible that TCA (due to its long half-life) may be enzymatically converted to DCA after samples have been collected. A simple method to negate the enzyme involvement (not the chemical processes) at the time of sampling would be ideal. Some possibilities include

freezing, denaturation of proteins in samples (which is actually achieved at the time of derivatization by the addition of the acidic solution). But, as with all the methods considered, balances between the benefits and disadvantages (including, increased time and labor) have to be weighed.

The most complicated matter in the analyses of TCE and its metabolites is the measurement of TCA (and DCA). Due to their low pKa (pKa's of DCA and TCA are approximately 1.5 and 0.5 at 25 ^{o}C, respectively), the two haloacetic acids are found predominantly as their anionic (ionized) form (Jia et al., 2003; Sarzanini et al., 1999; Urbansky, 2000). As a result, it is impossible to measure them directly with most of GC analytical methods currently available. Thus, in order to measure them by GC-ECD, it is prerequisite to include a step converting TCA and DCA to more volatile and stable forms (most commonly into their corresponding ester forms), as described in the previous publications.

In the derivatization process, HAAs are commonly converted to the corresponding volatile methyl esters, thus enabling the hydrophilic HAAs to be more readily available for headspace GC analysis. A mixture of sulfuric acid, methanol and water is one of the most common and simple ways for the esterification as described by Muralidhara and Bruckner (1999). Furthermore, for the analysis of HAAs (including DCA and TCA) in drinking water, other derivatization methods with diazomethane have been used for analysis of HAAs in drinking water as demonstrated by EPA method 552.1 (Ko et al., 2000). However, it should be duly noted with this method that the sample preparation procedures and GC analysis involved disadvantages of complexity,

labor intensity and lengthy sample pretreatment and analytical time, in addition to the potential explosiveness of diazomethane.

One of the pitfalls of the esterification of TCA and DCA to corresponding esters with a solution mixture of sulfuric acid, methanol and water was recognized by Ketcha *et al.* (1996). In developing a method for esterification of TCA and DCA, the conversion of TCA to DCA was observed in freshly-drawn blood upon the addition of acid for the derivatization resulting in artificially high DCA concentrations. Although, the amount of TCA converted to DCA by the addition of acid decreased with time, this conversion could be prevented by freezing blood samples overnight prior to derivatization. This indicated that reduced hemoglobin was involved in the acid-catalyzed conversion of TCA to DCA. Lead acetate has been added to samples to prevent the conversion of TCA to DCA (Narayanan *et al.,* 1999). Ketcha *et al* (1996), however, determined that the addition of lead acetate resulted in 80 % conversion of TCA to DCA after TCA was derivatized.

Instead of derivatizing TCA, adjustment of the pH to less than 0.5 by addition of acids was also utilized in U.S. EPA Method 552.2 (U.S. EPA, 1995). Since, the pKa of TCA is approximately 0.5, at pH 0.5 TCA exists as both protonated (50 %) and deprotonated (50 %) forms. Therefore, many acidification methods require many liquid-liquid extractions to recover almost all of anionic TCA to its protonated form. Furthermore, the selection of appropriate acids for acidifying (and for derivatizing) samples has to be considered. For example, Dalvi *et al* (2000) showed much higher levels of TCA are formed in the presence of chlorine ions, indicating that use of HCl for sample

acidification may convert DCA to TCA. Similarly, Shorney and Randtke (1994) reported increased speciation shifts when hydrochloric acid (rather than sulfuric acid) was used with methanol in the analysis of HAAs. Thus, the issues regarding derivatization of TCA (or DCA) and the possible conversion of TCA to DCA during the assay remain the major concerns about the analysis of TCE and its metabolites.

Selected GC-ECD, derivatization methods and other miscellaneous issues

The analytical method for TCE and its metabolites in this work was headspace GC coupled with ECD. The protocol was modified from the one described by Muralidhara and Bruckner (1999). Even though there are some shortcomings with this technique (for example, the issue of derivatization of TCA into its ester form as discussed earlier), its ease and the very short time required for treatment of samples, as well as the simultaneous detection of TCE and its metabolites in one run provided a convenient and rapid real-time analysis of the large number of blood and urine samples generated in time-course toxicokinetic profile studies. The conditions for headspace GC were: 1) the temperature gradient condition with a starting temperature of 120 ^{0}C for 3 min, with increases up to 170 ^{0}C by 25 ^{0}C/min, held for 3 min, 2) detector temperature, 360 ^{0}C; injector temperature, 200 ^{0}C, 3) 10' x 1/8" stainless steel column packed with 10 % customized coating of OV-17 (phenylpolysiloxane) on 80/100 μm mesh size matrix SUPELCOPORT™ (Supelco Inc, Bellefonte, PA) and 4) nitrogen as carrier gas (25 psi). The calibration curves were prepared using external standards and checked daily, then analyzed concurrently with the blood and urine samples.

U.S. FDA Guidance for Industry: Bioanalytical method validation, by the U.S. FDA mandates that autosampler stability should be checked (USFDA, 2001). This is to ensure that the concentration of analyte remains the same (or almost the same) from the time it is placed into the autosampler until the time it is actually analyzed. For example, in a typical time-course experiment in this project involving 6 rats, almost 100 samples are generated (excluding the number of standard samples for the calibration curve), which is comparable to the autosampler capacity (110 samples at one run). It takes usually up to 12 min from the time of one sample injection to next, so total time required to assay all the samples from even one experiment is long. Thus, the analytes must be stable for a prolonged period and the condition of GC and the detector have to remain constant.

CYP2E1 activity measurement

The activity of CYP2E1 can be measured with a few specific substrates with precautionary interpretation of the data in mind. As substrates/probes for CYP2E1 activity, *p*-nitrophenol (PNP) and chlorzoxazone (CLZ) are widely used as substrates. PNP undergoes 2-hydroxylation by CYP2E1, but other P450 enzymes including CYP3A4 in animal and humans are also believed to participate in its metabolism. Nevertheless, over 90 % of PNP hydroxylase activity is believed to be catalyzed by CYP2E1 (Tierney et al., 1992; Zerilli et al., 1997). Chlorzoxazone (CLZ), used as a centrally-acting muscle relaxant and a noninvasive *in vivo* probe, undergoes 6-hydroxylation by CYP2E1. CYP1A1 is also believed to be involved in its oxidation (Carriere et al., 1993). Thus, these results taken together indicate that PNP and CLZ can

be used as *in vitro* and *in vivo* measures of CYP2E1 activity, although they are not entirely CYP2E1-specific. However, the relative Km of CYP2E1 for PNP and CLZ compared with those of CYP1A1/CYP3A4, combined with the relative levels of these enzymes in the liver, suggest that CYP2E1 is the major isoform *in vivo* that oxidizes PNP and CLZ.

Toxicokinetic data analysis

The area-under-the-curve ($AUC_{0\rightarrow\infty}$) was calculated in this project by the linear trapezoidal method, with the terminal portion of the curve extrapolated to infinity by $C_{b,t}/\beta$ ($C_{b,t}$: the concentration at the last observation, β: the slope of the terminal phase determined by linear regression). The elimination half-life ($t_{\beta 1/2}$) was calculated as $\beta/0.693$. In order to calculate estimates of total body clearance (CL_b) and apparent volume of distribution at steady-state (Vss) using WinNonlin 4.1 (Pharsight Corp., Cary, NC), the individual blood concentration-time profiles (*iv* or *po*) were analyzed by two-compartmental methods. The blood concentration-time profiles of the metabolites formed were analyzed by non-compartmental methods (Perrier and Gibaldi, 1982). The renal clearance of TCA (CL_R) could be calculated as $CL_R = X_{u0\rightarrow\infty}/AUC_{0\rightarrow\infty}$ from the urinary excretion data (for example, $X_{u0\rightarrow\infty}$: the total amount of TCA recovered in the urine). Estimates of the peak blood concentration (C_{max}) and the time of occurrence (T_{max}) were also calculated with WinNonlin, where their initial estimates were obtained using the method of residuals.

CONCLUSIONS

Issues associated with the potential carcinogenicity of TCE and its metabolites have been debated for the past several decades. Determining the human relevance of animal carcinogenicity data and applying them to risk assessment of TCE and its metabolites has been a major source of controversy ever since. The U.S. EPA, with other federal agencies, is again reviewing carcinogenicity, toxicity and toxicokinetic data on TCE and its metabolites, in order to update its cancer and non-cancer risk assessments of TCE

The main objective of this study was to characterize the dose-dependency and effects of CYP2E1 induction on the TK of TCE and its major metabolites. PZ was selected as a CYP2E1 inducer in young adult male S-D rats. This age of rats exhibits the highest constitutive CYP2E1 activity and is most responsive to inducers. This animal model is intended to represent potentially sensitive subpopulations, which have environmentally- or genetically-determined elevated expression of hepatic microsomal CYP2E1 activity. Such subpopulations might be expected to form larger quantities of carcinogenic metabolites from a given dose of TCE than the "normal" populations. It is postulated that this may not be the case with low environmentally-relevant doses of TCE, since TCE is a blood-flow limited, rather than capacity-limited compound (i.e., even persons with the lowest levels in the general population of CYP2E1 have the isozyme in excess of that needed to metabolize all of trace levels of TCE). Proving this postulate can

have a profound impact on logic the USEPA uses to rationalize its adoption of the linearized, no-threshold cancer risk assessment model for TCE.

Therefore, following the literature reviews in Chapter 1, the effects of CYP2E1 induction by PZ on TCE metabolism are discussed in Chapter 2, by comparing the toxicokinetic parameters of TCE and its metabolites (TCOH, CH and TCA) between the control groups and CYP2E1-induced groups after administration of different doses of TCE. In Chapter 3, the influence of PZ-induction on TCA toxicokinetics after TCE, TCOH and TCA administration and its significance in TCE risk assessment are discussed.

Although it is important to consider the entire TCE metabolism pathway, an individual step may also be important for understanding the toxicity of TCE, if TCE toxicity is strongly dependent on the toxicokinetics of the specific metabolite (e.g., TCA). The aforementioned data and discussions will provide clues not only on the individual steps (e.g., from TCOH to TCA), but also on the overall picture of TCE metabolism. Better understanding of TCE metabolism, especially at low concentrations with the induction of CYP2E1 is needed. Data from this induction model can be applied to not only the risk assessment and regulation of TCE, but also to the broad range of halogenated hydrocarbons and other small organic molecules that utilize similar CYP2E1-mediated oxidative pathways. In order to obtain accurate information relevant to the risk assessment of TCE, DCA and TCA, it is also necessary to utilize robust analytical methods.

REFERENCES

Abbas R, Fisher JW. 1997. A physiologically based pharmacokinetic model for trichloroethylene and its metabolites, chloral hydrate, trichloroacetate, dichloroacetate, trichloroethanol, and trichloroethanol glucuronide in B6C3F1 mice. *Toxicol Appl Pharmacol* **147**(1):15-30.

Allen BC, Fisher JW. 1993. Pharmacokinetic modeling of trichloroethylene and trichloroacetic acid in humans. *Risk Anal* **13**(1):71-86.

APHA A, WPCF, ed. 1998. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association/American Water Works Association. Washington, DC.

ATSDR. 1989. *Toxicological profile for trichloroethylene*. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. pp. 139, Atlanta, GA (ATSDR/TP-88/24).

ATSDR. 1993. *Toxicological profile for trichloroethylene*. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA.

ATSDR. 1997. *Toxicological profile for trichloroethylene (Update).* US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA.

Bardag-Gorce F, Li J, French BA, French SW. 2005. The effect of ethanol-induced CYP2E1 on proteasome activity: The role of 4-hydroxynonenal. *Exp Mol Pathol* **78**(2):109-115.

Barton HA, Flemming CD, Lipscomb JC. 1996. Evaluating human variability in chemical risk assessment: Hazard identification and dose-response assessment for noncancer oral toxicity of trichloroethylene. *Toxicology* **111**(1-3):271-287.

Bhat HK, Kanz MF, Campbell GA, Ansari GA. 1991. Ninety day toxicity study of chloroacetic acids in rats. *Fundam Appl Toxicol* **17**(2):240-253.

B'Hymer C. 2003. Residual solvent testing: A review of gas-chromatographic and alternative techniques. *Pharm Res* **20**(3):337-344.

Brown SD, Muralidhara S, Bruckner JV, Bartlett MG. 2003. Trace level determination of trichloroethylene from liver, lung and kidney tissues by gas chromatography-magnetic sector mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* **783**(2):319-325.

Bruning T, Vamvakas S, Makropoulos V, Birner G. 1998. Acute intoxication with trichloroethene: Clinical symptoms, toxicokinetics, metabolism, and development of biochemical parameters for renal damage. *Toxicol Sci* **41**(2):157-165.

Buhler R, Lindros KO, Nordling A, Johansson I, Ingelman-Sundberg M. 1992. Zonation of cytochrome P450 isozyme expression and induction in rat liver. *Eur J Biochem* **204**(1):407-412.

Bull RJ, Sanchez IM, Nelson MA, Larson JL, Lansing AJ. 1990. Liver tumor induction in B6C3F1 mice by dichloroacetate and trichloroacetate. *Toxicology* **63**(3):341-359.

Calafat AM, Kuklenyik Z, Caudill SP, Ashley DL. 2003. Urinary levels of trichloroacetic acid, a disinfection by-product in chlorinated drinking water, in a human reference population. *Environ Health Perspect* **111**:151-154.

Camarasu CC, Mezei-Szuts M, Varga GB. 1998. Residual solvents determination in pharmaceutical products by GC-HS and GC-MS-SPME. *J Pharm Biomed Anal* **18**(4-5):623-638.

Carriere V, Goasduff T, Ratanasavanh D, Morel F, Gautier JC, Guillouzo A et al. 1993. Both cytochromes P450 2E1 and 1A1 are involved in the metabolism of chlorzoxazone. *Chem Res Toxicol* **6**(6):852-857.

Chiu WA, Caldwell JC, Keshava N, Scott CS. 2006. Key scientific issues in the health risk assessment of trichloroethylene. *Environ Health Perspect* **114**(9):1445-1449.

Chung HC, Kim SH, Lee MG, Kim SG. 2002. Increase in urea in conjunction with L-arginine metabolism in the liver leads to induction of cytochrome P450 2E1 (CYP2E1): The role of urea in CYP2E1 induction by acute renal failure. *Drug Metab Dispos* **30**(6):739-746.

Cummings BS, Parker JC, Lash LH. 2001. Cytochrome p450-dependent metabolism of trichloroethylene in rat kidney. *Toxicol Sci* **60**(1):11-19.

Czekaj P, Wiaderkiewicz A, Florek E, Wiaderkiewicz R. 2005. Tobacco smoke-dependent changes in cytochrome P450 1A1, 1A2, and 2E1 protein expressions in fetuses, newborns, pregnant rats, and human placenta. *Arch Toxicol* **79**(1):13-24.

Dalvi A, Al-Rasheed R, Javeed M. 2000. Haloacetic acids (HAAs) formation in desalination processes from disinfectants. *Desalination* **129**(3):261-271.

Damme B, Darmer D, Pankow D. 1996. Induction of hepatic cytochrome P4502E1 in rats by acetylsalicylic acid or sodium salicylate. *Toxicology* **106**(1-3):99-103.

Davidson IW, Beliles RP. 1991. Consideration of the target organ toxicity of trichloroethylene in terms of metabolite toxicity and pharmacokinetics. *Drug Metab Rev* **23**(5-6):493-599.

Davis ME. 1986. Effect of chloroacetic acids on the kidneys. *Environ Health Perspect* **69**:209-214.

DeAngelo AB, Daniel FB, Most BM, Olson GR. 1997. Failure of monochloroacetic acid and trichloroacetic acid administered in the drinking water to produce liver cancer in male F344/N rats. *J Toxicol Environ Health* **52**(5):425-445.

DeCaprio AP, Tarbell AM, Bott A, Wagemaker DL, Williams RL, O'Hehir CM. 2000. Routine analysis of 101 polychlorinated biphenyl congeners in human serum by parallel dual-column gas chromatography with electron capture detection. *J Anal Toxicol* **24**(6):403-420.

Dekant W, Schulz A, Metzler M, Henschler D. 1986. Absorption, elimination and metabolism of trichloroethylene: A quantitative comparison between rats and mice. *Xenobiotica* **16**(2):143-152.

Delinsky AD, Bruckner JV, Bartlett MG. 2005. A review of analytical methods for the determination of trichloroethylene and its major metabolites chloral hydrate, trichloroacetic acid and dichloroacetic acid. *Biomed Chromatogr* **19**(8):617-639.

Dixon AM, Brown SD, Muralidhara S, Bruckner JV, Bartlett MG. 2005. Optimization of SPME for analysis of trichloroethylene in rat blood and tissues by SPME-GC/MS. *Instrumentation Science and Technology* **33**:183–194.

Doran E, Whittington FW, Wood JD, McGivan JD. 2002. Cytochrome P450IIE1 (CYP2E1) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes. *Chem Biol Interact* **140**(1):81-92.

Fisher JW, Gargas ML, Allen BC, Andersen ME. 1991. Physiologically based pharmacokinetic modeling with trichloroethylene and its metabolite, trichloroacetic acid, in the rat and mouse. *Toxicol Appl Pharmacol* **109**(2):183-195.

Fisher JW, Mahle D, Abbas R. 1998. A human physiologically based pharmacokinetic model for trichloroethylene and its metabolites, trichloroacetic acid and free trichloroethanol. *Toxicol Appl Pharmacol* **152**(2): 339-59.

Fisher JW, Whittaker TA, Taylor DH, Clewell HJ, III, Andersen ME. 1989. Physiologically based pharmacokinetic modeling of the pregnant rat: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol Appl Pharmacol* **99**(3):395-414.

Forkert PG, Birch DW. 1989. Pulmonary toxicity of trichloroethylene in mice. Covalent binding and morphological manifestations. *Drug Metab Dispos* **17**(1):106-113.

Forkert PG, Lash L, Tardif R, Tanphaichitr N, Vandevoort C, Moussa M. 2003. Identification of trichloroethylene and its metabolites in human seminal fluid of workers exposed to trichloroethylene. *Drug Metab Dispos* **31**(3):306-311.

Forkert PG, Redza ZM, Mangos S, Park SS, Tam SP. 1994. Induction and regulation of CYP2E1 in murine liver after acute and chronic acetone administration. *Drug Metab Dispos* **22**(2):248-253.

Gonzalez-Leon A, Merdink JL, Bull RJ, Schultz IR. 1999. Effect of pre-treatment with dichloroacetic or trichloroacetic acid in drinking water on the pharmacokinetics of a subsequent challenge dose in B6C3F1 mice. *Chem Biol Interact* **123**(3):239-253.

Gonzalez FJ. 2005. Role of cytochromes P450 in chemical toxicity and oxidative stress: Studies with CYP2E1. *Mutat Res* **569**(1-2):101-110.

Guengerich FP. 2001. Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. *Chem Res Toxicol* **14**(6):611-650.

Guengerich FP. 2004. Cytochrome P450: What have we learned and what are the future issues? *Drug Metab Rev* **36**(2):159-197.

Guengerich FP. 2006. Cytochrome P450S and other enzymes in drug metabolism and toxicity. *AAPS J* **8**(1):E101-11.

Guengerich FP, Kim DH, Iwasaki M. 1991. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem Res Toxicol* **4**(2):168-179.

Hachenberg H, Schmidt AP. 1977. Gas Chromatographic Headspace Analysis. Heyden, London.

Hathway DE. 1980. Consideration of the evidence for mechanisms of 1,1,2-trichloroethylene metabolism, including new identification of its dichloroacetic acid and trichloroacetic acid metabolites in mice. *Cancer Lett* **8**(3):263-269.

Hoekstra EJ. 2003. Review of concentrations and chemistry of trichloroacetate in the environment. *Chemosphere* **52**(2):355-369.

Hotchkiss JA, Kim H, Hahn FF, Novak RF, Dahl AR. 1995. Pyridine induction of sprague-dawley rat renal cytochrome P4502E1: Immunohistochemical localization and quantitation. *Toxicol Lett* **78**(1):1-7.

HSDB, 2002. Hazardous Substances Data Bank. National Library of Medicine. Bethesda, MD (available at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB).

Hu Y, Ingelman-Sundberg M, Lindros KO. 1995. Induction mechanisms of cytochrome P450 2E1 in liver: Interplay between ethanol treatment and starvation. *Biochem Pharmacol* **50**(2):155-161.

IARC Working Group. 1995. Trichloroacetic acid. *IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* **63**:291-314. International Agency for Research on Cancer. Lyon, France.

IARC Working Group. 1995. Trichloroacetic acid. *IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* **63**:291-314. International Agency for Research on Cancer. Lyon, France.

IARC Working Group. 1995. Trichloroethylene. *IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. **63**:75-158. International Agency for Research on Cancer. Lyon, France.

IARC Working Group. 2004. Some drinking-water disinfectants and contaminants, including arsenic. monographs on chloramine, chloral and chloral hydrate, dichloroacetic acid, trichloroacetic acid and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone. *IARC Monograph on the Evaluation of the Carcinogenic Risks to Humans.* **84**:269-477. International Agency for Research on Cancer. Lyon, France.

Ikeda M, Miyake Y, Ogata M, Ohmori S. 1980. Metabolism of trichloroethylene. *Biochem Pharmacol* **29**(21):2983-2992.

IPCS. 1985. *Trichloroethylene*. World Health Organization, International Programme on Chemical Safety. Geneva (Environmental Health Criteria 50).

Jia M, Wu WW, Yost RA, Chadik PA, Stacpoole PW, Henderson GN. 2003. Simultaneous determination of trace levels of nine haloacetic acids in biological samples as their pentafluorobenzyl derivatives by gas chromatography/tandem mass spectrometry in electron capture negative ion chemical ionization mode. *Anal Chem* **75**(16):4065-4080.

Juuti S, Hoekstra E. 1998. The origins and occurrence of trichloroacetic acid. *Atmos Environ* **32**(17):3059-3060.

Kaneko T, Wang PY, Sato A. 1997. Assessment of the health effects of trichloroethylene. *Ind Health* **35**(3):301-324.

Kedderis GL. 1997. Extrapolation of in vitro enzyme induction data to humans in vivo. *Chem Biol Interact* **107**(1-2):109-121.

Ketcha MM, Stevens DK, Warren DA, Bishop CT, Brashear WT. 1996. Conversion of trichloroacetic acid to dichloroacetic acid in biological samples. *J Anal Toxicol* **20**(4):236-241.

Kim H, Weisel C. 1998. Dermal absorption of dichloro-and trichloroacetic acids from chlorinated water. *J Expo Anal Environ Epidemiol* **8**(4):555–575.

Kim H, Putt DA, Zangar RC, Wolf CR, Guengerich FP, Edwards RJ et al. 2001. Differential induction of rat hepatic cytochromes P450 3A1, 3A2, 2B1, 2B2, and 2E1 in response to pyridine treatment. *Drug Metab Dispos* **29**(3):353-360.

Kim SG, Novak RF. 1993. The induction of cytochrome P4502E1 by nitrogen- and sulfur-containing heterocycles: Expression and molecular regulation. *Toxicol Appl Pharmacol* **120**(2):257-265.

Ko YW, Gremm TJ, Abbt-Braun G, Frimmel FH, Chiang PC. 2000. Determination of dichloroacetic acid and trichloroacetic acid by liquid-liquid extraction and ion chromatography. *Fresenius J Anal Chem* **366**(3):244-248.

Kolb B, Ettre LS. 1997. Static Headspace-Gas Chromatography: Theory and Practice: Wiley-VCH. Weinheim, Germany.

Koop DR, Chernosky A, Brass EP. 1991. Identification and induction of cytochrome P450 2E1 in rat kupffer cells. *J Pharmacol Exp Ther* **258**(3):1072-1076.

Kraner JC, Lasker JM, Corcoran GB, Ray SD, Raucy JL. 1993. Induction of P4502E1 by acetone in isolated rabbit hepatocytes. Role of increased protein and mRNA synthesis. *Biochem Pharmacol* **45**(7):1483-1492.

Krasner SW, McGuire MJ, Jacangelo JG, Patania NL, Reagan KM, Aieta EM. 1989. Occurrence of disinfection by-products in US drinking water. *J Am Water Works Assoc* **81**(8):41-53.

Kwak MK, Kim SG, Kwak JY, Novak RF, Kim ND. 1994. Inhibition of cytochrome P4502E1 expression by organosulfur compounds allylsulfide, allylmercaptan and allylmethylsulfide in rats. *Biochem Pharmacol* **47**(3):531-539.

Larson JL, Bull RJ. 1989. Effect of ethanol on the metabolism of trichloroethylene. *J Toxicol Environ Health* **28**(4):395-406.

Larson JL, Bull RJ. 1992a. Metabolism and lipoperoxidative activity of trichloroacetate and dichloroacetate in rats and mice. *Toxicol Appl Pharmacol* **115**(2):268-277.

Larson JL, Bull RJ. 1992b. Species differences in the metabolism of trichloroethylene to the carcinogenic metabolites trichloroacetate and dichloroacetate. *Toxicol Appl Pharmacol* **115(**2):278-285.

Lash LH, Fisher JW, Lipscomb JC, Parker JC. 2000. Metabolism of trichloroethylene. *Environ Health Perspect* **108 Suppl 2**:177-200.

Lash LH, Xu Y, Elfarra AA, Duescher RJ, Parker JC. 1995. Glutathione-dependent metabolism of trichloroethylene in isolated liver and kidney cells of rats and its role in mitochondrial and cellular toxicity. *Drug Metab Dispos* **23(**8):846-853.

Lee KM, Bruckner JV, Muralidhara S, Gallo JM. 1996. Characterization of presystemic elimination of trichloroethylene and its nonlinear kinetics in rats. *Toxicol Appl Pharmacol* **139(**2):262-271.

Lipscomb JC, Garrett CM, Snawder JE. 1997. Cytochrome P450-dependent metabolism of trichloroethylene: Interindividual differences in humans. *Toxicol Appl Pharmacol* **142**(2):311-318.

Lipscomb JC, Mahle DA, Brashear WT, Garrett CM. 1996. A species comparison of chloral hydrate metabolism in blood and liver. *Biochem Biophys Res Commun* **227**(2):340-350.

Longo V, Ingelman-Sundberg M. 1993. Acetone-dependent regulation of cytochromes P4502E1 and P4502B1 in rat nasal mucosa. *Biochem Pharmacol* **46(**11):1945-1951.

Lovelock J. 2001. Homage to Gaia: The Life of an Independent Scientist. Oxford University Press. Oxford, Great Britain.

Lumpkin MH, Bruckner JV, Campbell JL, Dallas CE, White CA, Fisher JW. 2003. Plasma binding of trichloroacetic acid in mice, rats, and humans under cancer bioassay and environmental exposure conditions. Drug Metab Dispos **31(**10):1203-1207.

Mather GG, Exon JH, Koller LD. 1990. Subchronic 90 day toxicity of dichloroacetic and trichloroacetic acid in rats. *Toxicology* **64**(1):71-80.

Mayers DJ, Hindmarsh KW, Gorecki DK, Sankaran K. 1992. Sedative/hypnotic effects of chloral hydrate in the neonate: Trichloroethanol or parent drug? *Dev Pharmacol Ther* **19**(2-3):141-146.

Mayers DJ, Hindmarsh KW, Sankaran K, Gorecki DK, Kasian GF. 1991. Chloral hydrate disposition following single-dose administration to critically ill neonates and children. *Dev Pharmacol Ther* **16**(2):71-77.

Merdink JL, Gonzalez-Leon A, Bull RJ, Schultz IR. 1998. The extent of dichloroacetate formation from trichloroethylene, chloral hydrate, trichloroacetate, and trichloroethanol in B6C3F1 mice. *Toxicol Sci* **45**(1):33-41.

Mills GA, Walker V. 2000. Headspace solid-phase microextraction procedures for gas chromatographic analysis of biological fluids and materials. *J Chromatogr A* **902**(1):267-287.

Morel G, Cossec B, Lambert AM, Binet S. 1999. Evaluation of rat hepatic 2E1 activity in function of age, sex and inducers: Choice of an experimental model capable of testing the hepatotoxicity of low molecular weight compounds. *Toxicol Lett* **106**(2-3):171-180.

Muller G, Spassovski M, Henschler D. 1972. Trichloroethylene exposure and trichloroethylene metabolites in urine and blood. *Arch Toxikol* **29**(4):335-340.

Muller G, Spassowski M, Henschler D. 1975. Metabolism of trichloroethylene in man. III. interaction of trichloroethylene and ethanol. *Arch Toxicol* **33**(3):173-189.

Munch D, Hautman D. 1995. Method 551.1: Determination of chlorination disinfection byproducts, chlorinated solvents, and halogenated Pesticides/Herbicides in drinking water by liquid-liquid extraction and gas chromatography with electron-capture detection. *Methods for the determination of organic compounds in drinking water*-Supplement III, US Environmental Protection Agency, Washington, DC (EPA/600/R-95131).

Muralidhara S, Bruckner JV. 1999. Simple method for rapid measurement of trichloroethylene and its major metabolites in biological samples. *J Chromatogr B Biomed Sci Appl* **732**(1):145-153.

Murray M. 2006. Altered CYP expression and function in response to dietary factors: Potential roles in disease pathogenesis. *Curr Drug Metab* **7**(1):67-81.

Nakajima T. 1997. Cytochrome P450 isoforms and the metabolism of volatile hydrocarbons of low relative molecular mass. *J Occup Health* **39**:83–91.

Nakajima T, Wang RS, Elovaara E, Park SS, Gelboin HV, Vainio H. 1992a. A comparative study on the contribution of cytochrome P450 isozymes to metabolism of benzene, toluene and trichloroethylene in rat liver. *Biochem Pharmacol* **43**(2):251-257.

Nakajima T, Wang RS, Elovaara E, Park SS, Gelboin HV, and Vainio H. 1993. Cytochrome P450-related differences between rats and mice in the metabolism of benzene, toluene and trichloroethylene in liver microsomes. *Biochem Pharmacol* **45**(5):1079-1085.

Nakajima T, Wang RS, Katakura Y, Kishi R, Elovaara E, Park SS. 1992b. Sex-, age- and pregnancy-induced changes in the metabolism of toluene and trichloroethylene in rat liver in relation to the regulation of cytochrome P450IIE1 and P450IIC11 content. *J Pharmacol Exp Ther* **261**(3):869-874.

Nakajima T, Wang RS, Murayama N, Sato A. 1990. Three forms of trichloroethylene-metabolizing enzymes in rat liver induced by ethanol, phenobarbital, and 3-methylcholanthrene. *Toxicol Appl Pharmacol* **102**(3):546-552.

Narayanan L, Moghaddam AP, Taylor AG, Sudberry GL, Fisher JW. 1999. Sensitive high-performance liquid chromatography method for the simultaneous determination of low levels of dichloroacetic acid and its metabolites in blood and urine. *J Chromatogr B Biomed Sci Appl* **729**(1-2):271-277.

NCI. 1976. *Carcinogenesis bioassay of trichloroethylene*. US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, Bethesda, MD (NCI-CGTR-2, NIH 76-802).

Ni YC, Wong TY, Lloyd RV, Heinze TM, Shelton S, Casciano D et al. 1996. Mouse liver microsomal metabolism of chloral hydrate, trichloroacetic acid, and trichloroethanol leading to induction of lipid peroxidation via a free radical mechanism. *Drug Metab Dispos* **24**(1):81-90.

Nishimura M, Ueda N, Naito S. 2003. Effects of dimethyl sulfoxide on the gene induction of cytochrome P450 isoforms, UGT-dependent glucuronosyl transferase isoforms, and ABCB1 in primary culture of human hepatocytes. *Biol Pharm Bull* **26**(7):1052-1056.

Novak RF, Woodcroft KJ. 2000. The alcohol-inducible form of cytochrome P450 (CYP 2E1): Role in toxicology and regulation of expression. *Arch Pharm Res* **23**(4):267-282.

NRC (2006) Assessing the human health risks of trichloroethylene: Key scientific issues. Committee on human health risks of trichloroethylene, National Research Council, The National Academies Press, Washington, D.C. (available at http://www.nap.edu/catalog/11707.html)

NTP. 1983. *NTP technical report on the carcinogenesis studies of trichloroethylene (without epichlorohydrin) in F344/N rats and B6C3F1 mice (gavage studies).* Draft report. US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Toxicology Program. Research Triangle Park, NC (NIH Publication No. 83-1799).

NTP. 1988. *Toxicology and carcinogenesis studies of trichloroethylene (CAS No. 79-01-6) in four strains of rats (ACI, August, Marshall, Osborne-Mendel) (gavage studies).* US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Toxicology Program. Research Triangle Park, NC (NTP Technical Report Series No. 273; NIH Publication No. 88-2525).

NTP. 1990. *Carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No. 79-01-6) in F344/N rats and B6C3F1 mice (gavage studies).* US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Toxicology Program. Research Triangle Park, NC (NTP Technical Report Series No. 243).

Odum J, Green T, Foster JR, Hext PM. 1988. The role of trichloracetic acid and peroxisome proliferation in the differences in carcinogenicity of perchloroethylene in the mouse and rat. *Toxicol Appl Pharmacol* **92**(1):103-112.

Odum J, Foster JR, Green T. 1992. A mechanism for the development of clara cell lesions in the mouse lung after exposure to trichloroethylene. *Chem Biol Interact* 83(2):135-153.

Pankow D, Damme B, Schror K. 1994. Acetylsalicylic acid--inducer of cytochrome P-450 2E1? *Arch Toxicol* **68**(4):261-265.

Park KS, Sohn DH, Veech RL, Song BJ. 1993. Translational activation of ethanol-inducible cytochrome P450 (CYP2E1) by isoniazid. *Eur J Pharmacol* **248**(1):7-14.

Parkinson A, Mudra DR, Johnson C, Dwyer A, Carroll KM. 2004. The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. *Toxicol Appl Pharmacol* **199**(3):193-209.

Pawliszyn J. 2001. Solid phase microextraction. *Adv Exp Med Biol* **488**:73-87.

Peng R, Tennant P, Lorr NA, Yang CS. 1983. Alterations of microsomal monooxygenase system and carcinogen metabolism by streptozotocin-induced diabetes in rats. *Carcinogenesis* **4**(6):703-708.

Perrier D, Gibaldi M. 1982. General derivation of the equation for time to reach a certain fraction of steady state. *J Pharm Sci* **71**(4):474-475.

Poloyac SM, Tortorici MA, Przychodzin DI, Reynolds RB, Xie W, Frye RF et al. 2004. The effect of isoniazid on CYP2E1- and CYP4A-mediated hydroxylation of arachidonic acid in the rat liver and kidney. *Drug Metab Dispos* **32**(7):727-733.

Prout MS, Provan WM, Green T. 1985. Species differences in response to trichloroethylene. I. pharmacokinetics in rats and mice. *Toxicol Appl Pharmacol* **79**(3):389-400.

Raucy JL, Kraner JC, Lasker JM. 1993. Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. *Crit Rev Toxicol* **23**(1):1-20.

Renwick AG, Lazarus NR. 1998. Human variability and noncancer risk assessment--an analysis of the default uncertainty factor. *Regul Toxicol Pharmacol* **27**(1 Pt 1):3-20.

Sarzanini C, Bruzzoniti MC, Mentasti E. 1999. Preconcentration and separation of haloacetic acids by ion chromatography. *J Chromatogr A* **850**(1-2):197-211.

Schoedel KA, Tyndale RF. 2003. Induction of nicotine-metabolizing CYP2B1 by ethanol and ethanol-metabolizing CYP2E1 by nicotine: Summary and implications. *Biochim Biophys Acta* **1619**(3):283-290.

Schultz IR, Merdink JL, Gonzalez-Leon A, Bull RJ. 1999. Comparative toxicokinetics of chlorinated and brominated haloacetates in F344 rats. *Toxicol Appl Pharmacol* 158(2):103-114.

Shorney HL, Randtke SJ. 1994. Improved methods for haloacetic acid analysis. Proceedings Water Quality Technology Conference, San Francisco, CA, Nov:6-10.

Sidebottom H, Franklin J. 1996. The atmospheric fate and impact of hydrochlorofluorocarbons and chlorinated solvents. *Pure Appl Chem* **68**:1757–1769.

Snawder JE, Lipscomb JC. 2000. Interindividual variance of cytochrome P450 forms in human hepatic microsomes: Correlation of individual forms with xenobiotic metabolism and implications in risk assessment. *Regul Toxicol Pharmacol* **32**(2):200-209.

Soh Y, Rhee HM, Sohn DH, Song BJ. 1996. Immunological detection of CYP2E1 in fresh rat lymphocytes and its pretranslational induction by fasting. *Biochem Biophys Res Commun* **227**(2):541-546.

Steinberg AD, DeSesso JM. 1993. Have animal data been used inappropriately to estimate risks to humans from environmental trichloroethylene? *Regul Toxicol Pharmacol* **18**(2):137-153.

Styles JA, Wyatt I, Coutts C. 1991. Trichloroacetic acid: Studies on uptake and effects on hepatic DNA and liver growth in mouse. *Carcinogenesis* **12**(9):1715-1719.

Tamasi V, Hazai E, Porsmyr-Palmertz M, Ingelman-Sundberg M, Vereczkey L, Monostory K. 2003. GYKI-47261, a new AMPA [2-amino-3-(3-hydroxymethylisoxazole-4-yl)propionic acid] antagonist, is a CYP2E1 inducer. *Drug Metab Dispos* **31**(11):1310-1314.

Templin MV, Parker JC, Bull RJ. 1993. Relative formation of dichloroacetate and trichloroacetate from trichloroethylene in male B6C3F1 mice. *Toxicol Appl Pharmacol* **123**(1):1-8.

Templin MV, Stevens DK, Stenner RD, Bonate PL, Tuman D, Bull RJ. 1995. Factors affecting species differences in the kinetics of metabolites of trichloroethylene. *J Toxicol Environ Health* **44**(4):435-447.

Tierney DJ, Haas AL, Koop DR. 1992. Degradation of cytochrome P450 2E1: Selective loss after labilization of the enzyme. Arch Biochem Biophys **293**(1):9-16.

Tucker AN, Sanders VM, Barnes DW, Bradshaw TJ, White KL,Jr, Sain LE. 1982. Toxicology of trichloroethylene in the mouse. *Toxicol Appl Pharmacol* **62**(3):351-357.

Urbansky ET. 2000. Techniques and methods for the determination of haloacetic acids in potable water. *J Environ Monit* **2**(4):285-291.

US EPA. 1985. *Health assessment document for trichloroethylene.* EPA/600/8-82/006F. US Environmental Protection Agency, Washington, DC.

US EPA. 1986. *Guidelines for carcinogen risk assessment.* Federal Register 51(185): 33992-34003. US Environmental Protection Agency, Washington, DC. **51**(185): 33992–34003.

US EPA. 1995. US EPA method 552.2. *Determination of Haloacetic Acids and Dalapon in Drinking Water by Liquid-Liquid Extraction, Derivatization and Gas Chromatography with Electron-Capture Detection.Methods for the Determination of Organic Compounds in Drinking Water. Supplement III.* US Environmental Protection Agency, Washington, DC.

US EPA. 1999. *Guidelines for carcinogen risk assessment* (SAB Review draft. NCEA-F-0644*).* Risk Assessment Forum, US Environmental Protection Agency, Washington, DC (NCEA-F-0644).

US EPA, 2002. *Final draft for the addendum to drinking water criteria document for monochloroacetic acid and trichloroacetic acid.* EPA-822-R-02-036. Prepared for Health and Ecological Criteria Division, Office of Science and Technology, Office of Water, US Environmental Protection Agency, Washington, DC.

US EPA. 2005. *Guidelines for carcinogen risk assessment*. EPA/630/P-03/001B. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC.

US FDA. 2001. *Guidance for industry: Bioanalytical method validation*. Center for Drug Evaluation and Research (CDER), Food and Drug Administration. Rockville, MD.

Volkel W, Friedewald M, Lederer E, Pahler A, Parker J, Dekant W. 1998. Biotransformation of perchloroethene: Dose-dependent excretion of trichloroacetic acid, dichloroacetic acid, and N-acetyl-S-(trichlorovinyl)-L-cysteine in rats and humans after inhalation. *Toxicol Appl Pharmacol* **153**(1):20-27.

Wang P-Y, Kaneko T, Tsukada H, Nakano M, Sato A. 1997. Dose- and route-dependent alterations in metabolism and toxicity of chemical compounds in ethanol-treated rats: Difference between highly (chloroform) and poorly (carbon tetrachloride) metabolized hepatotoxic compounds. *Toxicol Appl Pharmacol* **142**:13-21.

Wang RS, Nakajima T, Tsuruta H, Honma T. 1996. Effect of exposure to four organic solvents on hepatic cytochrome P450 isozymes in rat. *Chem Biol Interact* **99**:239-252.

Wartenberg D, Reyner D, Scott CS. 2000. Trichloroethylene and cancer: Epidemiologic evidence. *Environ Health Perspect* **108** (Suppl 2):161-176.

Watanabe M, Takano T, Nakamura K. 1998. Effect of ethanol on the metabolism of trichloroethylene in the perfused rat liver. *J Toxicol Environ Health A* **55**(4):297-305.

Weisel CP, Kim H, Haltmeier P, Klotz JB. 1999. Exposure estimates to disinfection by-products of chlorinated drinking water. *Environ Health Perspect* **107**(2):103-110.

Williams D, LeBel G, Benoit F. 1997. Disinfection by-products in canadian drinking water. *Chemosphere* **34**(2):299-316.

Wilson CL, Safe S. 1998. Mechanisms of ligand-induced aryl hydrocarbon receptor-mediated biochemical and toxic responses. *Toxicol Pathol* **26**(5):657-671.

Winters DK, Cederbaum AI. 1992. Time course characterization of the induction of cytochrome P-450 2E1 by pyrazole and 4-methylpyrazole. *Biochim Biophys Acta* **1117**(1):15-24.

Woodard G, Lange SW, Nelson KW, Calvery HO. 1941. The acute oral toxicity of acetic, chloroacetic, dichloroacetic and trichloroacetic acids. *Journal of Industrial Hygiene and Toxicology* **23**:78–82.

Wu D, Cederbaum AI. 1993. Induction of liver cytochrome P4502E1 by pyrazole and 4-methylpyrazole in neonatal rats. *J Pharmacol Exp Ther* **264**(3):1468-1473.

Wu D, Cederbaum AI. 1994. Characterization of pyrazole and 4-methylpyrazole induction of cytochrome P4502E1 in rat kidney. *J Pharmacol Exp Ther* **270**(1):407-413.

Xu G, Stevens DK, Bull RJ. 1995. Metabolism of bromodichloroacetate in B6C3F1 mice. *Drug Metab Dispos* **23**(12):1412-1416.

Yoshida M, Fukabori S, Hara K, Yuasa H, Nakaaki K, Yamamura Y et al. 1996. Concentrations of trichloroethylene and its metabolites in blood and urine after acute poisoning by ingestion. *Hum Exp Toxicol* **15**(3):254-258.

Yu KO, Barton HA, Mahle DA, Frazier JM. 2000. In vivo kinetics of trichloroacetate in male fischer 344 rats. *Toxicol Sci* **54**(2):302-311.

Zerilli A, Ratanasavanh D, Lucas D, Goasduff T, Dreano Y, Menard C et al. 1997. Both cytochromes P450 2E1 and 3A are involved in the O-hydroxylation of p-nitrophenol, a catalytic activity known to be specific for P450 2E1. *Chem Res Toxicol* **10**(10):1205-1212.

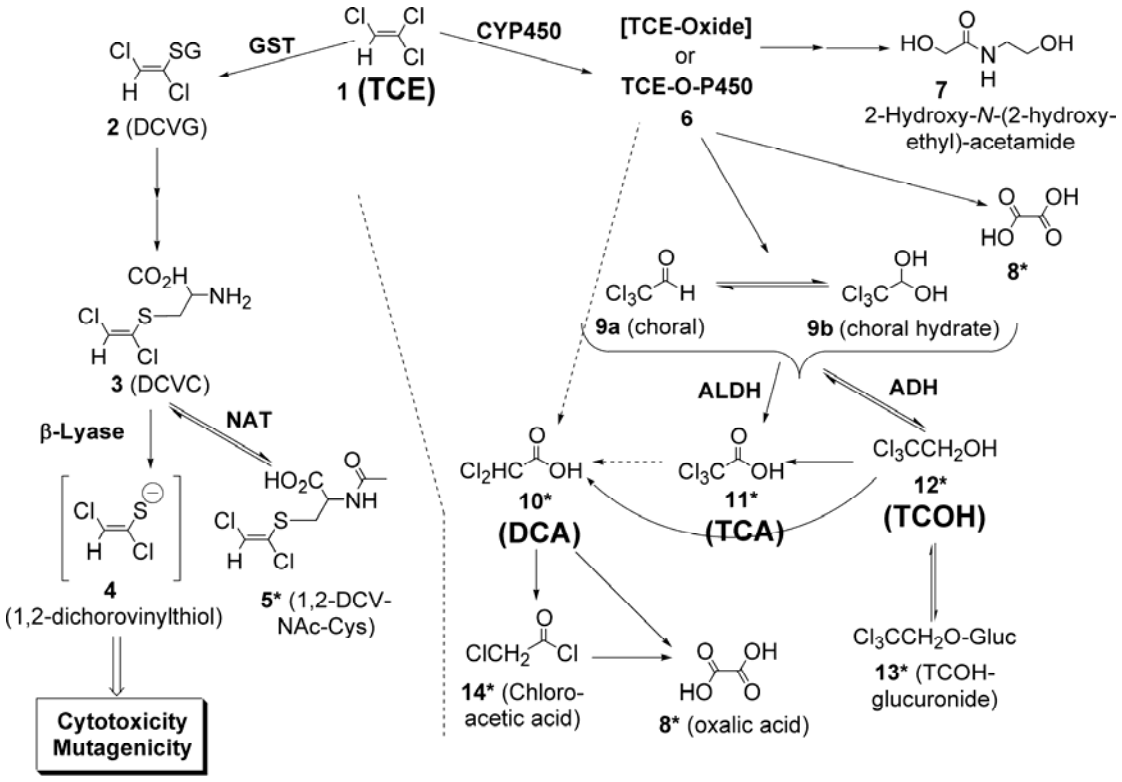

Figure 1-1. A schematic diagram of TCE metabolism pathway (known urinary metabolites are designated with asterisks) modified from Figure 1 of Lash et al. (2000) *Env Health Persp Supple* **108** (Suppl 2): p. 177.

CHAPTER 2. Cytochrome P450 2E1 Induction by Pyridazine Produces Qualitative and Quantitative Changes in the Metabolism of Trichloroethylene to Potentially Hepatocarcinogenic Metabolites[1]

[1] S. Lee, C. A. White, S. Muralidhara, and J. V. Bruckner. To be submitted to Drug Metabolism and Disposition.

Cytochrome P450 2E1 Induction by Pyridazine Produces Qualitative and Quantitative Changes in the Metabolism of Trichloroethylene to Potentially Hepatocarcinogenic Metabolites

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Abbreviations:

TCE, trichloroethylene; PZ, pyridazine; TCA, trichloroacetic acid; S-D, Sprague-Dawley (rats); AAALAC, American Association for Accreditation of Laboratory Animal Care; AUC, area under the concentration versus time curve.

ABSTRACT:

Cytochrome P4502E1 (CYP2E1), which catalyzes the oxidation of many small volatile organic chemicals (VOCs) such as 1,1,1-trichloroethylene (TCE), is induced by a variety of xenobiotics, as well as by certain disease states. It is widely accepted that CYP2E1 induction results in increased production of bioactive metabolites from TCE, leading to the potential for increased cancer risks. Trichloroacetic acid (TCA) is generally believed to be a proximate mouse hepatocarcinogen. One objective of this project is to test the hypothesis that CYP2E1 induction results in relatively minor increases in TCE metabolism at low doses. Another objective is to characterize the effect of CYP2E1 induction by pyridazine (PZ) on TCE's metabolic profile, including the toxicokinetics (TK) of its primary downstream metabolites, chloral hydrate (CH), trichloroethanol (TCOH) and TCA. Young male Sprague-Dawley rats (175 ~ 200 g) were pretreated with PZ (200 mg/kg, i.p.) in saline or saline (controls) daily for 3 days. The animals were then administered TCE (10, 50 or 200 mg/kg, p.o.). PZ pretreatment resulted in moderate decrease in TCE AUCs with the 50 and 200 mg/kg doses. The PZ and control TCE AUCs were not significantly different at the lowest (10 mg/kg) dose. The CYP2E1 induction enhanced the CH AUC for the higher TCE doses. The magnitude of the increase in the CH AUC over controls was quite modest at the lowest TCE dose. PZ elicited no significant increases in plasma TCOH levels the lowest TCE dose. Enhanced biotransformation of TCE to TCA by PZ was manifested by ~ 2-fold increase in TCA C_{max} values at 50 and 200 mg/kg doses of TCE, as well as shorter T_{max}'s. The most striking influence of PZ on TCE TK was enhancement of its clearance from the

bloodstream. This phenomenon was evidenced by ~ 2 to 3-fold decreases in TCA t½ and AUC values at 10, 50 and 200 mg TCE/kg. PZ may exert this effect by enhancing TCA renal clearance. More rapid clearance may result in lower liver cancer risks from TCE in P450-induced populations. Findings in this investigation also offer support for the hypothesis that elevated CYP2E1 activity has diminishing influence on TCE metabolic activation, the lower the exposure to this well metabolized blood flow-limited chemical.

Introduction

1,1,2-Trichloroethylene (TCE) is a volatile organic chemical (VOC) that has been widely utilized as an organic solvent and a degreaser for metal parts (ATSDR, 1997). Due to its extensive use, TCE is now a frequent drinking water contaminant in the U.S., and is the most commonly-found pollutant of groundwater at Superfund sites (Fay and Mumtaz, 1996; Fay, 2006). TCE volatizes into the atmosphere and enters surface and groundwater by leaching from disposal operations and hazardous waste sites. TCE is also often found in indoor air due to the use of TCE-containing consumer products and volatilization from the water supply (Weisel and Jo, 1996; Wu and Schaum, 2000). TCE, perchloroethylene (PCE) and several other VOCs have frequently been detected in the blood of a large percentage of non-occupationally-exposed adults monitored across the U.S. (Churchill et al., 2001; Blount et al., 2006).

TCE is metabolically activated by two pathways, oxidation and glutathione conjugation, to bioactive metabolites (Lash et al., 2000; Clewell et al., 2001). The oxidative pathway predominates quantitatively in the liver, where the majority of TCE biotransformation occurs. Nakajima et al. (1990, 1993) found that Cytochrome P4502E1 (CYP2E1), a high-affinity/low-capacity isoform, was primarily responsible for metabolism of low TCE concentrations in rat liver. CYP2B1/2 was most important at high TCE concentrations, with CYP1A1/2 and CYP2C11 making minor contributions. CYP2E1 is a constitutive isozyme in both rat and human liver. Guengerich et al. (1991) found that CYP2E1 in human liver oxidizes TCE and a number of other VOCs. P450-

mediated oxidation of TCE yields chloral and chloral hydrate (CH). The latter is both oxidized to trichloroacetic acid (TCA) and reduced to trichloroethanol (TCOH). Much of the TCOH is conjugated with glucuronide and excreted in the urine and bile. Dichloroacetic acid (DCA) is also formed from TCE in mice, but its origins are unclear. Only traces of DCA are sometimes found in rats and humans. Both TCA and DCA appear to contribute to TCE hepatocarcinogenesis in mice (Bull et al., 2002). The principal mode of action of TCA in the liver is as a peroxisome proliferator-activated receptor alpha (PPARα) agonist. PPARα stimulation alters cell signaling, potentially enhancing DNA synthesis and inhibiting apoptosis in the precancerous clones and depressing replication of normal hepatocytes (Bull, 2000). Nevertheless, this is a non-genotoxic mechanism of carcinogenesis.

Variations in levels and activity of hepatic microsomal CYP2E1 may: alter the bioavailability and therapeutic efficacy of drugs metabolized by the isoform; provide protection by enhancing the metabolic clearance of toxic xenobiotics; or enhance the bioactivation of some xenobiotics to cytotoxic and/or mutagenic metabolites. Guengerich et al. (1991), for example, showed that human CYP2E1 was responsible for the metabolic activation of some 15 suspected carcinogens, including vinyl chloride, styrene, benzene, chloroform and TCE. CYP2E1 induction occurs in response to a variety of drugs (e.g., isoniazid, aspirin, chlorzoxazone, caffeine, tamoxifen) and certain conditions (e.g., fasting, acute renal failure, obesity, diabetes, chronic alcohol consumption) (Lieber, 1997; Gonzalez, 2005; Cederbaum, 2006). Polymorphisms in the CYP2E1 gene are another source of intersubject variability in activity and levels of the isoform in different ethnic groups (Stevens et al., 1994; McCarver et al., 1998). Ten

polymorphic loci on the human CYP2E1 gene have been reported, with most of them in the promoter and intron regions (Harada et al., 2001). Snawder and Lipscomb (2000) found a 12-fold variation in CYP2E1 protein content in hepatic microsomes from 40 organ donors, due to genetic and/or environmental factors.

It is widely recognized that induction of CYP2E1 can enhance TCE metabolism and potentiate the toxicity of high doses of the VOC. Cornish and Adefuin (1966) and Carlson (1974) were among the first researchers to demonstrate that pretreatment of rats with different P450 inducers enhanced acute liver injury by single, high doses of TCE. Buben and O'Flaherty (1985) concluded from a dose-response study in mice that hepatotoxicity caused by high doses of TCE and perchloroethylene (PCE) was due to their metabolites. TCE and PCE hepatocytotoxicity are usually of minor concern, however, due to the VOCs' low potency as cytotoxins. The influence of CYP2E1 inducers on formation of potentially carcinogenic metabolites at moderate and low TCE exposure levels are of primary concern in occupational and environmental situations, respectively. There is limited empirical evidence that CYP2E1 induction can have a significant effect on metabolic clearance of moderate and high doses of TCE in rats, but have little influence on low doses (Kaneko et al., 1994). Kedderis (1997) predicted that a 10-fold increased in the maximal rate of hepatic metabolism of TCE would result in only a 2 % increase in metabolite formation by a human inhaling 10 ppm of the chemical for 4 h. A physiologically-based pharmacokinetic (PBPK) model was used to make this prediction in the absence of laboratory data.

It has been assumed that CYP2E1 induction will enhance the formation of potentially carcinogenic metabolites from TCE and therefore increase cancer risks. *In vitro* metabolism data provide much of the support for this assumption. There are very few relevant data from *in vivo* experiments. Kaneko et al. (1994) demonstrated that CYP2E1 induction by ethanol had little effect on low TCE doses by monitoring cumulative urinary excretion of TCA and TCOH. These data provide no information of ethanol's effect on the time-course [e.g., areas under the blood concentration versus time curves (AUCs) or peak blood concentration (C_{max}s)] of these or other key metabolites. Thus, one of the primary objectives of the current investigation was to characterize the action of pyridazine, a potent CYP2E1 inducer (Kim and Novak, 1993), on the internal dosimetry of the parent compound (TCE) and its major biotransformation products. Such dosimetry data are essential for estimation of cancer risks for different exposure scenarios. The second primary objective was to test the hypothesis that the influence of CYP2E1 induction on TCE metabolism is inversely related to TCE dose. This hypothesis, if true, would counter the assumption that elevated CYP2E1 activity/level increases formation of potentially carcinogenic metabolites and the attendant cancer risks of low, environmentally-relevant exposures to TCE.

Materials and Methods

Chemicals: 1,1,2-Trichloroethylene (TCE) (> 99.9 % of purity); trichloroethanol (TCOH), trichloroacetic acid (TCA), chloral hydrate (CH) and dichloroacetic acid (DCA) (all > 99.9 % purity); and pyridazine (PZ) were purchased from Aldrich Chemical Co (Milwaukee, WI)). Isooctane (ACS spectrophotometric grade) was obtained from Sigma Aldrich (St. Louis, MO). Sulfuric acid and methanol were obtained from J.T. Baker (Phillipsburg, NJ) and Sigma Aldrich, respectively. Alkamuls EL-620® (formerly Emulphor®), a polyethoxylated vegetable oil supplied by Rhone-Poulenc (Cranbury, NJ), was used to prepare stable aqueous TCE emulsions.

Animals: Male Sprague-Dawley (S-D) rats of 100 – 125 g were purchased from Charles River Laboratories (Raleigh, NC). The animals were housed 2 rats per cage in their own limited-access room of an AAALAC-accredited animal facility. The room was maintained at 21°C and 50 ± 10 % humidity with a 12-h light/dark cycle. Full spectrum fluorescent lights were on daily from 0600 – 1800 h. The rats were supplied Purina Rat Chow No. 5001® and tap water *ad libitum* during an acclimation period of at least 1 week. The study protocol was approved by the University of Georgia Animal Care and Use Committee.

Dosage and Sample Collection Regimens: Groups of 6 male S-D rats were injected with 200 mg PZ/kg i.p. in saline for 3 days between 0900 and 1000 h. Kim and Novak (1993) reported this dosage regimen to produce a 4- to 5-fold increase in hepatic

microsomal *p*-nitrophenol (PNP) hydroxylase activity in male Harlan S-D rats. Our S-D rats from Charles River exhibited a 2.5-fold increase in PNP hydroxylation under these conditions (data not shown). Control rats were injected i.p. with saline for 3 consecutive days. A cocktail of ketamine HC1 (100 mg/ml): acepromazine maleate (20 mg/ml): xylazine HC1 (10 mg/ml) in a proportion of 3:2:1 (v/v/v) was then injected i.m. in a volume of 0.8 ml/kg to produce surgical anesthesia. A PE-50 cannula (OD = 0.97 mm, ID = 0.58 mm) was implanted in the left carotid artery of each animal on the third day, soon after administration of the last PZ/saline dose. The cannula was filled with heparinized saline (1,000 U/ml) to maintain its patency. Each cannula was tunneled s.c. and exited at the nape of the neck, so the animal could move freely and serial blood samples be taken upon recovery. Food was withheld during the 24-h recovery period to minimize intersubject variability in GI absorption of TCE. Water was available during this time. Control and PZ-pretreated groups were gavaged with 10, 50 or 200 mg TCE/kg in a 5 % aqueous Alkamuls EL-620® emulsion in a total dosing volume of 1 ml/kg. Serial micro (10 to 50 µl) blood samples were taken from the carotid cannula for up to 24 h post dosing. An equivalent volume of heparinized saline was injected after each sampling into the cannula to replace lost blood volume. Access to food was allowed during the 24-h monitoring period.

Sample Analyses. The blood samples were collected on ice and transferred to 20-ml gas chromatography headspace (GC) vials containing 200 µl of esterification solution comprised of distilled water, concentrated sulfuric acid and methanol in a ratio of 6:5:1 (v/v/v). The headspace vials were capped with polytetrafluoroethylene (PTFE)-

coated rubber septa and aluminum cap, then tightly crimped. The contents were ultrasonicated for 1 min. The procedure converted TCA and DCA to their volatile methyl esters (Muralidhara and Bruckner, 1999). TCE, TCOH and CH were sufficiently volatile at the GC temperatures employed. TCE, TCA, DCA, CH and TCOH could thus be quantified in each 10–50-µl blood sample by headspace analysis. The vials were placed into a TurboMatrix 110® thermostat-controlled autosampler attached to a Perkin-Elmer Clarus 500 GC equipped with an electron capture detector. The GC headspace sampler was maintained at a constant 125°C. The temperature of the column was kept at 120°C for 3 min, then increased 25°C/min up to 170°C and maintained there for 3 min for each sample. The injector and detector temperatures were 200 and 360°C, respectively. Analyses were carried out on a 10′ X ⅛″ stainless steel column packed with a 10 % customized column coating of OV-17 (phenylpolysiloxane) on the 80/100-µm mesh size matrix Supelcoport® (Supelco Inc., Bellefonte, PA). Nitrogen was used as the carrier gas at 25 psi. TCA, DCA, CH and TCOH standards were prepared daily in HPLC grade water and analyzed concurrently with the blood samples (There were no difference between the results of the standards with water or blood as matrices). Isooctane was utilized for TCE. The limits of detection and quantitation for each analyte were ~ 5 and 20 ng/ml, respectively.

Calculation of Kinetic Parameters: Blood TCE concentration versus time profiles were evaluated using WinNonlin Professional Version 4.1 (Pharsight Co., Mountain View, CA). The individual TCE time-courses of orally-dosed rats were analyzed by compartmental models using standard equations, for calculation of relevant

parameters [i.e., terminal elimination half-life ($T_{\beta½}$), total body clearance (CL), volume of distribution (Vd) and area-under blood concentration versus time curves (AUCs)]. Individual CH, TCOH and TCA blood time profiles were analyzed by non-compartmental methods using standard equations (Perrier and Gibaldi, 1982). Maximum blood concentrations (C_{max}) and times to C_{max} (T_{max}) were observed means values.

Statistical Analyses: Student's t-test was used to determine the statistical significance ($p < 0.05$) of differences in each pharmacokinetic parameter as a function of TCE dose and PZ-pretreatment.

Results

Orally-administered TCE exhibits dose-dependent kinetics in the 10 – 200 mg/kg dosage range and is eliminated more rapidly in PZ-pretreated animals. The blood TCE time-profiles for the 10, 50 and 200 mg TCE/kg p.o. groups are shown in Figs. 1A, B and C, respectively, and as expected difference in distribution phase is apparent with the increase of TCE dose. TCE is absorbed very rapidly from the GI tract of fasted rats administered the chemical in an aqueous emulsion. This is not evident in Figs. 1A – C, due to the compression of the time scale on the X axis. An increase in the observed T_{max} is evident, however, with increase in dose (Table 1). The control TCE AUCs increase disproportionately with dose above 10 mg/kg, indicative of the onset of metabolic saturation. The increases in $T_{\beta½}$ values with dosage are another indicator of metabolic saturation. PZ pretreatment results in modest decreases in half-lives, but manifests smaller AUC values for the two higher TCE dosage-levels. The PZ-induced reduction in TCE AUCs becomes less pronounced with decrease in TCE dose (Fig. 2A, Table 1). TCE AUC, C_{max} and $T_{\beta½}$ values for the control and PZ groups are significantly different at the highest (200 mg/kg) TCE dose.

Blood TCA time-courses in PZ-pretreated rats were quite different from those that were anticipated. It would be expected that CYP2E1 induction would result in increased TCA formation, manifested by higher blood TCA levels and AUCs. Blood TCA concentrations were significantly higher in induced animals than in controls during the initial 3 h in the groups ingesting 10, 50 and 200 mg TCE/kg (Figs. 1A, B). This is

reflected in the PZ-pretreated groups with shorter T_{max} values and higher C_{max}s at 50 mg TCE/kg (Table 2). However, the decrease in blood TCA levels was prominent after 2 h post dosing in PZ-treated groups. TCA concentrations in controls exceeded those in the PZ-pretreated groups for the duration of the 24-h sampling period at all three TCE dosage levels (Figs. 1A – C). This situation was most pronounced for the highest TCE dose (Fig. 2B). The control TCA AUC was ~ 3-fold higher and the $T_{\beta½}$ was significantly longer in this instance (Table 2). This pattern was similar but showed less pronounced differences for the two lower TCE doses.

Blood DCA concentrations, as noted in the Materials and Methods, were analyzed in all blood samples. DCA was consistently quantifiable only in blood samples from the 200 mg TCE/kg PZ-pretreatment group (data not shown). Trace levels of DCA were only found sporadically in other treatment and dosing groups.

Evaluation of the TCOH time-course data revealed a TCE dose-dependent increase in TCOH AUCs, as well as PZ-induced increases in blood TCOH concentrations. Apparent saturation of TCOH production was evidenced by 2-fold increases in the TCOH AUCs with 5- and 4-fold incremental increases in the amount of TCE administered in control groups (Table 3). Disproportionate increases in C_{max} were also exhibited with the increase in TCE dosage. At the highest TCE dose, PZ-pretreatment resulted in modest increases over controls in TCOH AUC and C_{max} values. These modest increases over controls in blood TCOH levels can be visualized in plots of blood TCOH time-courses in Fig. 3. The PZ-induced increases in TCOH AUCs were inversely related to the TCE

dose (Fig. 4A). The control and PZ AUC $T_{\beta½}$ and C_{max} values were not different at the lowest (10 mg/kg) TCE dosage.

Effects of the TCE dosage and PZ pretreatment on TK parameter estimates for CH showed were not as consistent as was the case with the other metabolites. CH AUC C_{max} and T_{max} values increased disproportionately (3-fold increases in the CH AUCs with 5- and 4-fold incremental increases with TCE dose) in controls, indicative of saturation of TCE oxidation to this intermediate metabolite (Table 4). PZ pretreatment resulted in an expected larger AUC at the intermediate (50 mg/kg) and higher (200 mg/kg) TCE dosage level (Figs. 4B, C). PZ did not significantly alter $T_{\beta½}$, C_{max} or T_{max} values of CH from controls at any TCE dose. $T_{\beta½}$ of CH, a metabolite of TCE, was expected to be shorter than that of parent compound, but shorter $T_{\beta½}$ of CH than TCE $T_{\beta½}$ was noted at 50 and 200 TCE/kg. Although the estimated $T_{\beta½}$ values of CH were statistically significant shorter than those of TCE at 50 and 200 mg TCE/kg dose level, this was attributed to the more significantly lower blood concentration of CH (evidenced by much smaller C_{max}s than those of TCE), which can easily be converted to next metabolites. Inspection of the CH time-courses in Fig. 3 revealed considerable fluctuations in blood concentrations of the metabolite in both the control and PZ groups, especially at the highest TCE dose.

Discussion

Findings in the current study provide insight into the absorption of orally-administered TCE, especially in the presence of elevated activities of CYP2E1. The chemical is very rapidly absorbed into the arterial circulation from the GI tract of fasted rats. The T_{max}'s ranged from 2.8 min for the lowest dose to 9.7 min for the highest dose (Table 1). Lee et al. (2000) previously observed that the rapidity of oral absorption of TCE decreased with increase in dose. TCE rapidly diffuses through the gastric and intestinal membranes, as it is a small, unchanged, lipophilic molecule. Giving the VOC as an aqueous emulsion should promote its absorption. TCE will quickly volatilize from an emulsion's micelles within the warm luminal environment (Lee et al., 1997), resulting in relatively large quantities of the chemical coming into direct content with epithelial membranes. D'Souza et al. (1985) report that > 90 % of TCE is absorbed systemically when administered in a similar manner to fasted rats. Rats are frequently fasted to minimize inter-subject variability in absorption and bioavailability. Fatty foods, in particular, retard the absorption of lipophilic chemicals such as TCE. Vegetable oils are known to act as a reservoir in the gut for carbon tetrachloride ($CC1_4$), delaying its absorption until they are emulsified, cleaved by lipases and absorbed (Kim et al., 1990).

TCE data from the present investigation also provide information about the metabolism and elimination of the VOC. Evaluation of the dose-dependency of the AUC, $T_{\beta½}$ and C_{max} values reveals saturation of TCE metabolism. The 5-fold increase in TCE dose from 10 to 50 mg/kg resulted in a 12-fold increase in the TCE AUC (Table 1). Lee et al. (2000) report the onset of metabolic saturation between oral bolus doses of 8 and 16

mg TCE/kg in male S-D rats. It should be recognized that metabolic saturation is not an "all or nothing" event. Its onset is gradual and its course progressive, with higher and higher TCE doses resulting in smaller and smaller increments in metabolites. TCE's terminal elimination of half-life becomes progressively longer with increase in dose, also evidenced at higher doses in this study. This occurs despite increases in the amount of the VOC that is exhaled (Dekant et al., 1986). Although no tissue deposition data were collected in the present study, TCE is known to be distributed to tissues largely according to their blood flow rate and fat content (Davidsohn and Beliles, 1991). TCE that escapes exhalation and metabolism at high dosage levels is largely deposited in adipose tissue. High lipid:blood partition coefficient of TCE and slow rate of blood flow to the adipose tissue result in prolongation of TCE's residence time in the body, despite its propensity for metabolism (Bruckner et al., 2006).

It is a widely-held principle of toxicology that induction of enzymes responsible for metabolic activation (i.e., conversion of a parent compound to a more cytotoxic or mutagenic metabolite) may result in increased formation of reactive metabolites and a resulting increased likelihood of toxicity. This principle, as described in the Introduction, is known to be applicable to high doses of TCE and halocarbons. Researchers have clearly shown that pretreatment of rodents with a variety of P450 inducers will potentiate the toxicity of hepatotoxic solvents. Folland et al. (1976) describes a case involving a woman and other workers at an isopropyl alcohol bottling plant who became ill after a subsequent exposure to a quantity of $CC1_4$ that by itself was not toxic. The woman developed liver injury and kidney failure, because repeated exposures to isopropanol markedly induced CYP2E1 in her liver, resulting in a marked increase in metabolism of

$CC1_4$ to reactive, cytotoxic free radicals. Manno et al. (1996) described a case of potentiation of $CC1_4$ hepatotoxicity an alcoholic in an occupational setting. It is important to recognize that workers may frequently be subjected to relatively high exposures to $CC1_4$, TCE and other VOCs.

There is emerging empirical evidence that the effects of CYP2E1 inducers on moderate to high doses of TCE and other well-metabolized VOCs may not be applicable to low-dose exposure situations. Kaneko et al. (1994) utilized an ethanol dosage regimen that increased the metabolism of TCE and 1,1,1-trichloroethane (TRI) by rat liver microsomes 5-fold. The systemic clearance and metabolism of TRI, a poorly metabolized congener, were significantly increased. With such a compound, whose intrinsic clearance is lower than its hepatic blood flow rate, the maximum rate of its metabolism (V_{max}) is independent of dose (i.e., P450 induction induces TRI's metabolism, even at low exposure levels). Kaneko and co-workers' ethanol dosage regimen did not affect the elimination of low to moderate doses of TCE from the animals' blood or their urinary excretion of TCOH or TCA. Enzyme induction should have little or no effect on the metabolism of low concentrations of extensively-metabolized chemicals (Sato, 1991; Wang et al., 1996; Lipscomb et al., 2003). Hepatic blood flow limits the extent of metabolism of such chemicals. Kedderis (1997) utilized a PBPK model to predict that 10-fold increase in V_{max} would result in only a 2 % increase in the amount of TCE metabolized by a human inhaling 10 ppm TCE for 4 h. Lipscomb et al. (2003) subsequently used a PBPK model that forecast a 2 % increase in the quantity of TCE oxidized by people ingesting 2 L of water containing 5 μg TCE/L (5 ppb). These

modelers did not have laboratory data for verification of the accuracy/validity of their calculations.

The current investigation provides evidence to support the hypothesis that the influence of hepatic CYP2E1 induction on TCE metabolism is inversely related to TCE dose. It should be recalled that our PZ dosing regimen produced a 2.5-fold increase in CYP2E1 activity. This is less than the 4- to 5-fold elevation Kim and Novak (1993) reported, likely due to a ~ 2-fold induction by the 24-h fasting before the oral exposure of TCE we employ in controls and PZ-pretreated animals. PZ pretreatment of the 50 and 200 mg TCE/kg groups generally produces larger AUCs and higher C_{maxs} for TCE metabolites, TCOH and CH, than were manifested in controls (Tables 3 and 4). Occasional exceptions, for which there are no ready explanations, are present. The increases in CH and TCOH AUC values in the 50 and 200 mg TCE/kg groups are relatively modest. More pronounced changes would be anticipated at higher TCE doses (Kaneko et al., 1994). The influence of PZ on TCA kinetics is discussed below. A key finding in this phase of the project is the lack of significant effects of PZ pretreatment on TCOH and CH AUC or C_{max} values at the lowest TCE dosage (10 mg/kg). The very slight, but consistent changes at 10 mg/kg would likely disappear at even lower TCE doses. The lack of a statistically significant effect of CYP2E1 induction on biotransformation of 10 mg TCE/kg, which is far greater than environmentally-relevant TCE level, is also manifested by the absence of alterations of TCE kinetic parameters (Table 1).

Assessment of human health risks from TCE has been challenging, because TCE's metabolism, TK and mode(s) of action (MOA) are inherently complex.

Assessment of cancer risks of trace, environmentally-relevant levels of TCE is a subject of major public health concern. The EPA's standard default policy, in the absence of adequate experimental evidences to the contrary, has been to utilize a linear, multistage model to extrapolate from high-dose rodent cancer bioassay data to predict human cancer risks from environmental exposures. This very conservative approach assumes there is no threshold dose for cancer causation and results in high cancer risk estimates. It has recently been opined by a panel convened by the National Academy of Sciences that there is insufficient knowledge of TCE's MOA and low dose TK to adopt a biologically-based dose-response model, rather than the linear default mode (NRC, 2006). A key argument in favor of use of the conservative linear model is that it will be protective of subpopulations that have environmentally- or genetically-based high TCE metabolic activation capacity. Data from the present study support the aforementioned PBPK modeling efforts that refute this argument/assumption. Humans whose CYP2E1 gene is expressed should have CYP2E1 activity far in excess of that necessary to metabolize all of very low TCE doses. Therefore, it is reasonable to conclude that genetically-or environmentally-determined increased in CYP2E1-mediated metabolic capacity are inconsequential for most TCE environmental exposure scenarios. This conclusion should also apply to other extensively-metabolized environmental contaminants such as vinyl chloride, benzene, chloroform, etc.

There is very little information from *in vivo* experiments on the influence of CYP2E1 induction on TCE's oxidation to CH and its two major "downstream" metabolites, TCOH and TCA. Most of our current knowledge comes from measurements of the metabolites in liver microsomes from pretreated animals (Nakajima et al., 1990,

1993; Lash et al., 2000), cultured hepatocytes (Woodcraft and Novak, 1998) or other *in vitro* systems. Some investigations (Larson and Bull, 1989; Kaneko et al., 1994) have assessed effects of CYP2E1 inducers such as ethanol on TCE metabolism by monitoring urinary excretion of TCOH and TCA. There are very few blood or tissue time-course data for metabolites of TCE or other VOCs. Blood time-course studies of the influence of P450 inducers on parent drugs and their metabolites in laboratory animal and human blood are relatively common (e.g., Fromm et al., 1996; Lin et al., 1999; Monsarrat et al., 1998). Internal dosimetry data on TCE's metabolites are essential for constructing and validating PBPK models, as well as for extrapolating from high-dose rodent experiments to low-dose human exposure scenarios for non-cancer and cancer risk assessments. This is exemplified by our unanticipated finding of a marked reduction in blood TCA levels in PZ-pretreated rats. Such a marked reduction in concentrations of this mouse hepatocarcinogenic metabolite implies that liver cancer risks may be substantially reduced under such circumstances. Subsequent publications from the present study describe further investigations of the phenomenon, as well as evaluation of the ability of other CYP2E1 inducers to exert such an unexpected effect.

Footnotes

This work was supported by DOE Cooperative Agreement DE-FC09-02CH11109.

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References

ATSDR (1997) *Toxicological Profile for Trichloroethylene (Update).* US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA.

Blount BC, Kovelski RJ, McElprang DO, Ashley DL, Marrow JC, Chambers DM, and Cardinali FL (2006) Quantitation of volatile organic compounds in whole blood using solid-phase microextraction and gas chromatography-mass spectrophotometry. *J Chromatogr B* **832**:292-301.

Bruckner JV, Anand SS, and Warren DA (2006) Toxic effects of solvents and vapors, in *Casarette and Doull's Toxicology: The Basic Science of Poisons*, 7th ed (Klaassen CD ed) pp 874-876, McGraw-Hill, New York.

Buben JA and O'Flaherty EJ (1985) Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: A dose-effect study. *Toxicol Appl Pharmacol* **78**:105-122.

Bull RJ (2000) Mode of action of liver tumor induction by trichloroethylene and its metabolites, trichloroacetate and dichloroacetate. *Environ Health Perspect* **108** (Suppl 2):241-259.

Bull RJ, Orner JA, Cheng RS, Stillwell L, Stauber AJ, Sasser LB, Lingohr BD, and Thrall BC (2002) Contribution of dichloroacetate and trichloroacetate to liver tumor induction in mice by trichloroethylene. *Toxicol Appl Pharmacol* **182**:55-65.

Carlson GP (1974) Enhancement of the hepatotoxicity of trichloroethylene by inducers of drug metabolism. *Res Commun Chem Pathol Pharmacol* **7**:637-640.

Cederbaum AI (2006) CYP2E1-biochemical and toxicological aspects and role in alcohol-induced liver injury. *Mt Sinai J Med* **73**:657-672.

Churchill JE, Ashley DL, and Kaye WE (2001) Recent chemical exposures and blood volatile organic compound levels in a large population-based sample. *Arch Environ Health* **56**:157-166.

Clewell HJ III, Barton HA, Maull EA, and Andersen ME (2001) Under what conditions is trichloroethylene likely to be a carcinogen in humans? *Human Ecol Risk Assess* **7**:687-716.

Cornish HH and Adefuin J (1966) Ethanol potentiation of halogenated aliphatic solvent toxicity. *Am Ind Hyg Assoc J* **27**:57-61.

Davidsohn IW and Beliles RP (1991) Consideration of the target organ toxicity of trichloroethylene in terms of metabolite toxicity and pharmacokinetics. *Drug Metab Rev* **23**:493-599.

Dekant W, Schulz A, Metzler M, and Henschler D (1986) Absorption, elimination and metabolism of trichloroethylene: A quantitative comparison between rats and mice. *Xenobiotica* **16**:143-152.

D'Souza RW, Bruckner JV, and Feldman S (1985) Oral and intravenous trichloroethylene pharmacokinetics in the rat. *J Toxicol Environ Health* **15**:587-601.

Fay RM (2006) Environmental pollutant mixtures in completed exposure pathways at hazardous waste sites. *Environ Toxicol Pharmacol* in press.

Fay RM and Mumtaz MM (1996) Development of a priority list of chemical mixtures occurring at 1188 hazardous waste sites, using the HazDat database. *Food Chem Toxicol* **34**:1163-1165.

Folland DS, Schaffner W, Ginn HE, Crofford OB, and McMurray DR (1976) Carbon tetrachloride toxicity potentiated by isopropyl alcohol. Investigation of an industrial outbreak. *JAMA* **236**:1853-1856.

Fromm MF, Busse D, Kroemer HK, and Eichelbaum M (1996) Differential induction of prehepatic and hepatic metabolism of verapamil by rifampin. *Hepatology* **24**:796-801.

Gonzalez FJ (2005) Role of cytochrome P450 in chemical toxicity and oxidative stress: Studies with CYP2E1. *Mutat Res* **569**:101-110.

Guengerich FP, Kim D-H, and Iwasaki M (1991) Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem Res Toxicol* **4**:168-179.

Harada S, Agarwal DP, Nomura F, and Higuchi S (2001) Metabolic and ethnic determinants of alcohol drinking habits and vulnerability to alcohol-related disorder. *Alcohol Clin Exp Res* **25**: 71S-75S.

Kaneko T, Wang-PY, and Sato A (1994) Enzymes induced by ethanol differently affect the pharmacokinetics of trichloroethylene and 1,1,1-trichloroethane. *Occup Environ Med* **51**:113-119.

Kedderis GL (1997) Extrapolation of in vitro enzyme induction data to humans in vivo. *Chem-Biol Interact* **107**:109-121.

Kim HJ, Bruckner JV, Dallas CE, and Gallo JM (1990) Effect of dosing vehicles on the pharmacokinetics of orally administered carbon tetrachloride in rats. *Toxicol Appl Pharmacol* **102**:50-60.

Kim SG and Novak RF (1993) The induction of cytochrome P4502E1 by nitrogen- and sulfur-containing heterocyclics: Expression and molecular regulation. *Toxicol Appl Pharmacol* **120**:257-265.

Larson JL and Bull RJ (1989) Effect of ethanol on the metabolism of trichloroethylene. *J Toxicol Environ Health* **28**:395-406.

Lash LH, Fisher JW, Lipscomb JC, and Parker JC (2000) Metabolism of trichloroethylene. *Environ Health Perspect* **108** (Suppl 2):177-200.

Lee KM, Muralidhara S, Dallas CE, and Bruckner JV (1997) Lack of volatilization and escape of orally administered trichloroethylene from the gastrointestinal tract of rats. *Toxicol Industr Health* **13**:81-89.

Lee KM, Muralidhara S, White CA, and Bruckner JV (2000) Mechanisms of the dose-dependent kinetics of trichloroethylene: Oral bolus dosing of rats. *Toxicol Appl Pharmacol* **164**:55-64.

Lieber CS (1997) Cytochrome P-4502E1: Its physiological and pathological role. *Physiol Rev* **77**:517-544.

Lin JH, Chiba M, Chen IW, Nishime JA, deLuna FA, and Yamazaki M (1999) Effect of dexamethasone on the intestinal first-pass metabolism of indinavir in rats: Evidence of cytochrome P-450 3A and p-glycoprotein induction. *Drug Metab Dispos* **27**:1187-1193.

Lipscomb JC, Teuschler LK, Swartout J, Popken D, Cox T, and Kedderis GL (2003) The impact of cytochrome P450 2E1-dependent metabolic variance on a risk-relevant pharmacokinetic outcome in humans. *Risk Anal* **23**:1221-1238.

Manno M, Razzadore M, Grossi M, and Sbrana C (1996) Potentiation of occupational carbon tetrachloride toxicity by ethanol abuse. *Hum Exp Toxicol* **15**:294-300.

McCarver DG, Byun R, Hines RN, Hichme M, and Wegenek W (1998) A genetic polymorphism in the regulatory sequences of human CYP2E1: Association with increased chlorzoxazone hydroxylation in the presence of obesity and ethanol and intake. *Toxicol Appl Pharmacol* **152**:276-281.

Monsarrat B, Chatelut E, Royer I, Alvinerie P, Dubois J, and Dezeuse A (1998) Modification of paclitaxel metabolism in a cancer patient by induction of cytochrome P450 3A4. *Drug Metab Dispos* **26**:229-233.

Muralidhara S and Bruckner JV (1999) Simple method for rapid measurement of trichloroethylene and its major metabolites in biological samples. *J Chromatogr B Biomed Sci Appl* **732**(1):145-153.

Nakajima T, Wang R-S, Murayama N, and Sato A (1990) Three forms of trichloroethylene-metabolizing enzymes in rat liver induced by ethanol, phenobarbital, and 3-methylcholanthrene. *Toxicol Appl Pharmacol* **102**:546-552.

Nakajima T, Wang RS, Elovaara E, Park SS, Gelboin HV, and Vainio H (1993) Cytochrome P450-related differences between rats and mice in the metabolism of benzene, toluene and trichloroethylene in liver microsomes. *Biochem Pharmacol* **45**:1079-1085.

NRC (2006) Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues. National Research Council, The National Academy Press, Washington, DC.

Perrier D and Gibaldi M (1982) General derivation of the equation for time to reach a certain fraction of steady state. *J Pharm Sci* **71**:474-475.

Sato A (1991) The effect of environmental factors on the pharmacokinetic behavior of organic solvent vapors. *Ann Occup Hyg* **35**:525-541.

Snawder JE and Lipscomb JC (2000) Individual variance in cytochrome P450 forms in human hepatic microsomes: Correlation of individual forms with xenobiotic metabolism and implications in risk assessment. *Regul Toxicol Pharmacol* **32**:200-209.

Stephens EA, Taylor JA, Kaplan N, Yang C-H, Hsieh LL, Lucier GW, and Bell DA (1994) Ethnic variation in the CYP2E1 gene: Polymorphism analysis of 695 African-Americans, European-Americans and Taiwanese. *Pharmacogenetics* **4**:185-192.

Wang P-Y, Kaneko T, Tsukada H, Nakano M, and Sato A (1997) Dose- and route-dependent alterations in metabolism and toxicity of chemical compounds in ethanol-treated rats: Difference between highly (chloroform) and poorly (carbon tetrachloride) metabolized hepatotoxic compounds. *Toxicol Appl Pharmacol* **142**:13-21.

Wang RS, Nakajima T, Tsuruta H, and Honma T (1996) Effect of exposure to four organic solvents on hepatic cytochrome P450 isozymes in rat. *Chem Biol Interact* **99**:239-252.

Wartenberg D, Reyner D, and Scott CS (2000) Trichloroethylene and cancer: Epidemiologic evidence. *Environ Health Perspect* **108** (Suppl 2):161-176.

Weisel CP and Jo WK (1996) Injection, inhalation and dermal exposures to chloroform and trichloroethylene from tap water. *Environ Health Perspect* **104**:48-51.

Wong O (2004) Carcinogenicity of trichloroethylene: An epidemiologic assessment. *Clin Occup Environ Med* **53**:537-589.

Woodcroft KJ and Novak RF (1998) Xenobiotic-enhanced expression of cytochromes P450 2E1 and 2B in primary cultured rat hepatocytes. *Drug Metab Dispos* **26**:372-378.

Wu C and Schaum J (2000) Exposure assessment of trichloroethylene. *Environ Health Perspect* **108** (Suppl 2):359-363.

Legends for figures

FIG. 1. Male S-D rats were injected with saline (control) or 200 mg PZ/kg i.p. for 3 days. They were then gavaged with 10, 50 or 200 mg TCE/kg, serial blood samples taken for 24 h via an indwelling cannula, and the samples analyzed for their TCE and TCA content. TCE and TCA time profiles for the 10, 50 and 200 mg TCE/kg p.o. groups of controls (dashed lines) and PZ-induced (solid lines) are pictured in plates A, B and C, respectively. Points represent means ± S.E. for groups of 4 – 5 rats. Lines were drawn point to point to connect between the mean values. Some of the error bars not apparent, because S.E. was so small to fit within the data point. Designation of significant differences is omitted for sake of clarity.

FIG 2. Effect of 3 days of 200 mg PZ/kg i.p. pretreatment on blood TCE and TCA $AUC_{0\rightarrow 24}$ values for control (clear bars) and PZ-induced (shaded bars) groups of male S-D rats gavaged with 10, 50 or 200 mg TCE/kg. Insets show 10 and 50 mg TCE/kg values more clearly. Bar heights represent means (± S.E., n = 4 – 5). Asterisks indicate statistically significant difference between control and PZ-pretreated groups.

FIG. 3. Male S-D rats were injected with saline (control) or 200 mg PZ/kg i.p. for 3 days. They were then gavaged with 10, 50 or 200 mg TCE/kg, serial blood samples taken for 24 h via an indwelling cannula, and the samples analyzed for their TCOH and CH content. CH and TCOH time profiles for the 10, 50 and 200 mg TCE/kg p.o. groups of controls (dashed lines) and PZ-induced (solid lines) are pictured in plates A, B and C,

respectively. Points represent means ± S.E. for groups of 4 – 5 rats. Lines were drawn point to point to connect between the mean values. Error bars not shown fit within the data point. Some of the error bars not apparent, because S.E. was so small to fit within the data point. Designation of significant differences is omitted for sake of clarity.

FIG 4. Effect of 3 days of 200 mg PZ/kg i.p. pretreatment on blood TCOH and CH $AUC_{0\rightarrow24}$ values for control (clear bars) and PZ-induced (shaded bars) groups of male S-D rats gavaged with 10, 50 or 200 mg TCE/kg. Bar heights represent means (± S.E., *n* = 4 – 5). Asterisks indicate statistically significant difference between control and PZ-pretreated groups.

Figures and Tables

FIG 1.

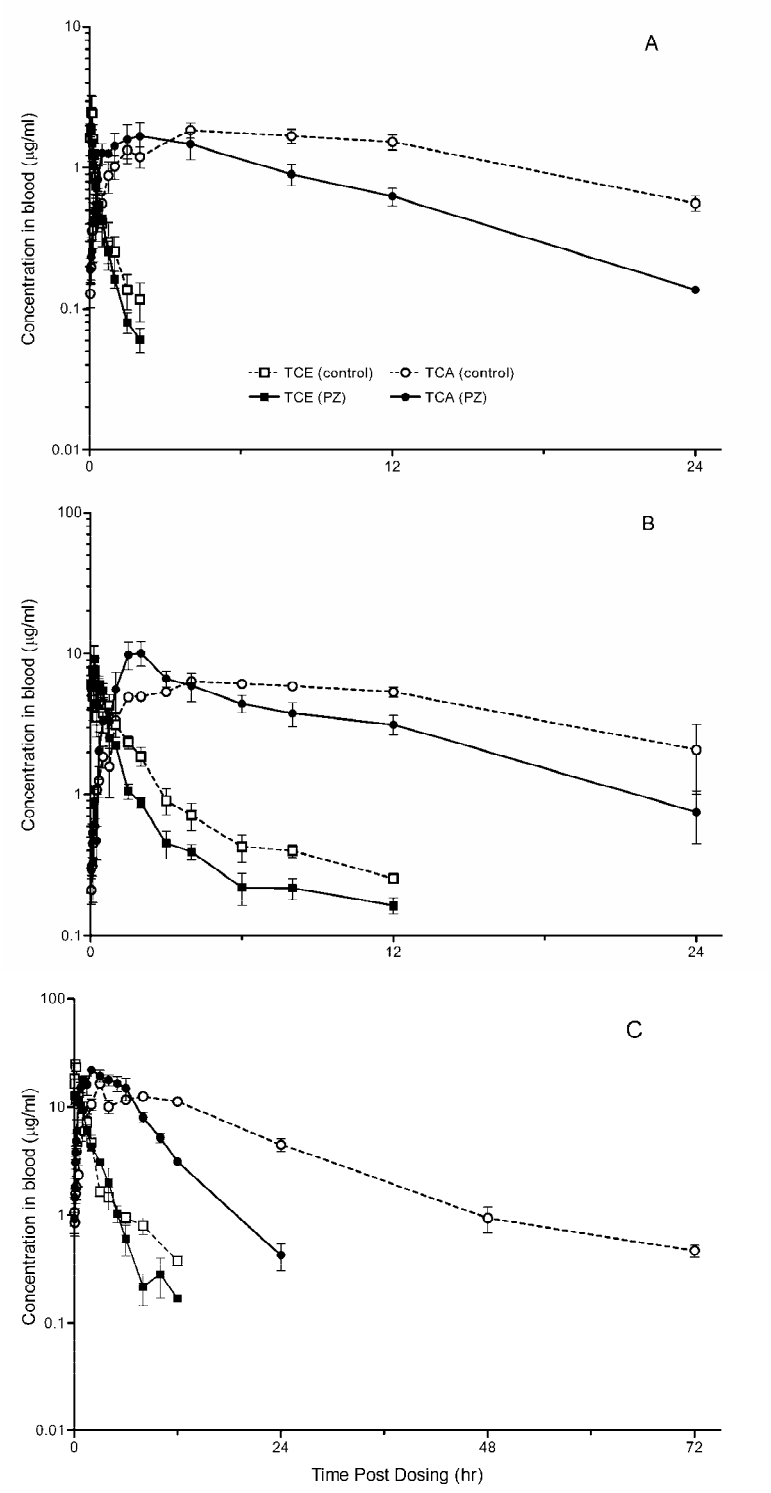

FIG. 2

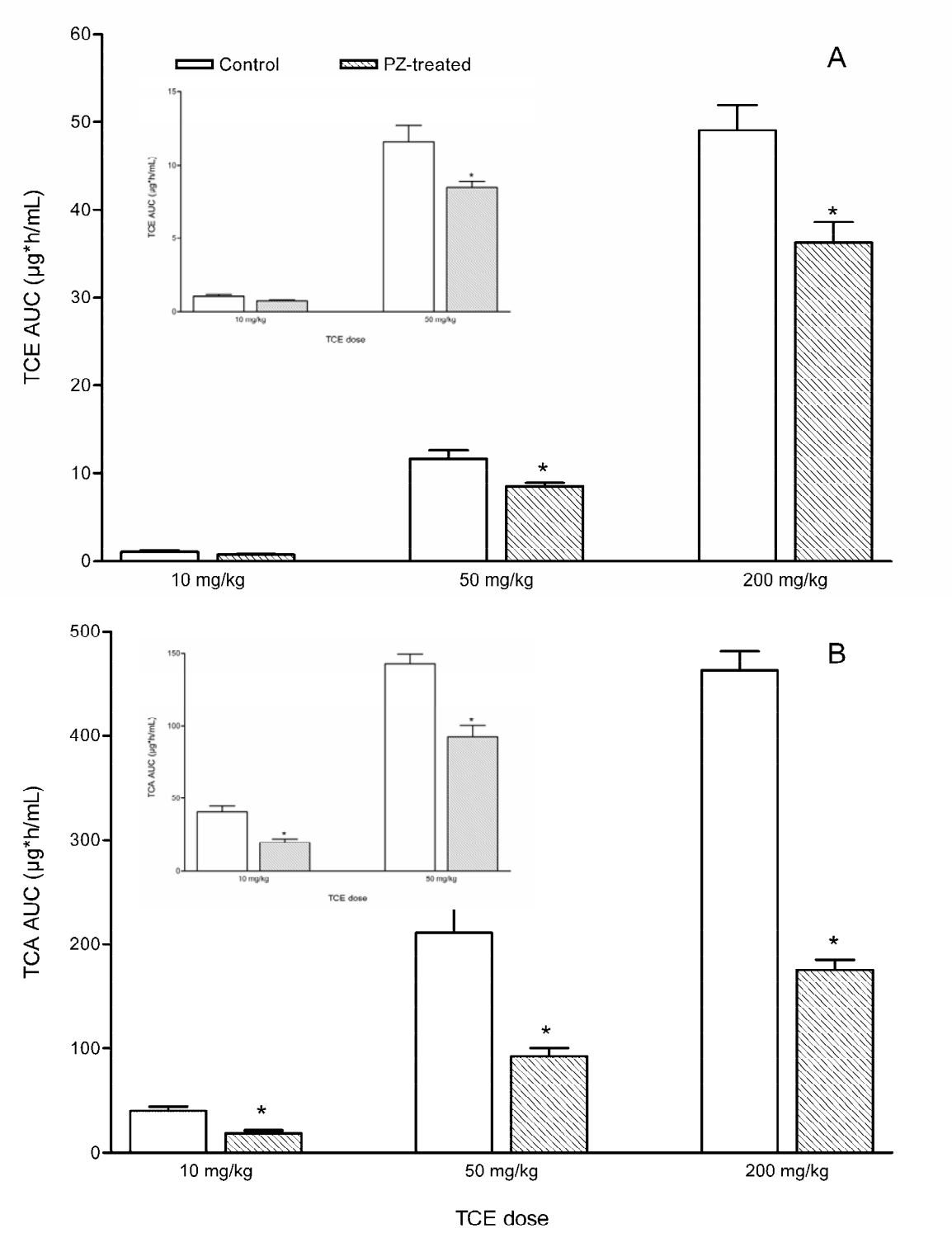
A
Control
PZ-treated
TCE AUC (µg*h/mL)
60
50
40
30
20
10
0
10 mg/kg
50 mg/kg
200 mg/kg
*
TCE AUC (µg*h/mL)
15
10
5
0
10 mg/kg
50 mg/kg
TCE dose
B
TCA AUC (µg*h/mL)
500
400
300
200
100
0
10 mg/kg
50 mg/kg
200 mg/kg
*
TCA AUC (µg*h/mL)
150
100
50
0
10 mg/kg
50 mg/kg
TCE dose
TCE dose

FIG. 3

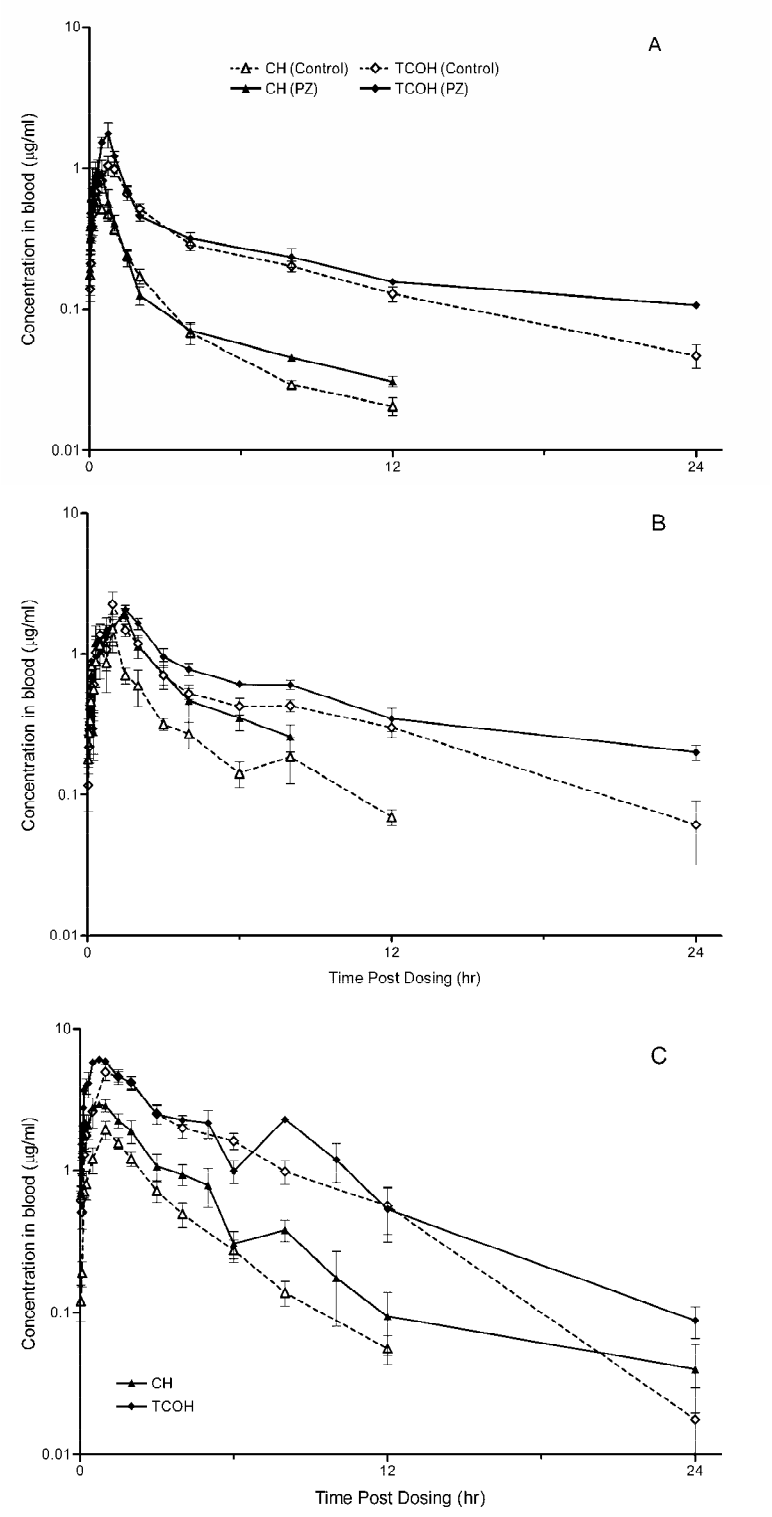
A
CH (Control)
TCOH (Control)
CH (PZ)
TCOH (PZ)
Concentration in blood (µg/ml)
10
1
0.1
0.01
0
12
24
B
Concentration in blood (µg/ml)
Time Post Dosing (hr)
C
CH
TCOH
Concentration in blood (µg/ml)
Time Post Dosing (hr)

FIG. 4.

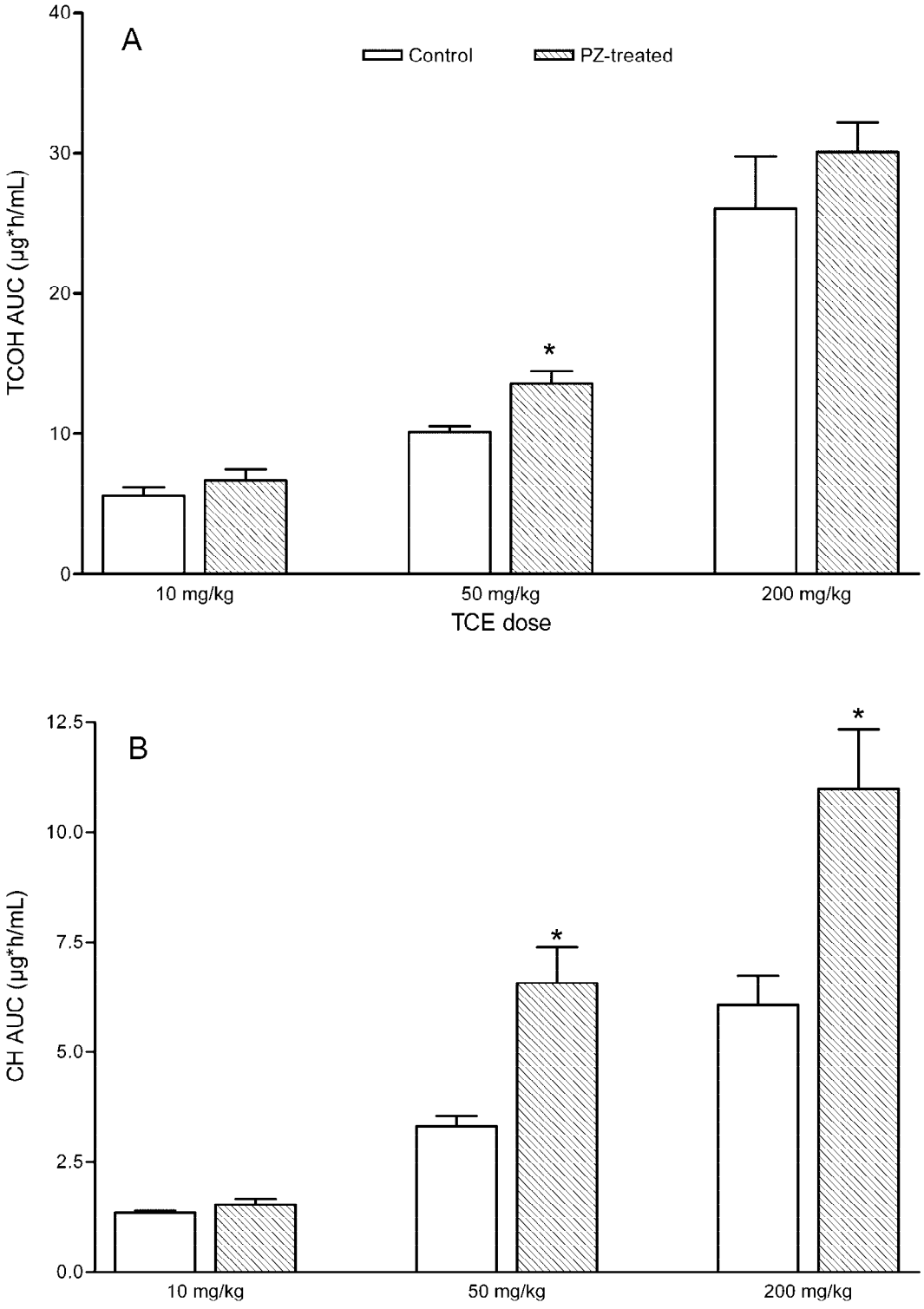
A
Control
PZ-treated
TCOH AUC (µg*h/mL)
40
30
20
10
0
*
10 mg/kg
50 mg/kg
200 mg/kg
TCE dose
B
CH AUC (µg*h/mL)
12.5
10.0
7.5
5.0
2.5
0.0
*
*
10 mg/kg
50 mg/kg
200 mg/kg

Table 1. TCE toxicokinetic parameter estimates for control and PZ-pretreated rats

	TCE AUC (μg*h/ml)		$T_{\beta 1/2}$ (h)		Cmax (μg/ml)		Tmax (min)	
TCE Dose (mg/kg)	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced
10	1.0 ± 0.2	0.7 ± 0.1	1.2^{a} ± 0.1	0.9^{a} ± 0.1	3.1 ± 0.7	2.3 ± 0.2	4.4 ± 1.3	2.8 ± 0.5
50	11.6^{A} ± 1.1	8.5^{B} ± 0.4	3.7^{b} ± 0.3	3.1^{b} ± 0.3	10.7 ± 1.1	11.1 ± 1.3	7.6 ± 1.2	7.0 ± 1.6
200	49.0^{A} ± 2.9	36.3^{B} ± 2.2	4.0 ± 3.5	4.0 ± 0.3	24.6^{A} ± 2.2	13.2^{B} ± 0.3	9.7 ± 2.2	4.4 ± 0.8

Male S-D rats were injected with saline (controls) or 200 mg PZ/kg i.p. for 3 days. They then were gavaged with 10, 50 or 200 mg TCE/kg. Serial micro-blood samples were taken from the freely-moving animals via a carotid artery cannula for 24 h post dosing and analyzed for their content of TCE and its metabolites (TCA, TCOH and CH) by headspace GC. Different lower case letters indicate a statistically significant difference between TCE dosage level values. Different upper case letters indicate a significant difference between control and PZ-pretreated group values ($p < 0.05$). Results are expressed as mean ± S.E. ($n = 4 \sim 5$).

Table 2. TCA toxicokinetic parameter estimates for control and PZ-pretreated rats

	TCA AUC (μg*h/ml)		$T_{\beta 1/2}$ (h)		Cmax (μg/ml)		Tmax (min)	
TCE Dose (mg/kg)	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced
10	$33.1^{A} \pm 2.6$	$19.3^{B} \pm 2.6$	$10.1^{A} \pm 0.3$	$5.7^{B} \pm 0.3$	1.8 ± 0.2	1.9 ± 0.3	$300^{A} \pm 54$	$84^{B} \pm 19$
50	$126.2^{A} \pm 10.0$	$84.6^{B} \pm 9.0$	$10.2^{A,b} \pm 1.0$	$6.3^{B} \pm 0.8$	$6.3^{A} \pm 1.6$	$12.1^{B} \pm 1.9$	$240^{A} \pm 0$	$128^{B} \pm 19$
200	$463.2^{A} \pm 18.0$	$175.5^{B} \pm 9.4$	$13.2^{A} \pm 0.6$	$4.2^{B} \pm 0.3$	$16.6^{A} \pm 1.2$	$23.6^{B} \pm 1.2$	$180^{A} \pm 0$	$138^{B} \pm 15$

Table 3. TCOH toxicokinetic parameter estimates for control and PZ-pretreated rats

	TCOH AUC (µg*h/ml)		$T_{\beta 1/2}$ (hr)		Cmax (µg/ml)		Tmax (min)	
TCE Dose (mg/kg)	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced
10	5.0 ± 0.5	5.4 ± 0.8	8.1 ± 0.5	7.1 ± 1.1	1.2 ± 0.1	1.9 ± 0.3	43 ± 6	39 ± 3
50	10.1^{A} ± 0.4	13.6^{B} ± 0.9	5.6 ± 0.5	6.5 ± 0.5	3.1 ± 0.6	2.6 ± 0.1	54 ± 4	60 ± 0
200	26.1 ± 3.7	30.1 ± 2.1	2.9 ± 0.2	4.1 ± 0.2	5.6 ± 0.4	6.2 ± 0.1	77 ± 9	51 ± 4

Table 4. CH toxicokinetic parameter estimates for control and PZ-pretreated rats

	CH AUC (μg*h/ml)		$T_{\beta 1/2}$ (hr)		Cmax (μg/ml)		Tmax (min)	
TCE Dose (mg/kg)	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced
10	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	2.2 ± 0.1	0.9 ± 0.1	1.2 ± 0.2	13 ± 3	17 ± 2
50	3.3^{A} ± 0.3	6.1^{B} ± 0.8	2.6 ± 0.3	2.3 ± 0.5	1.7 ± 0.2	2.3 ± 0.2	48 ± 6	33 ± 7
200	6.1^{A} ± 0.7	11.0^{B} ± 1.4	2.1 ± 0.1	2.7 ± 0.6	2.2 ± 0.2	3.0 ± 0.2	72 ± 6	48 ± 6

CHAPTER 3. Different TCA Toxicokinetics after TCE, TCOH and TCA administration Due to CYP2E1 Induction by Pyridazine in Male Sprague-Dawley Rats [2]

[2] S. Lee, C. A. White, S. Muralidhara, and J. V. Bruckner. To be submitted to Drug Metabolism and Disposition.

Different TCA Toxicokinetics after TCE, TCOH and TCA administration Due to CYP2E1 Induction by Pyridazine in Male Sprague-Dawley Rats

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Abbreviations:

TCA, trichloroacetic acid; TCE, trichloroethylene; PZ, pyridazine; S-D, Sprague-Dawley (rats); AAALAC, American Association for Accreditation of Laboratory Animal Care; AUC, area under the concentration versus time curve; TK, toxicokinetics.

ABSTRACT:

Trichloroacetatic acid (TCA) is a toxicologically important metabolite of 1,1,2-trichloroethylene (TCE) and perchloroethylene (PCE), as well as a byproduct of chlorination of drinking water. TCA is generally believed to be a proximate mouse hepatocarcinogen. Previous experiments revealed that pretreatment of male Sprague-Dawley (S-D) rats with pyridazine (PZ), a cytochrome P4502E1 (CYP2E1) inducer, resulted in a marked, unexpected increase in clearance of TCA from the bloodstream after the administration of TCE p.o. The objective of the current investigation was to determine the cause of this phenomenon, as well as to assess the influence of PZ on the toxicokinetics (TK) of TCA and trichloroethanol (TCOH), the other end metabolite of TCE.

Young (bw ≈ 200 g) male S-D rats were given 200 mg PZ/kg in saline i.p. for 3 consecutive days. Controls received i.p. saline injections. Groups of rats then received: 10 or 50 mg TCE/kg p.o.; 10 or 50 mg TCA/kg i.v.; 50 mg TCOH/kg i.v. Serial blood samples were then taken for up to 48 h and analyzed by headspace gas chromatography for their TCE, TCA and/or TCOH. Additional groups of PZ-pretreated and control rats were administered 10 or 50 mg TCA/kg i.v. and their urine collected for delineation of cumulative urinary TCA excretion. PZ pretreatment apparently did not significantly alter the *in vivo* metabolism of TK of TCOH. A portion of the TCOH dose was converted to TCA, but this was markedly inhibited by PZ. PZ had a profound impact on the TK of i.v. TCA, as evidenced by marked decreases from controls in TCA AUCs and shorter half-lives. These effects were found to be due largely to enhanced urinary TCA excretion. A

less pronounced increase at the higher (50 mg/kg i.v.) dose is suggestive of saturation of some processes, possibly transport of TCA from blood into the urinary filtrate by an organic anion transporter. The mechanism(s) by which TCA enters the urinary filtrate is unknown. The substantial decreases observed here in internal TCA are contrary to what would be anticipated with pretreatment with PZ, a CYP2E1 inducer. This phenomenon, if shared by other common CYP2E1 inducers, may have a profound impact on standard assumptions made by the U.S. EPA in its cancer risk assessments of TCE, PCE and other solvents that are metabolically activated by CYP2E1.

Introduction

Trichloroacetic acid (TCA) is presently of considerable interest to the scientific and regulatory communities. It is a toxicologically-significant end metabolites of the oxidative pathways fro 1,1,2-trhchloroethylene (TCE), perchloroethylene (PCE) and other chlorinated volatile organic chemical (VOC) environmental contaminants in rodents and humans (Green and Prout, 1985; Odum *et al.,* 1988; Lash et al., 2000). TCA, along with dichloroacetic acid (DCA) and chloroform are major byproducts of chlorination of drinking water (Weisel et al., 1999). TCA has been used as a soil sterilant and a selective herbicide for control of many annual and perennial grasses in agriculture. TCA is also used as an etching agent for metal surfaces, a solvent in the plastics industry, and even as an antiseptic, hemostatic, and keratolytic in medicine (Hoekstra, 2003; HSDB, 2002). TCA exposure is currently widespread in the U.S. It was found in 76 % of the urine specimens of 402 U.S. residents surveyed (Calafat et al., 2003).

The primary toxicological concern about TCA is its potential carcinogenicity. Results of a number of studies indicate that TCA and/or DCA are proximate B6C3F1 mouse liver carcinogen(s) in TCE-exposed animals (Bull, 2000; Bull et al., 2002). Bull et al. (1990) has previously shown that TCA produces liver adenomas and carcinomas in both sexes of B6C3F1 mice when given in their drinking water. Peroxisome proliferation is thought to be the major, non-genotoxic mechanism of action of TCA (Maloney and Waxman, 1999; Bull, 2000, NRC, 2006). Direct interaction of TCA with the nuclear proxisome proliferator-activated receptor alpha (PPARα) modifies signals, involving cell proliferation, inhibition and apoptosis, in different populations of hepatocytes. Transcript

profiling shows that 93 % of all gene expression changes in wild-type mice are dependent on PPARα, including gene involved in cell growth (Laughter et al., 2004). A lack of such concordance in PPARα-null mice led the researchers to conclude that activation of PPARα by TCA plays a dominant role in TCE-induced hepatocarcinogenesis. TCA is also known to induce DNA hypomethylation, which may increase oncogene expression (Ge et al., 2001; Tao et al., 1998).

Much about the toxicokinetics (TK) of TCA has been well characterized. Orally-administered TCA is rapidly and extensively absorbed by mice (Gonzalez-Leon *et al.,* 1999; Xu et al., 1995). TCA injected i.v. quickly exits the vasculature of rats, as reflected by a short distribution phase and rapid equilibration with tissues (Schultz et al., 1999; Yu et al, 2000). Schultz et al. report that TCA's steady-state volume of distribution approximates total body water. A number of research groups (e.g., Merdink et al., 1995) observe that TCA has a particularly long half-life in the blood of rodents. Substantial interspecies differences in half-life are described [e.g., 8 h after 65 mg TCA/kg iv in rats (Schultz et al, 1999) versus 51 h following 200 ppm TCE/kg oral exposure in humans (Fisher et al., 1998)]. The TCA binding capacity of human plasma *in vitro* is considerably higher than that of rat plasma. Mouse plasma TCA binding is much lower than that in the rat (Lumpkin et al., 2003; Templin et al., 1995).

TCA is slowly cleared from the systemic circulation due to a combination of factors including its strong plasma protein binding, large volume of distribution, poor metabolism, possible reabsorption from the bladder and/or urine, and enterohepatic recirculation of trichloroethanol (TCOH) and its conversion to TCA (Hobara et al., 1988; Lumpkin et al., 2003; Schultz et al., 1999; Stenner et al., 1997; Yu et al., 2000).

Relatively little, however, is known about TCA's elimination. Very little is excreted in the feces (Yu et al., 2000). Renal clearance appears to be moderate, though it is not known whether TCA is freely filtered in the glomerulus and/or serves as a substrate for active renal tubular transporters.

An investigation was recently conducted to elucidate the influence of pyridazine (PZ), a potent Cytochrome P450 2E1 (CYP2E1) inducer, on blood profiles of chloral hydrate (CH) and its major "downstream" metabolites, TCOH and TCA, in TCE-dosed rats (Lee et al., 2006). Blood concentrations of CH and TCOH were moderately increased in the PZ-pretreated rats that ingested moderate TCE doses. Unexpectedly, blood TCA concentrations in these animals diminished more rapidly to much lower levels than in controls. Such an effect could have important consequences in cancer risk assessments of TCE, PERC and TCA as TCA internal dosimetry serves as the basis of species-, dose- and route of exposure extrapolations. The objective of the current study is to clarify the effects of PZ on TCA and TCOH kinetics, in order to gain a better understanding of the basis for the marked reduction in TCA's bioavailability.

Materials and Methods

Chemicals: 1,1,2-Trichloroethylene (TCE) (> 99.9 % of purity); trichloroethanol (TCOH), trichloroacetic acid (TCA), chloral hydrate (CH) and dichloroacetic acid (DCA) (all > 99.9 % purity); and pyridazine (PZ) were purchased from Aldrich Chemical Co (Milwaukee, WI)). Isooctane (ACS spectrophotometric grade) was obtained from Sigma Aldrich (St. Louis, MO). Sulfuric acid and methanol were obtained from J.T. Baker (Phillipsburg, NJ) and Sigma Aldrich, respectively. Alkamuls EL-620® (formerly Emulphor®), a polyethoxylated vegetable oil supplied by Rhone-Poulenc (Cranbury, NJ), was used to prepare stable aqueous TCE emulsions.

Animals: Male Sprague-Dawley (S-D) rats of 100 – 125 g were purchased from Charles River Laboratories (Raleigh, NC). The animals were housed 2 rats per cage in their own limited-access room of an AAALAC-accredited animal facility. The room was maintained at 21°C and 50 ± 10 % humidity with a 12-h light/dark cycle. Full spectrum fluorescent lights were on daily from 0600 – 1800 h. The rats were supplied Purina Rat Chow No. 5001° and tap water *ad libitum* during an acclimation period of at least 1 week. The study protocol was approved by the University of Georgia Animal Care and Use Committee.

Dosage and Sample Collection Regimens: Groups of 6 male S-D rats were injected with 200 mg PZ/kg i.p. in saline for 3 days between 0900 and 1000 h. Kim and Novak (1993) reported this dosage regimen to produce a 4- to 5-fold increase in hepatic

microsomal *p*-nitrophenol (PNP) hydroxylase activity in male Harlan S-D rats. Our S-D rats from Charles River exhibited a 2.5-fold increase in PNP hydroxylation under these conditions (data not shown). Control rats were injected i.p. with saline for 3 consecutive days. A cocktail of ketamine HC1 (100 mg/ml): acepromazine maleate (20 mg/ml): xylazine HC1 (10 mg/ml) in a proportion of 3:2:1 (v/v/v) was then injected i.m. in a volume of 0.8 ml/kg to produce surgical anesthesia. An Indwelling PE-50 cannulae (OD = 0.97 mm, ID = 0.58 mm) were implanted into the left jugular vein for i.v. TCA or TCOH administration and into the left carotid artery for serial blood sampling from each animal on the third day, soon after i.p. injection of the last PZ/saline dose. Groups of rats given TCE orally had only a carotid cannula implanted installed. The cannulae were filled with heparinized saline (1,000 U/ml) to maintain their patency. An equivalent volume of heparinized saline was injected via the cannula after each sampling to replenish blood volumes. Each cannula was tunneled s.c. and exited at the nape of the neck, so the animal could move freely and serial blood samples be taken with a minimum of stress upon recovery. Food was withheld during a 24-h recovery period, but water was provided a*d libitum*.

Experiments were conducted to assess the influence of PZ-pretreatment on blood TCA concentration profiles. Data from a previous study (Lee et al., 2006) were used to illustrate the effect on PZ or the kinetics of TCA generated from TCE. PZ-pretreated and control groups had been gavaged with 10 or 50 mg TCE/kg in a 5 % aqueous Alkamuls EL-620® emulsion. In a second experiment, PZ and control groups received 10 or 50 mg TCA/kg in saline by injection into the jugular vein cannula. Other PZ-pretreated and control groups were given 50 mg TCOH/kg in saline i.v. in the third experiment. The

total dosing volume was 1 ml/kg for all the experiments. Serial arterial micro (10 to 50 μl) blood samples were taken from all the animals for 48 or 96 h post dosing and TCA concentrations quantified as described below. The estimation of TK parameters is also detailed below.

Cumulative urinary excretion of TCA was monitored in a separate study. Twenty rats were housed individually for a 2-day acclimation period in Nalgene 650-0100® metabolic cages for small rats. The cages were designed for separate collection of urine and feces. Food and water were available *ad libitum* during the 2 days of acclimation in the cages. Ten animals were injected with 200 mg/kg i.p. daily for 3 days. The others were given i.p. injections of saline for 3 days and served as controls. Soon after i.p. injection of the last PZ/saline dose, each animal was anesthesized, surgically implanted with a jugular vein cannula, as described before. Animals were placed back into metabolism cages upon recovery, food was withheld during a 24-h recovery period, but water was provided a*d libitum*.

One set of 5 control and 5 PZ-pretreated rats received 10 mg TCA/kg via the jugular cannula. Another set of 5 control and 5 PZ rats received 50 mg TCA/kg i.v. Voided urine was collected on ice from the individual animals 2, 4, 8, 12, 24, and 48 h post dosing (72 h was final urine collection time point for control groups given 50 mg TCA/kg i.v.). The volume for each collection period was recorded. The urine samples were transferred to 1.5 ml microfuge tubes and stored at – 80 ^{o}C until analysis. Access to food and water was provided during the collection period.

Sample Analyses. Urine samples were diluted with HPLC-grade water up to 1:1000. Less concentrated samples at later time-points were diluted 1:250 or 1:500. Blood and diluted urine samples were transferred to 20-ml gas chromatography (GC) headspace vials, containing 200 µl of esterification solution comprised of distilled water, concentrated sulfuric acid and methanol in a ratio of 6:5:1 (v/v/v), topped with aluminum caps with polytetrafluoroethylene (PTFE)-coated rubber septa and then tightly crimped. The contents were ultrasonicated for 1 min. The procedure converted TCA (and DCA, if any) in the blood and urine to their volatile methyl esters (Muralidhara and Bruckner, 1999). TCE, TCOH and CH were sufficiently volatile at the GC temperatures employed. TCE, TCA, DCA, CH and TCOH could thus be quantified in each 10 to 50-µl blood sample by headspace analysis. The vials were placed into a TurboMatrix 110® thermostat-controlled autosampler attached to a Perkin-Elmer Clarus 500 GC equipped with an electron capture detector. The GC headspace sampler was maintained at a constant 125° C. The temperature of the column was kept at 120° C for 3 min, then increased 25° C/min up to 170° C and maintained there for 3 min for each sample. The injector and detector temperatures were 200 and 360° C, respectively. Analyses were carried out on a 10′ X ⅛″ stainless steel column packed with a 10 % customized column coating of OV-17 (phenylpolysiloxane) on the 80/100-µm mesh size matrix Supelcoport® (Supelco Inc., Bellefonte, PA). Nitrogen was used as the carrier gas at 25 psi. TCA, DCA, CH and TCOH standards were prepared daily in HPLC grade water and analyzed concurrently with the blood samples. (There was no difference between the results of the standards with water or blood as matrices). Isooctane was utilized for TCE. The limits of detection and quantitation for each analyte were ~ 5 and 20 ng/ml, respectively.

Calculation of Kinetic Parameters: Blood TCA concentration versus time profiles were evaluated using WinNonlin Professional Version 4.1 (Pharsight Co., Mountain View, CA). The individual TCA time-courses of i.v.-dosed rats were analyzed by compartmental models using standard equations, for calculation of relevant parameters [i.e., terminal elimination half-life ($t_{\beta½}$), total body clearance (CL), volume of distribution (Vd) and area-under blood concentration versus time curves (AUCs)]. Individual TCA blood time-course profiles after TCE or TCOH administration were analyzed by noncompartmental methods using standard equations (Perrier and Gibaldi, 1982). Maximum blood concentrations (C_{max}) and times to C_{max} (T_{max}) were observed values. In TCOH i.v. administration studies, fraction of initial TCOH dose converted to TCA [(Fm ($_{TCOH \rightarrow TCA}$)] was calculated from the equation of Fm = {[TCA AUC (after TCOH i.v. administration, min*μmole/ml) X TCA CL (TCA i.v. administration, ml/min/kg)]}/ [TCOH i.v. dose, μmole/kg)]. TCOH formation clearance (CL_F), then was calculated from the equation of CL_F = Fm X CL_{TCOH}. In TCA urinary elimination experiments of TCA i.v. administration, fraction of initial dose excreted in urine (F_{Elim}) of TCA (of each animal) was calculated from [cumulative excreted amount of TCA in urine] divided by [dose X body weight].

Statistical Analyses: One- or two-way analysis of variance ANOVA was used to determine the statistical significance of differences in TK parameters as a function of PZ-treatment and dose, with $p < 0.05$ as the level of significance.

Results

PZ-pretreatment of rats has pronounced effects on blood TCA TK under a variety of exposure conditions. TK parameter estimates are listed in Table 1, while blood TCA time-courses are pictured in Fig 1, 3 and 4. A 5-fold increase in the oral TCE dose resulted in 3.5- and 6.4-fold increases of the TCA Cmax in control and PZ-treated animals, respectively (Table 1). PZ-pretreatment produced a doubling of the TCA Cmax at 50 mg TCE/kg p.o. The TCA Tmax values are significantly shorter in PZ-pretreated rats than controls at each TCE dosage-level. These shorter Tmax's and higher Cmax's are apparent in Fig. 1. It is also apparent here that TCA is eliminated more quickly from the bloodstream of the PZ-pretreated rats at each TCE dose. The overall influence of these changes on TCA AUC values is illustrated in Fig. 2.

The results of the TCA i.v. injection experiments were consistent with a PZ-induced increase in systemic TCA clearance. TCA $T_{\beta½}$ and clearance (CL) values in controls did not exhibit dose-dependence (Table 1). CL was significantly higher with increase in TCA dose in the PZ-treated than in the control groups. The PZ-pretreatment regimen resulted in a significant increase in clearance of both i.v. doses of TCA. This was reflected by TCA half-lives that were one-half as long as those in controls. The relatively rapid rate of TCA elimination in the PZ groups is obvious in Figs 3 A and B. The impact of PZ on TCA AUCs was even more prominent at 50 than at 10 mg TCA/kg i.v. (Fig 2), which is consistent with the observed non-linear increase in clearance.

PZ-pretreatment has a pronounced effect on blood TCA concentrations in rats given 50 mg TCOH/kg i.v. Blood TCA levels in control animals confirm that TCOH, an

end metabolite of TCE, is converted to TCA to some extent. The PZ treatment regimen substantially reduces the TCA Cmax and half-life in the TCOH-dosed animals (Table 1). The striking decrease from controls in TCA blood concentrations over time can be seen in Fig. 4A. There is a ~ 50 fold-reduction from controls in the PZ-group's TCA AUC value. TCOH fraction metabolized to TCA [Fm ($_{TCOH \rightarrow TCA}$)] and clearance formation (CL_F) in PZ-treated groups also showed 20- and 10-fold decreases from those of controls, respectively (Table 1). As described in the Discussion, this diminished formation of TCA from TCOH appears to be in tandem with increased renal clearance of TCA.

Assessment of urinary excretion of TCA reveals that PZ pretreatment did indeed accentuate this process. Cumulative urinary excretion plots of TCA in control (dashed lines) and PZ-pretreated (solid lines) rats given 10 or 50 mg TCA/kg, i.v. are shown in Figs. 5A and B. It appears here that the difference between control and pretreated animals is larger at the lower dosage-level. Examination of the data in Table 2 reveals that PZ-pretreatment resulted in ~ 3.6-fold and 1.8-fold increases in the amount of TCA excreted during the first 2 h in the 10 and 50 mg TCA/kg i.v. groups, respectively. A similar pattern is true for: the amount of TCA excreted during the initial 2 hr, expressed as % of initial dose administered; and the cumulative amount of TCA excreted during the 48-h monitoring period. TCA is eliminated more rapidly during the first four hours after dosing, but continuous to be excreted for the entire 48-h interval.

Discussion

A basic premise in toxicology is that induction of hepatic microsomal cytochrome P450s will result in potentiation of the hepatotoxicity (and possibly hepatocarcinogenicity) of a number of VOCs by increasing their metabolic activation to reactive, cytotoxic metabolites. Much of the support for this premise has come from *in vitro* experiments with liver microsomes. Researchers have reported potentiation of acute liver injury in rodents and humans subjected to a variety of CYP2E1 inducers before subsequent exposures to high doses of VOCs such as TCE, benzene and carbon tetrachloride ($CC1_4$) (Cornish and Adefuin, 1966; Folland et al., 1976; Manno et al., 1996; Marrubini et al., 2003). There have also been reports of the influence of CYP2E1 induction on urinary excretion of VOC metabolites (Kaneko et al., 1994; Kenyon et al., 1996). There are very few instances in which the influence of inducers on the time-courses of the parent compound and its key metabolites has been delineated. These internal dosimetry data (e.g., blood or target tissue AUCs and C_{max}'s) are essential for calculation of non-cancer and cancer risks.

The influence of pretreatment of rats with PZ on the blood time-courses of TCE and its key metabolites was recently characterized in our laboratory (Lee et al., 2006). This experimental approach resulted in an unexpected and heretofore unreported effect, namely a marked decrease in TCA AUC and C_{max} values in the CYP2E1-induced rats after high dose (200 mg/kg) of TCE exposure. CYP2E1 catalyzes the oxidation of TCE to a short-lived, ternary intermediate and/or epoxide and on the CH (Bull, 2000; Cai and Guengerich, 2000), but it is not clear what effect, if any, CYP2E1 induction has on

subsequent metabolic steps in the oxidative pathway or on metabolite binding and excretory processes. *In vivo* blood time-course data from the preceding study by Lee et al. (2006) indicates that PZ pretreatment of S-D rats results in small to moderate increases in formation of CH, TCOH and TCA from moderate TCE doses, although TCA is cleared much more rapidly from the bloodstream. Thus, the decision was made to further investigate the influence of PZ on TCA's systemic clearance and urinary elimination.

Findings in the current study clearly demonstrate that PZ pretreatment produces more rapid clearance of TCA from S-D rats' blood, as a result of more rapid and extensive urinary excretion of TCA. PZ-pretreatment results in ~ 2-fold reductions in TCA half-life and > 2-fold increases in CL at each TCA dosage-level (Table 1). The PZ-induced increase in clearance rate was somewhat larger for the higher (50 mg/kg) i.v. TCA dose. The PZ-induced increases, in the amount of TCA excreted during the first 2 h and the cumulative amount excreted over 48 h, were substantially greater at the lower (10 mg/kg) TCA dose. This finding is suggestive of a saturable process, whose capacity is being approached at the higher (50 mg/kg i.v.) dose of TCA. PZ may be acting by enhancing an organic anion transporter in renal tubules, but no information on this potential mechanism was located in the literature.

The TK of TCA is consistent with that of a mobile water-soluble compound. As TCA is fully charged at physiological pH, it would not be expected to diffuse across membranes. A substantial portion of i.v.-injected TCA quickly exited the vasculature of S-D rats in the present study. Shultz et al. (1999) and Yu et al. (2000) also observed a short, pronounced distribution phase and rapid equilibration with tissues of F-344 rats.

Halestrap and Price (1999) have shown that most mammalian cells have monocarboxylate/proton co-transporters that rapidly convey lactate, pyruvate, acetate and ketone bodies into cells. Such transporters were found in a variety of tissues including liver, kidney and brain, as well as skeletal, smooth and cardiac muscles. Conversely, knowledge about transport/export of such compounds from cells is negligible. Shultz et al. (1999) reported that TCA's steady-state volume of distribution approximated total body water in rats. We also observed that the estimated TCA volume of distribution (Vd) was unaffected by PZ treatment in both 10 and 50 mg TCA/kg i.v. (~ 0.5 and 0.75 L/kg, respectively). There was no evidence of dose-dependent TCA TK in our study, or in one in which F-344 rats were given 1, 10 or 50 mg TCA/kg i.v. by Yu et al. (2000).

TCA is poorly metabolized by rats (Lash et al., 2000). The majority of i.v. TCA is excreted unchanged in the urine. Fecal excretion by rats is minimal. No DCA or other metabolites were found in blood or urine, which is consistent with the previous studies (Temlin et al., 1995; Yu et al., 2000). It has been suggested that TCA is a source of DCA (Larson and Bull, 1992), but experimental evidence of this conversion is lacking. As much as 12 % of i.v. doses of TCA have been exhaled as CO_2 by mice and rats (Green and Prout, 1985; Styles et al., 1991). Nevertheless, it appears unlikely that PZ induction of TCA formation or biliary elimination would make a significant contribution to the marked increase in TCA clearance we observed.

Plasma protein binding is an important contributor to TCA's relatively long terminal half-life. Human plasma exhibits the highest TCA binding capacity and mouse plasma the lowest (Templin et al., 1995). Rat plasma binding capacity is intermediate, ranging from 38.6 % at TCA concentrations of 100 – 500 μM to 66.6 % at 0.1 μM *in*

vitro (Lumpkin et al., 2003). The partitioning of TCA from blood into tissues becomes more pronounced when administered doses of TCA or TCE are increased (Schultz et al., 1999; Lumpkin et al., 2006). TCA binding to tissues appears to be minimal. The latter research group report substantially higher TCA levels in blood than in liver, kidney or other tissues of rats gavaged with a wide range of TCE doses. It should be recognized that TCA bound to plasma proteins is not available for uptake into tissues or renal excretion. It is possible that renal organic anion transporters, if operative, may have a higher affinity for TCA than does albumin. If glomerular filtration is the mechanism of urinary excretion of TCA, only unbound TCA in blood would be available for filtration. Yu et al. (2000) opined that free TCA is indeed filtered in the glomeruli. Furthermore, they calculated with a kinetic model that unbound TCA molecules are filtered several times, due to subsequent re-absorption of some of them in the urinary tract (i.e., renal tubules and bladder). Data in support of this excretory mechanism are still lacking. If this process does occur, displacement of TCA from albumin by PZ could contribute to increased glomerular filtration and urinary excretion of TCA. It is not known, however, whether PZ binds to plasma proteins, nor whether its binding affinity for plasma proteins exceeds that of TCA.

PZ does not appear to affect the metabolism of TCOH *in vivo*, as there are no significant differences in TK parameters between PZ-treated and control groups given 50 mg TCOH/kg i.v. The blood TCOH elimination curves in the two groups are parallel. Some of the xenobiotic inducers of several other CYP isoforms, known as microsomal enzyme inducers (MEI), also induce certain uridine diphosphate (UDP)

glucuronosyltransferases [UGT] (Parkinson, 2001; Shelby and Klaassen, 2006), but no evidence was found in the literature that this is the case for CYP2E1 inducers, including PZ. In rats, glucuronides are preferentially excreted into the urine for aglycones with molecular weights less than 250 (e.g., TCOH) (Parkinson, 2001). Renal and biliary organic anion transport systems are responsible for secretion of such glucuronides into the urine and bile, respectively. Stenner et al. (1997) reported that biliary TCOH contributes significantly to blood TCA levels. β-Glucuronidase in the gut flora hydrolyzes the conjugate to release TCOH. The TCOH is reabsorbed, apparently converted back to CH, and oxidized to TCA. Our findings in Fig. 4 suggest the existence of the conversion process of TCOH to TCA. CH, however, was not observed at all after 50 mg TCOH/kg i.v. dosing in both control and PZ groups (Lee et al., 2006), suggesting TCOH was converted to TCA directly. In control groups, blood TCA levels in rats injected with 50 mg TCOH/kg i.v. increase for the first few h after TCOH dosing, remain constant for some 12 h, and decline slowly thereafter, where shows identical TK profiles from TCA i.v. administration. However, PZ pretreatment results in markedly lower TCA levels. It is evident that PZ interferes with formation of TCA from TCOH, and increases the urinary excretion of TCA, at the same time

In summary, effects of PZ on the kinetics of TCE's two major end-metabolites have been characterized in a previous study. PZ-pretreatment appeared to produce modest increases in blood levels of CH, TCOH and TCA (Lee et al., 2006). The increase in TCA was transient, in that TCA was cleared much more rapidly in the PZ groups than in controls. The current investigation provided insight into the increased clearance of

TCA from the systemic circulation. It was clearly shown that PZ markedly enhanced the urinary elimination of TCA. The process (es) by which TCA enters the urine is (are) unknown, so the mechanism by which PZ alters urinary TCA excretion remains to be determined. PZ had no apparent influence on TCOH metabolism or systemic clearance. TCOH conversion to TCA was markedly inhibited by PZ-pretreatment. The substantially lower internal doses (i.e., AUCs and Cmaxs) of TCA in TCE-exposed animals should have a substantial impact on liver cancer risk estimates, if the effect is shared by other common CYP2E1 inducers. Decreased internal doses of the proximate liver carcinogen are the opposite of what would be expected with pre-exposure to a CYP2E1 inducer.

Footnotes

This work was supported by DOE Cooperative Agreement DE-FC09-02CH11109. This work was presented at the annual SOT meeting in San Diego (2006) abstract number 729.

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References

Bull RJ (2000) Mode of action of liver tumor induction by trichloroethylene and its metabolites, trichloroacetate and dichloroacetate. *Environ Health Perspect* **108** (Suppl 2):241–259.

Bull RJ, Orner JA, Cheng RS, Stillwell L, Stauber AJ, Sasser LB, Lingohr BD, and Thrall BC (2002) Contribution of dichloroacetate and trichloroacetate to liver tumor induction in mice by trichloroethylene. *Toxicol Appl Pharmacol* **182**:55-65.

Bull RJ, Sanchez IM, Nelson MA, Larson JL, and Lansing AJ (1990) Liver tumor induction in B6C3F1 mice by dichloroacetate and trichloroacetate. *Toxicology* **63**:341-359.

Cai H and Guengerich FP (2000) Acylation of protein lysines by trichloroethylene oxide. *Chem Res Toxicol* **13**:327-335.

Calafat AM, Kuklenyik Z, Caudill SP, and Ashley DL (2003) Urinary levels of trichloroacetic acid, a disinfection by-product in chlorinated drinking water, in a human reference population. *Environ Health Perspect* **111**:151-154.

Cornish HH and Adefuin J (1966) Ethanol potentiation of halogenated aliphatic solvent toxicity. *Am Ind Hyg Assoc J* **27**:57-61.

Fisher JW, Mahle D, and Abbas R (1998) A human physiologically based pharmacokinetic model for trichloroethylene and its metabolites, trichloroacetic acid and free trichloroethanol. *Toxicol Appl Pharmacol* **152**: 339-59.

Folland DS, Schaffner W, Ginn HE, Crofford OB, and McMurray DR (1976) Carbon tetrachloride toxicity potentiated by isopropyl alcohol. Investigation of an industrial outbreak. *JAMA* **236**:1853-1856.

Ge R, Yang S, Kramer PM, Tao L, and Pereira MA (2001) The effect of dichloroacetic acid and trichloroacetic acid on DNA methylation and cell proliferation in B6C3F1 mice. *J Biochem Mol Toxicol* **15**:100-106.

Gonzalez-Leon A, Merdink JL, Bull RJ, and Schultz IR (1999) Effect of pre-treatment with dichloroacetic or trichloroacetic acid in drinking water on the pharmacokinetics of a subsequent challenge dose in B6C3F1 mice. *Chem Biol Interact* **123**:239-253.

Green T and Prout MS (1985) Species differences in the response to trichloroethylene II. Biotransformation in rats and mice. *Toxicol Appl Pharmacol* 79:401–411.

Halestrap AP and Price NT (1999) The proton-linked monocarboxylate transporter (MCT) family: Structure, function and regulation. *Biochem J* **343**:281-299.

Hobara T, Kobayashi H, Kawamoto T, Iwamoto S, and Sakai T (1988) The absorption of trichloroethylene and its metabolites from the urinary bladder of anesthetized dogs. *Toxicology* **48**:141-153.

Hoekstra EJ (2003) Review of concentrations and chemistry of trichloroacetate in the environment. *Chemosphere* **52**:355-369.

HSDB (2002) Hazardous Substances Data Bank. National Library of Medicine. Bethesda, MD. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

Kaneko T, Wang-PY, and Sato A (1994) Enzyme induced by ethanol differently affect the pharmacokinetics of trichloroethylene and 1,1,1-trichloroethane. *Occup Environ Med* **51**:113-119.

Kenyon EM, Kraichely RE, Hudson KT and Medinsky MA (1996) Differences in rates of benzene metabolism correlate with observed genotoxicity. *Toxicol Appl Pharmacol* **136**:49-56.

Kim SG and Novak RF (1993) The induction of cytochrome P4502E1 by nitrogen- and sulfur-containing heterocycles: Expression and molecular regulation. *Toxicol Appl Pharmacol* **120**:257-265.

Larson JL and Bull RJ (1992) Metabolism and lipoperoxidative activity of trichloroacetate and dichloroacetate in rats and mice. *Toxicol Appl Pharmacol* **115**:268-277.

Lash LH, Fisher JW, Lipscomb JC, and Parker JC (2000) Metabolism of trichloroethylene. *Environ Health Perspect* **108** (Suppl 2):177-200.

Laughter AR, Dunn CS, Swanson CL, Howroyd P, Cattley RC, and Corton JC (2004) Role of the peroxisome proliferator-activated receptor alpha (PPARα) in responses to trichloroethylene and metabolites, trichloroacetate and dichloroacetate in mouse liver. *Toxicology* **203**:83-98.

Lee S, White CA, Muralidhara S, and Bruckner JR (2006) Cytochrome P450 2E1 induction by pyridazine produces qualitative and quantitative changes in the metabolism of trichloroethylene to potentially hepatocarcinogenic metabolites. *Drug Metab Dispos* in preparation.

Lumpkin MH, Bruckner JV, Campbell JL, Dallas CE, White CA, and Fisher JW (2003) Plasma binding of trichloroacetic acid in mice, rats, and humans under cancer bioassay and environmental exposure conditions. *Drug Metab Dispos* **31**:1203-1207.

Lumpkin MH, White CA, Muralidhara S, Fisher JW, Dallas CE, and Bruckner JV (2006) Dose-, species-, and time-dependency of blood, liver and other tissue trichloroacetic acid levels as dosimeters of trichloroethylene exposure. *Toxicol Appl Pharmacol* submitted.

Maloney EK and Waxman DJ (1999) Trans-activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. *Toxicol Appl Pharmacol* **161**:209-218.

Manno M, Razzadore M, Grossi M, and Sbrana C (1996) Potentiation of occupational carbon tetrachloride toxicity by ethanol abuse. *Hum Exp Toxicol* **15**:294-300.

Marrubini G, Castoldi AF, Coccini T, and Manzo L (2003) Prolonged ethanol ingestion enhances benzene myelotoxicity and lowers urinary concentrations of benzene metabolite levels in CD-1 male mice. *Toxicol Sci* **75**:16-24.

Merdink JL, Gonzalez-Leon A, Bull RJ, and Schultz IR (1998) The extent of dichloroacetate formation from trichloroethylene, chloral hydrate, trichloroacetate, and trichloroethanol in B6C3F1 mice. *Toxicol Sci* **45**:33-41.

Muralidhara S and Bruckner JV (1999) Simple method for rapid measurement of trichloroethylene and its major metabolites in biological samples. *J Chromatogr B Biomed Sci Appl* **732**:145-53.

NRC (2006) Assessing the Human Health Risks of Trichloroethylene: Kay Scientific Issues. National Research Council, The National Academy Press, Washington, DC.

Odum J, Green T, Foster JR, and Hext PM (1988) The role of trichloracetic acid and peroxisome proliferation in the differences in carcinogenicity of perchloroethylene in the mouse and rat. *Toxicol Appl Pharmacol* **92**:103-112.

Parkinson A (2001) Biotransformation of xenobiotics, in *Casarette and Doull's Toxicology: The Basic Science of Poisons*, 6th ed (Klaassen CD ed) pp 197-200, McGraw-Hill, New York.

Schultz IR, Merdink JL, Gonzalez-Leon A, and Bull RJ (1999) Comparative toxicokinetics of chlorinated and brominated haloacetates in F344 rats. *Toxicol Appl Pharmacol* **158**:103-114.

Shelby MK and Klaassen CD (2006) Induction of rat UDP-glucuronosyltransferases in liver and duodenum by microsomal enzyme inducers that activate various transcriptional pathways. *Drug Metab Dispos* **34**:1772-1778.

Stenner RD, Merdink JL, Stevens DK, Springer DL, and Bull RJ (1997) Enterohepatic recirculation of trichloroethanol glucuronide as a significant source of trichloroacetic acid. metabolites of trichloroethylene. *Drug Metab Dispos* **25**:529-535.

Styles JA, Wyatt I, and Coutts C (1991) Trichloroacetic acid: Studies on uptake and effects on hepatic DNA and liver growth in mouse. *Carcinogenesis* **12**:1715-1719.

Tao L, Kramer PM, Ge R, and Pereira MA (1998) Effect of dichloroacetic acid and trichloroacetic acid on DNA methylation in liver and tumors of female B6C3F1 mice. *Toxicol Sci* **43**:139-144.

Templin MV, Stevens D, Stenner RD, Bonate P, Tuman D, and Bull RJ (1995) Factors affecting species differences in the kinetics of metabolites of trichloroethylene. *J Toxicol Environ Health* **44**:435–447.

Xu G, Stevens DK, and Bull RJ (1995) Metabolism of bromodichloroacetate in B6C3F1 mice. *Drug Metab Dispos* **23**:1412-1416.

Weisel CP, Kim H, Haltmeier P, and Klotz JB (1999) Exposure estimates to disinfection by-products of chlorinated drinking water. *Environ Health Perspect* **107**:103-110.

Yu KO, Barton HA, Mahle DA, and Frazier JM (2000) In vivo kinetics of trichloroacetate in male Fischer 344 rats. *Toxicol Sci* **54**:302–311.

Legends for figures

FIG. 1. Blood TCA concentrations in S-D rats following oral administration of 10 mg (squares) or 50 mg (circles) TCE/kg. The PZ-pretreated groups (solid lines) received 200 mg PZ/kg i.p. for 3 days before their TCE ingestion. Controls (dashed lines) were injected with saline i.p. for 3 days. Symbols represent mean concentrations (μg/ml) ± S.E. (n = 4 ~ 5). Lines were drawn point to point to help distinguish the elimination curves. Some S.E.'s were too small to be visible.

Fig. 2. TCA areas under the blood concentration versus time curves (AUCs) in control S-D rats (clear bars) and groups of rats pretreated (shaded bars) with 200 mg PZ/kg i.p for 3 days before subsequent dosing with 10 or 50 mg TCA/kg i.v.; 10 or 50 mg TCE/kg p.o.; or 50 mg TCOH/kg i.v. Bar heights represent means (μg*h/ml) ± S.E. for groups of 4 or 5 rats. Asterisks represent statistically significant differences between controls and PZ-pretreated groups. The mean TCA AUC value for PZ-pretreated TCOH group is not visible on this scale (2.5 ± 0.3 μg*h/ml). Insets show TCA AUC's values from 10 and 50 mg TCE/kg p.o.; 50 mg TCOH/kg i.v. administration more clearly.

FIG. 3. Blood TCA concentrations versus time curves of groups of S-D rats injected with (A) 10 or (B) 50 mg TCA/kg i.v. PZ-treated groups (solid lines) received 200 mg PZ/kg i.p. for 3 days before TCA administration. Controls (dashed lines) were injected with saline i.p. for 3 days. Symbols represent means (μg/ml) ± S.E. (n = 4 ~ 5).

Lines were drawn point to point to help visualize the elimination curves. Some S.E.'s were too small to be visible.

FIG. 4. Blood concentration time-profiles of (A) TCA and (B) free TCOH in S-D rats given 50 mg TCOH/kg i.v. The pretreated groups (solid lines) received 200 mg PZ/kg i.p. for 3 consecutive days. Controls (dashed lines) were similarly received saline. Symbols represent means (μg/ml) ± S.E. (n = 4 ~ 5). Lines were drawn point to point to help identify the elimination curves.

FIG. 5. Cumulative urinary TCA excretion curves for groups of S-D rats injected with (A) 10 or (B) 50 mg TCA/kg i.v. The PZ-pretreated groups (solid lines) received 200 mg PZ/kg i.p. for 3 consecutive days and controls (dashed lines) were similarly received saline. Cumulative TCA excretion is presented as % of the initial dose administered. Each point represents the means ± S.E. for a group of 4 or 5 rats. Lines were connected between the mean values to help distinguish one accumulation curve from another. Some S.E.'s were too small to be visible.

Figures and Tables

FIG 1.

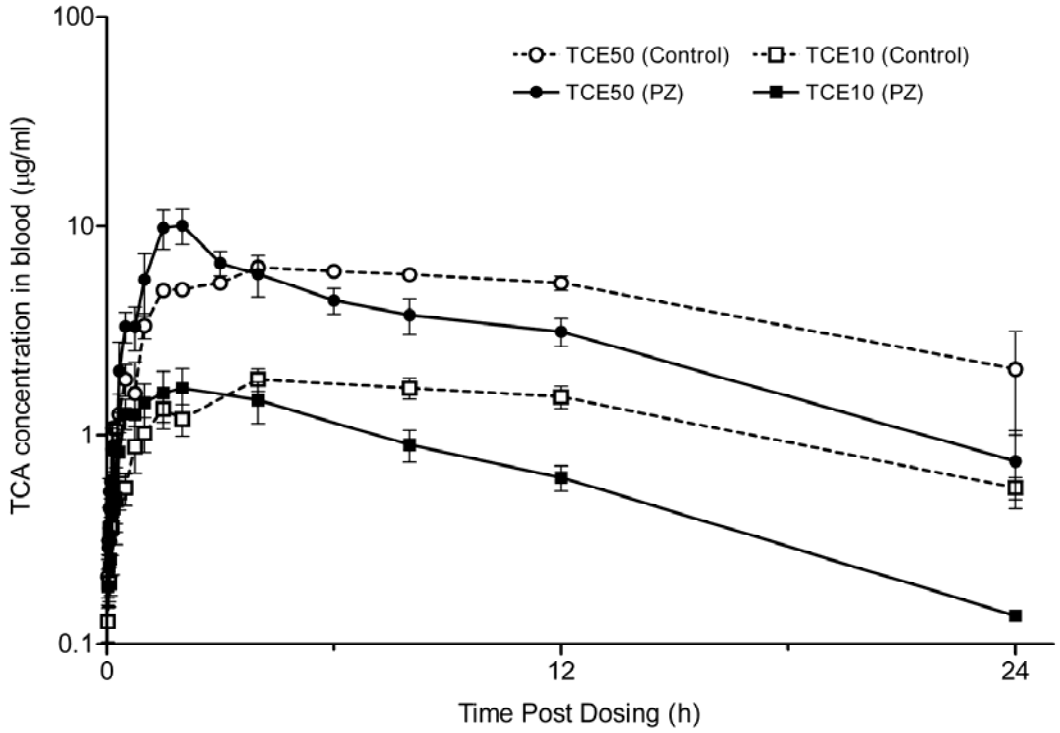

FIG 2.

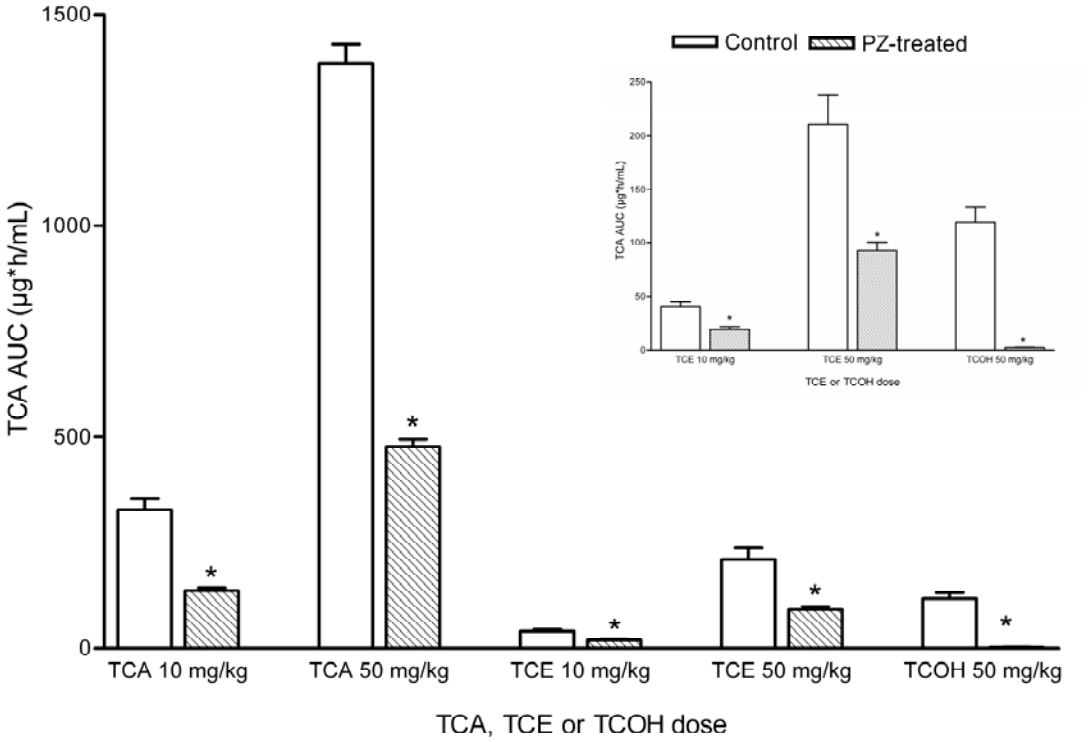

FIG 3.

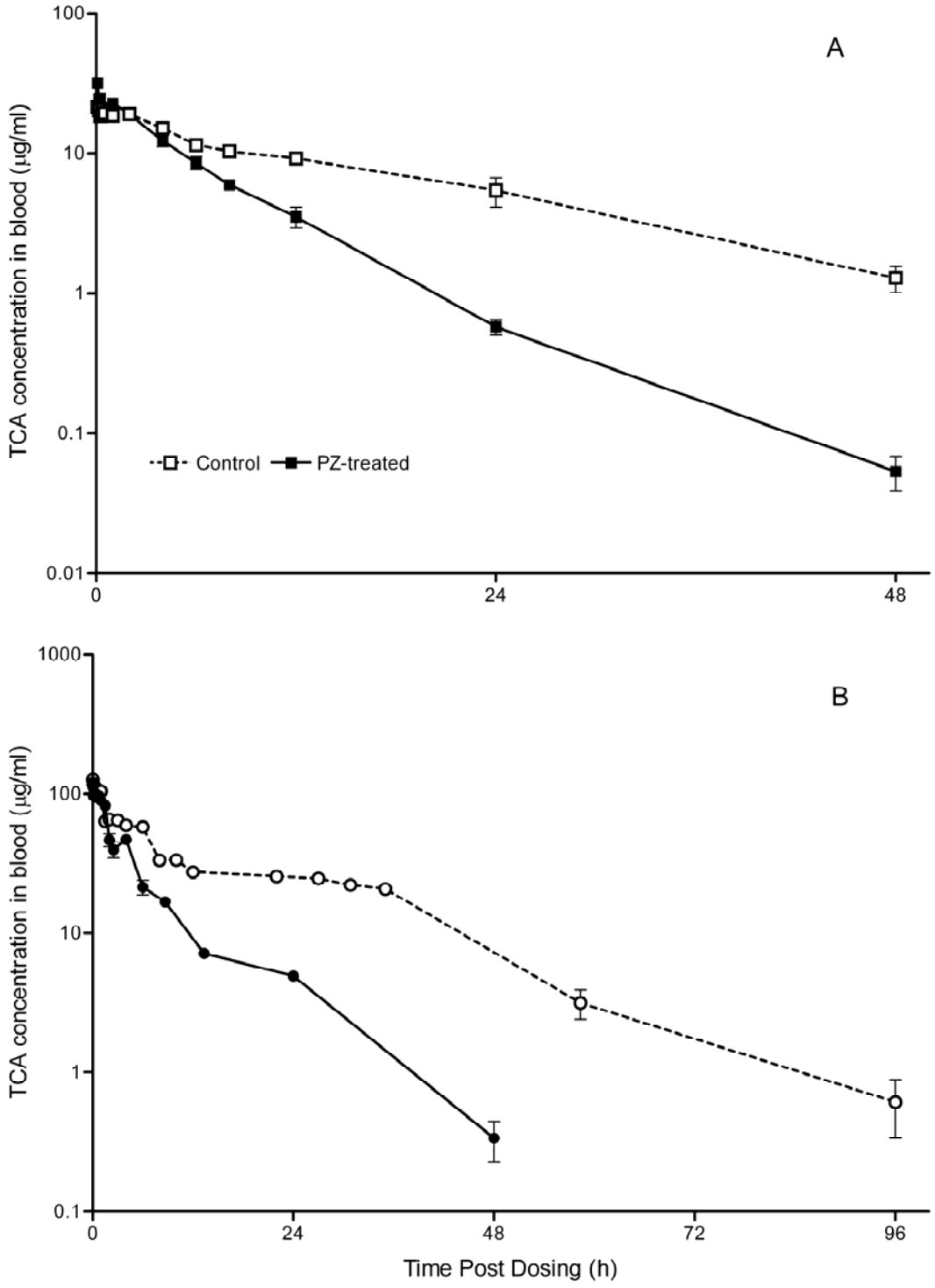
A
B
TCA concentration in blood (µg/ml)
Control
PZ-treated
Time Post Dosing (h)
100
10
1
0.1
0.01
1000
0
24
48
72
96

FIG 4.

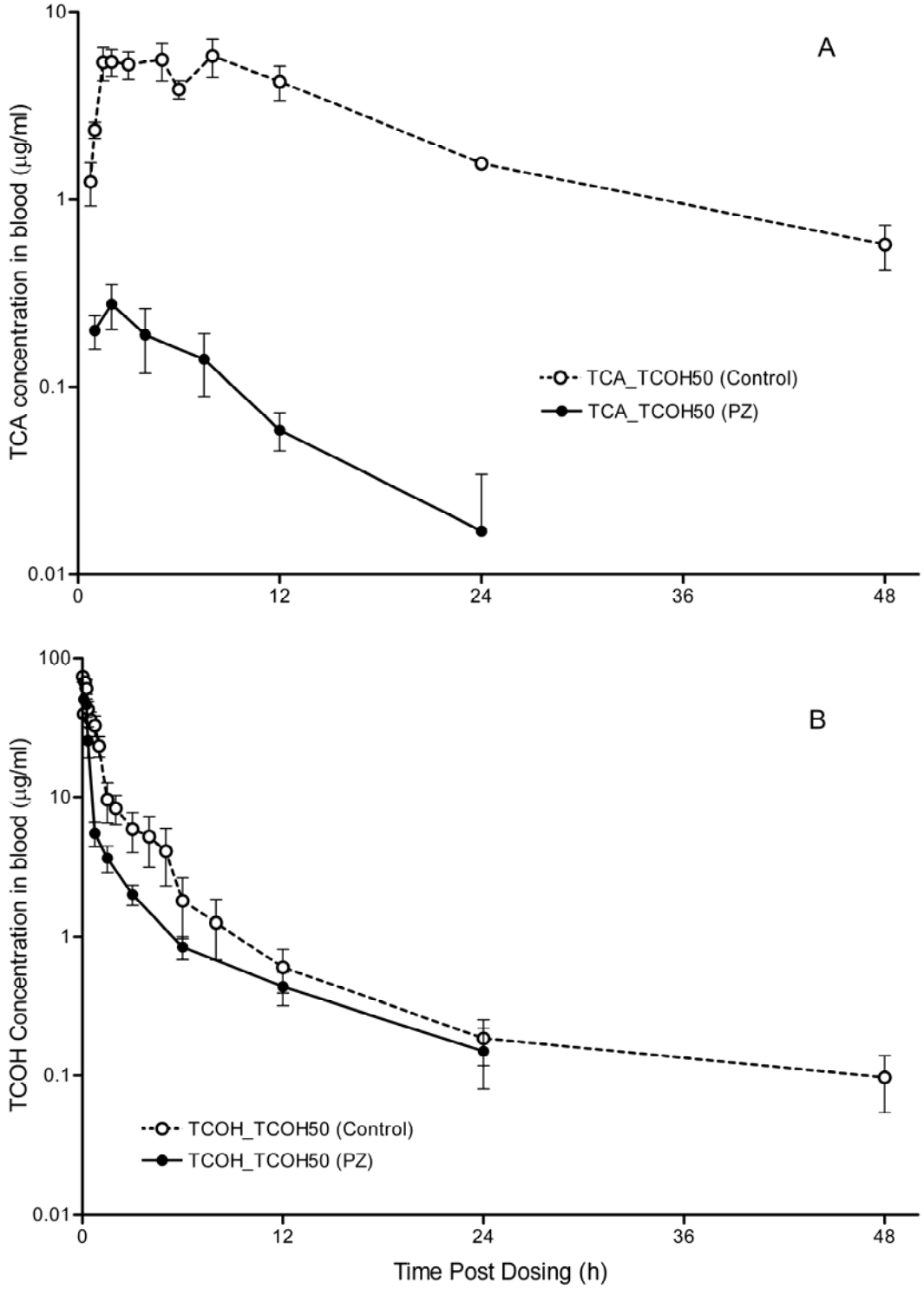
A
TCA concentration in blood (µg/ml)
10
1
0.1
0.01
0
12
24
36
48
TCA_TCOH50 (Control)
TCA_TCOH50 (PZ)
B
TCOH Concentration in blood (µg/ml)
100
10
1
0.1
0.01
0
12
24
36
48
TCOH_TCOH50 (Control)
TCOH_TCOH50 (PZ)
Time Post Dosing (h)

FIG 5.

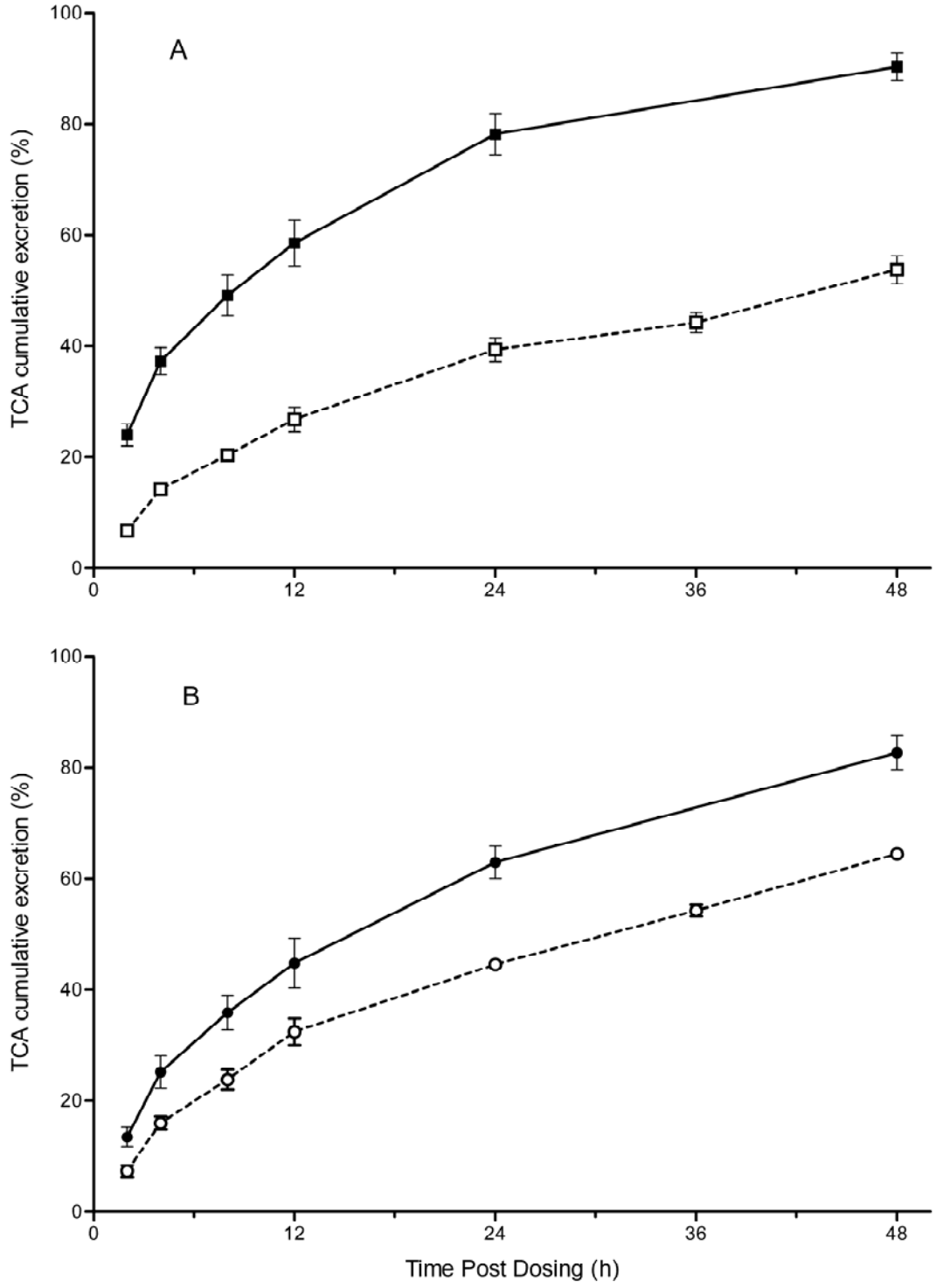
A
B
TCA cumulative excretion (%)
TCA cumulative excretion (%)
0
20
40
60
80
100
0
12
24
36
48
Time Post Dosing (h)

Table 1. Blood TCA TK parameter estimates in PZ-pretreated and control rats

Dosage Level	$T_{\beta 1/2}$ (hr)		CL/CL_F (ml/min/kg)		C_{max} (μg/ml)		T_{max} (min)	
	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced
TCA i.v. 10 mg/kg	$13.0^A \pm 1.0$	$5.6^B \pm 0.5$	$0.5^A \pm 0.1$	$1.2^B \pm 0.1$	23.1 ± 1.9	29.6 ± 2.0	ND	ND
TCA i.v. 50 mg/kg	$14.5^A \pm 0.1$	$7.5^B \pm 1.1$	$0.6^A \pm 0.1$	$1.8^B \pm 0.1$	117.0 ± 7.1	121.0 ± 8.4	ND	ND
TCE p.o. 10 mg/kg	$10.1^A \pm 0.7$	$5.7^B \pm 0.3$	NA	NA	1.8 ± 0.2	1.9 ± 0.3	$300^A \pm 54$	$84^B \pm 19$
TCE p.o. 50 mg/kg	$10.2^A \pm 1.0$	$6.3^B \pm 0.8$	NA	NA	6.3 ± 0.1	12.1 ± 1.9	$240^A \pm 0$	$128^B \pm 19$
TCOH i.v. 50 mg/kg	$14.5^A \pm 0.7$	$4.9^B \pm 0.7$	$4.7^A \pm 0.7$	$0.4^B \pm 0.1$	8.0 ± 0.4	0.4 ± 0.1	$330^A \pm 62$	$105^B \pm 15$

Male S-D rats were injected with saline (controls) or 200 mg PZ/kg i.p. for 3 days. On the third day, a carotid artery and/or a jugular venous cannula were implanted surgically and the animals allowed to recover for 24 h. Groups were then gavaged with 10 or 50 mg TCE/kg. Other groups received 10 or 50 mg TCA/kg i.v. The last group of animals was injected i.v. with 50 mg TCOH/kg. Serial micro-blood samples were taken from the arterial cannula of each rat for up to 96 h post dosing and analyzed for their TCA content by headspace GC. Different lower case letters indicate a statistically significant difference between TCA or TCE dosage level values. Different upper case letters indicate a significant difference between control and PZ-pretreated group values ($p < 0.05$). CL of TCOH i.v. dosing groups indicates CL_F. Results are expressed as mean ± S.E. for groups of 4 or 5 rats.

Table 2. Comparison of the urine profiles between the controls and PZ-pretreated groups after 10 or 50 mg TCA/kg, i.v. administration in male S-D rats

	Excreted amount of TCA in urine (μg, 2 h)		Excreted amount of TCA in urine (%, 2 h)		Cumulative excreted amount of TCA in urine (μg, 48 h)		F_{Elim} in urine (48 h)	
	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced	Control	PZ-induced
TCA i.v. 10 mg/kg	108.9 ± 11	391.1 ± 30	6.76^{A} ± 0.7	$24.0^{B,a}$ ± 2.0	866.4 ± 49	1475.3 ± 53	0.54^{A} ± 0.03	0.90^{B} ± 0.03
TCA i.v. 50 mg/kg	628.3 ± 90	1167.0 ± 145	7.34^{A} ± 1.0	$13.5^{B,b}$ ± 1.7	5513.4 ± 88	7257.5 ± 324	0.64^{A} ± 0.01	0.83^{B} ± 0.02

Male S-D rats were housed individually in metabolism cages and injected with saline (controls) or 200 mg PZ/kg ip for 3 days. Different groups then received 10 or 50 mg TCA/kg in saline i.v. Voided urine samples were collected and their volumes measured 2, 4, 8, 12, 24 and 48 h post dosing (urine samples of control groups given 50 mg TCA/kg i.v. were collected until 72 h post dosing. TCA concentrations were measured by headspace GC. The values for the cumulative excreted amount of TCA in urine (μg) and F_{Elim} in urine of control groups for 72 h after i.v. administration of 50 mg TCA/kg were 7757.8 ± 132.4 and 0.91 ± 0.02, respectively. Different lower case letters indicate a statistically significant difference between TCA dosage groups. Different upper case letters indicate a significant difference between control and PZ-pretreated group values ($p < 0.05$). Results are expressed as mean ± S.E. for groups of 4 or 5 rats.

CHAPTER 4. SUMMARY AND CONCLUSIONS

Trichloroethylene (TCE) is a common environmental contaminant at many hazardous waste sites around the country. Issues associated with the potential carcinogenicity of TCE and its metabolites have been debated for the past couple decades and determining the human relevance of animal carcinogenicity data and applying them to risk assessment of TCE and its metabolites has been controversial ever since. Assessing cancer risks of trace environmentally-relevant levels of TCE also has been a subject of major public health debate and scientific topic of interest. Assessment of TCE human health risks is challenging because of its inherently complex metabolism, toxicokinetics (TK) and mode(s) of action (MOA) and the widely varying perspectives on many critical scientific issues.

Because of this range of issues, the U.S. Environmental Protection Agency (EPA) solicited scientific input in development of 2001 draft health risk assessment of TCE, which was aimed at embracing diverse perspectives. Its efforts culminated with 16 state-of-the-science (SOS) articles, published together as an Environmental Health Perspectives Supplement in 2000. Since that time, a significant amount of new data has been accumulated. Nonetheless, a number of controversial scientific issues relevant to assessing TCE health risks remain debatable, including the pharmacokinetics of TCE and its metabolites, mode(s) of action and effects of TCE metabolites, and TCE cancer

epidemiology. Among them, the role of different susceptibilities of individuals and/or subpopulations in TCE health risks assessments were set to be investigated, since the correlation has been well established between the different TCE susceptibilities and smoking, alcohol consumption, or medications (aspirin). Population with diabetes, different genetic factors, as well as different ages can also exhibit different susceptibilities.

The main objective of this dissertation was to provide specific CYP2E1 induction models which could help explain different susceptibilities in TCE exposure. Thus, the animal experiments using CYP2E1 induction models with specific inducers in young male S-D rats were designed to account for the various physiological/pathological conditions in environmentally relevant low-level TCE exposure scenarios.

It is believed in general that induction of enzymes (one of many causes leading to differences in susceptibility) responsible for metabolic activation of xenobiotic substrates would lead to increase the rate and extent of the metabolism *in vitro* and *in vivo* (though, many physiological parameters can alter these effects *in vivo*). The induction may result in increased formation of reactive metabolites and subsequently increased potential of toxicity, which was presumed also to be applicable to high doses of TCE and other halocarbons exposure. There are emerging evidences that the effects of CYP2E1 inducers on moderate to high doses of TCE and other well-metabolized VOCs may not be applicable to low-dose exposure situations. Thus, in case of the environmentally relevant low-level TCE exposure, it was hypothesized that CYP2E1 induction would not result in the increased formation of carcinogenic metabolites (TCA and/or DCA), because TCE

itself is a well metabolized compound, and the enzymatic capacity of CYP2E1 is inherently in excess compared with the trace levels of TCE (flow limited), thus, CYP2E1 induction would not increase subsequent cancer risks from low-level TCE exposure.

The specific aims of this dissertation were, therefore, primarily to generate relevant data from PZ induction model on 1) the changes in TCE toxicokinetics after TCE administration, 2) the changes of toxicokinetic profiles of TCE metabolites (especially, TCOH and TCA), and its extrapolation for future application in TCE and TCA risk assessments.

Following the literature review in Chapter 1, the effects of PZ-induction of CYP2E1 on TCE metabolism were discussed in Chapter 2, by comparing the toxicokinetic parameter estimates of TCE and its metabolites (TCOH and TCA) between the control and PZ-induced animals after TCE administration with different doses (10, 50 and 200 mg/kg p.o.). A key finding of the study in Chapter 2 was the lack of significant effects of the pretreatment of PZ, a known CYP2E1 inducer, on TCOH and CH at the lowest (10 mg/kg) TCE dosage (where AUC or C_{max} values were not affected). These very slight changes at 10 mg/kg TCE i.v. would likely disappear at even lower environmentally relevant TCE doses. The lack of a significant effect of CYP2E1 induction on biotransformation of 10 mg TCE/kg was also manifested by the absence of alterations of TCE kinetic parameters. But the most unexpected findings of the study in Chapter 2 came from the changes in TCA TK in PZ groups. As stated, it was expected that CYP2E1 induction would also result in increased TCA formation (manifested by the

parameters, such as higher blood TCA AUC). Blood TCA concentrations were significantly higher (reflected by the higher C_{max} and shorter T_{max} values) in PZ induced animals than in controls during the early time points in the groups ingesting 10 and 50 mg TCE/kg. However, this pattern was not evident for the PZ-pretreated and control groups with 200 mg TCE/kg p.o., exhibited by the ~ 18-fold decrease of TCA AUC in PZ-groups from that of controls. Furthermore, elimination half-life ($T_{\beta½}$) of TCA in PZ groups was decreased by ~ 14-fold. Interestingly, blood concentrations of DCA, was consistently quantifiable only in PZ-pretreatment group at 200 mg TCE/kg p.o.

In Chapter 3, the influence of PZ-induction of CYP2E1 (which seems to bear no direct relevance, at first glance) on TCA toxicokinetics after the administration of three different substrates (i.e., TCE, TCOH and TCA), and its significance in TCE and TCA cancer risk assessments were discussed. The data indicated that the increase in TCA was transient, with TCA being cleared much more rapidly in PZ groups than in controls, while PZ-pretreatment appeared to produce modest changes in blood levels of CH, TCOH and TCA after 10 or 50 mg TCE/kg p.o. But, most significantly, the study described in Chapter 3 provided insight into the increased clearance of TCA from the systemic circulation, where data clearly demonstrated that PZ markedly enhanced TCA systemic clearance as well as the urinary elimination of TCA. The mechanism(s) by which TCA enters the urine is (are) unknown, although the filtration process is believed to account for significant portion of TCA excretion, thus how PZ (or other inducers) interact with urinary TCA excretion remains to be investigated. While, PZ had no

apparent influence on TCOH metabolism or systemic clearance, TCOH-derived formation of TCA was markedly interfered by PZ-pretreatment.

The EPA's standard default policy, in the absence of adequate experimental evidences to the contrary, has been to utilize a linear, multistage model to extrapolate from high-dose rodent cancer bioassay data to predict human cancer risks from environmental exposures. This very conservative approach assumes there is no threshold dose for cancer causation and results in high cancer risk estimates. However, as demonstrated by the studies in Chapters 2 and 3, this traditional, conservative approach can not always be justified even in case of the induction model, where increased formation of reactive metabolites and subsequently increased potential of toxicity were assumed. These are some of the emerging evidences that the effects of the exposures to moderate to high doses of TCE and other well-metabolized VOCs may not be applicable to low-dose exposure scenarios. The substantially reduced internal dosimetry of TCA (represented by changes in toxicokinetic parameters, such as, AUC, Cmax, Tmax, etc) due to PZ-pretreatment in TCE-exposed animals should have a significant impact on liver cancer risk estimates, if the effect is shared by other common CYP2E1 inducers (e.g., acetone). Decreased internal doses of the proximate liver carcinogen are the opposite of what would be expected with pre-exposure to a CYP2E1 inducer.

Better understanding of TCE metabolism especially at the low concentration under the induction models remains to be further investigated, which is critical for the elucidation of the susceptibility, and extrapolation of animal data to humans at low level

exposures. These induction models can be applicable to the broad range of halogenated hydrocarbons and other small VOCs in near future, where more pertinent potential applications in environments regulation can be utilized. As the roles of physiologically-based pharmacokinetic (PBPK) models in the application of TCE health risks assessments are important as ever, generating more relevant and accurate induction data from *in vivo* animal studies should be forthcoming.

Appendix A. Data for Chapter 2

Appendix A-1a. Blood PK monitoring of control & TCE (10 mg/kg, po): Feb. 19, 2005

TCE Conc (μg/mL)

Time	animal number							
min	B	C	D	E	F	Ave	STDEV	SEM
2	3.0328	0.7672	2.6635	0.9894	1.7673	1.8440	0.9976	0.4073
4	2.0684	1.6203	4.5097	0.8245	5.0367	2.8119	1.8543	0.7570
6	2.5452	1.5666	4.1727	0.5628	5.1084	2.7911	1.8577	0.7584
8	1.8569	1.6418	2.5882	0.3119	2.7711	1.8340	0.9748	0.3980
10	1.4841	1.0002	1.3694	0.2043	2.6886	1.3493	0.9007	0.3677
15	0.7041	0.4890	1.7049	0.1477	1.8655	0.9822	0.7615	0.3109
20	0.6324	0.4359	0.6109	0.0832	1.3020	0.6129	0.4436	0.1811
30	0.5951	0.2323	0.5392	0.0846	0.8733	0.4649	0.3114	0.1271
45	0.3140	0.1606	0.4632	0.0660	0.7413	0.3490	0.2664	0.1087
60	0.3241	0.1792	0.3212	0.0803	0.5363	0.2882	0.1725	0.0704
90	0.2122	0.1233	0.1706	0.0000	0.2466	0.1506	0.0960	0.0392
120	0.2280	0.0889	0.1534	0.0000	0.1907	0.1322	0.0900	0.0367
180	0.0760	0.0545	0.1190	0.0000	0.1921	0.0883	0.0721	0.0295
240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

CH Conc (μg/mL)

min	B	C	D	E	F	Ave	STDEV	SEM
2	0.2800	0.1001	0.1581	0.3127	0.0232	0.1748	0.1214	0.0495
4	0.2198	0.3388	0.4650	0.7363	0.1712	0.3862	0.2264	0.0924
6	0.8727	0.5702	0.6718	0.7240	0.2670	0.6211	0.2261	0.0923
8	0.9924	0.5622	0.4977	0.7574	0.3627	0.6345	0.2455	0.1002
10	0.7646	0.3787	1.1680	0.7030	0.5470	0.7122	0.2954	0.1206
15	0.8079	0.1924	0.8656	0.6663	0.6329	0.6330	0.2645	0.1080
20	0.5307	0.8700	0.3073	0.4515	0.7150	0.5749	0.2211	0.0902
30	0.5287	0.5528	0.3740	0.4660	0.6367	0.5116	0.0983	0.0401
45	0.4382	0.2989	0.6050	0.4411	0.5818	0.4730	0.1243	0.0508
60	0.3514	0.3735	0.3738	0.3015	0.4388	0.3678	0.0494	0.0202
90	0.2336	0.2168	0.2426	0.3119	0.1924	0.2395	0.0448	0.0183
120	0.1788	0.2002	0.2362	0.1297	0.1111	0.1712	0.0511	0.0209
180	0.0618	0.0871	0.2165	0.1094	0.1465	0.1243	0.0602	0.0246
240	0.0395	0.0833	0.0424	0.1117	0.0638	0.0681	0.0301	0.0123
360	0.0470	0.0371	0.0859	0.0804	0.0662	0.0633	0.0210	0.0086
480	0.0203	0.0264	0.0337	0.0337	0.0313	0.0291	0.0057	0.0023
720	0.0168	0.0183	0.0174	0.0334	0.0165	0.0205	0.0072	0.0030
1440	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

TCOH Conc (μg/mL)

Time	animal number							
	B	C	D	E	F	Ave	STDEV	SEM
2	0.1503	0.0991	0.1375	0.2313	0.0746	0.1386	0.0600	0.0245
4	0.1226	0.1706	0.2175	0.4083	0.1322	0.2102	0.1169	0.0477
6	0.4957	0.3454	0.3337	0.5629	0.1919	0.3859	0.1461	0.0597
8	0.6589	0.3657	0.2484	0.7825	0.2974	0.4706	0.2362	0.0964
10	0.5810	0.2431	0.7154	0.7942	0.4776	0.5623	0.2160	0.0882
15	0.8998	0.1915	0.5539	0.9970	0.7595	0.6803	0.3200	0.1307
20	0.7228	1.0047	0.2640	0.8350	1.0422	0.7737	0.3129	0.1277
30	0.7974	0.5220	0.4542	0.9923	1.3041	0.8140	0.3489	0.1424
45	0.9100	0.6776	1.2674	0.7706	1.6051	1.0461	0.3846	0.1570
60	0.7194	0.8179	0.9271	1.0043	1.4623	0.9862	0.2872	0.1173
90	0.5198	0.6196	0.5910	0.6081	0.9714	0.6620	0.1773	0.0724
120	0.4751	0.4772	0.4354	0.4614	0.7215	0.5141	0.1171	0.0478
180	0.2230	0.3322	0.4444	0.4247	0.6068	0.4062	0.1424	0.0581
240	0.2115	0.2674	0.2478	0.3501	0.3561	0.2866	0.0640	0.0261
360	0.1620	0.1940	0.1979	0.2819	0.2772	0.2226	0.0538	0.0220
480	0.1446	0.1872	0.2030	0.2162	0.2563	0.2015	0.0408	0.0167
720	0.1092	0.0853	0.1190	0.1821	0.1480	0.1287	0.0374	0.0153
1440	0.0537	0.0140	0.0400	0.0537	0.0732	0.0469	0.0219	0.0089

TCA Conc (μg/mL)

min	B	C	D	E	F	Ave	STDEV	SEM
2	0.2283	0.0601	0.0968	0.1287	0.1268	0.1281	0.0625	0.0255
4	0.1315	0.1851	0.1963	0.3457	0.1221	0.1962	0.0897	0.0366
6	0.4500	0.3833	0.3805	0.4275	0.1466	0.3576	0.1216	0.0496
8	0.6106	0.3232	0.2593	0.4040	0.2142	0.3623	0.1561	0.0637
10	0.5402	0.2208	0.5477	0.6088	0.3495	0.4534	0.1624	0.0663
15	0.8729	0.1195	0.3863	0.6200	0.4577	0.4913	0.2796	0.1141
20	0.6944	0.7861	0.2161	0.5862	0.3856	0.5337	0.2319	0.0947
30	0.7933	0.4209	0.2942	0.8279	0.4539	0.5580	0.2384	0.0973
45	1.5013	0.6798	1.4208	0.4487	0.3562	0.8814	0.5429	0.2216
60	1.2311	1.1334	1.6012	0.7831	0.3551	1.0208	0.4726	0.1929
90	1.3769	1.9578	1.9548	0.5212	0.8666	1.3355	0.6433	0.2626
120	2.0304	0.7658	1.2863	1.0563	0.8448	1.1967	0.5080	0.2074
180	2.6003	1.8725	1.0890	1.1334	0.7264	1.4843	0.7500	0.3354

240	2.4182	1.8556	2.0187	1.6602	1.0116	1.7929	0.5182	0.2317
360	2.4993	2.0709	1.8068	1.5219	0.8218	1.7441	0.6287	0.2812
480	2.0713	1.7857	1.5054	1.6670	0.9353	1.5929	0.4218	0.1886
720	2.0886	1.5952	1.4588	1.1182	0.8233	1.4168	0.4812	0.2152
1440	0.7955	0.6241	0.4785	0.5547	0.3494	0.5605	0.1663	0.0679

Appendix A-1b. Pharmacokinetic parameters of TCE and its metabolites after Control and TCE (10 mg/kg, po) administration: Feb. 19, 2005

TCE two compartmental model

	B	C	D	E	F	Ave	STDEV	SEM	
AUC	1.23	0.73	1.39	0.64	0.76	0.95	0.34	0.15	ug*h/ml
Beta_HL	1.05	1.30	1.08	1.05	1.60	1.22	0.24	0.11	h
CL_F	2.26	3.82	2.00	4.36	3.65	3.22	1.03	0.46	ml/h/kg
V2_F	33.87	22.99	20.19	16.08	16.47	21.92	7.26	3.25	L/kg
Tmax	2.0	8.0	4.0	2.0	6.0	4.4	2.6	1.3	min
Cmax	3.03	1.64	4.51	0.99	5.11	3.06	1.77	0.72	ug/ml

CH: non-compartmental model

	B	C	D	E	F	Ave	STDEV	SEM	
AUC	1.19	1.25	1.49	1.37	1.33	1.33	0.12	0.05	ug*hrml
HL	1.07	1.64	0.99	2.01	1.62	1.47	0.43	0.19	hr
Tmax	8	20	10	8	20	13.2	6.26	2.56	min
Cmax	0.99	0.87	1.17	0.76	0.72	0.90	0.18	0.08	ug/ml

TCOH: non-compartmental model

	B	C	D	E	F	Ave	STDEV	SEM	
AUC	4.02	3.98	4.55	5.67	6.58	4.96	1.13	0.51	ug*hrml
HL	7.60	9.18	7.21	7.39	9.49	8.17	1.07	0.48	hr
Tmax	45	20	45	60	45	43.0	14.40	5.88	min
Cmax	0.91	1.01	1.27	1.00	1.6	1.16	0.28	0.12	ug/ml

TCA Non compartmental model

	B	C	D	E	F	Ave	STDEV	SEM	
AUC_24h	42.57	33.05	31.95	31.52	26.35	33.09	5.90	2.64	ug*hrml
HL	10.59	10.04	8.98	10.03	10.86	10.10	0.72	0.32	hr
Tmax	180	360	240	480	240	300.0	120.0	53.67	min
Cmax	2.60	2.07	2.02	1.67	1.01	1.87	0.59	0.26	ug/ml

Appendix A-2a. Blood PK monitoring of PZ induction (200 mg/kg, ip, 3 days) and TCE (10 mg/kg, po): Feb. 13, 2005

TCE Conc (μg/mL)

	animal number							
min	A	B	C	J	L	Ave	STDEV	SEM
2	1.9927	1.4051	2.1268	1.4434	2.1843	1.8304	0.3775	0.1688
4	0.6642	3.0273	1.8649	1.9863	1.9671	1.9020	0.8383	0.3749
6	1.3029	2.4844	1.4434	0.2363	1.9991	1.4932	0.8451	0.3780
8	0.5301	1.8202	1.2454	0.5429	0.9963	1.0270	0.5383	0.2407
10	1.1496	1.4690	0.9069	1.0474	1.5456	1.2237	0.2742	0.1226
15	0.6233	1.0717	0.5250	0.6923	0.7664	0.7358	0.2078	0.0929
20	0.3794	0.6285	0.3602	0.2785	1.0474	0.5388	0.3130	0.1400
30	0.4803	0.5122	0.3321	0.3640	0.4547	0.4287	0.0772	0.0345
45	0.2887	0.3807	0.1405	0.3053	0.1584	0.2547	0.1023	0.0458
60	0.1571	0.2376	0.1213	0.1763	0.1188	0.1622	0.0486	0.0217
90	0.0626	0.1277	0.0830	0.0805	0.0460	0.0800	0.0306	0.0137
120	0.0434	0.1047	0.0613	0.0575	0.0345	0.0603	0.0271	0.0121
240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

CH Conc (μg/mL)

Time	animal number							
2	0.0876	0.2418	0.3596	0.1209	0.1632	0.1946	0.1088	0.0486
4	0.0846	0.7404	0.4865	0.1330	0.1843	0.3258	0.2798	0.1251
6	0.1390	0.8885	0.6830	0.1420	0.4019	0.4509	0.3318	0.1484
8	0.0907	1.3055	1.5624	0.3657	0.1692	0.6987	0.6847	0.3062
10	0.1088	1.3599	0.4503	0.6286	0.3173	0.5730	0.4791	0.2143
15	0.5712	1.4131	0.7585	0.8915	0.4213	0.8111	0.3812	0.1705
20	0.6902	1.1441	0.8014	0.9658	0.6407	0.8485	0.2071	0.0926
30	1.6089	1.1339	0.8196	0.6818	0.4176	0.9324	0.4579	0.2048
45	0.2617	0.8020	0.4158	0.9380	0.4479	0.5731	0.2842	0.1271
60	0.5524	0.4926	0.2798	0.4207	0.3095	0.4110	0.1165	0.0521
90	0.1481	0.2678	0.3294	0.2260	0.1880	0.2318	0.0703	0.0314
120	0.1197	0.1783	0.0629	0.1438	0.1239	0.1257	0.0421	0.0188
240	0.0447	0.0858	0.0641	0.0840	0.0749	0.0707	0.0169	0.0076
480	0.0417	0.0435	0.0441	0.0477	0.0502	0.0455	0.0034	0.0015
720	died	0.0308	0.0369	0.0248	died	0.0308	0.0060	0.0027
1440		0.0000	0.0000	0.0000		0.0000	0.0000	0.0000

TCOH Conc (μg/mL)

Time	animal number							
	A	B	C	J	L	Ave	STDEV	SEM
2		0.2307	0.3238	0.4081	0.2395	0.3005	0.0831	0.0372
4	0.1663	0.5256	0.4103	0.2750	0.1929	0.3140	0.1517	0.0678
6	0.1974	0.6254	0.5389	0.4147	0.2550	0.4063	0.1818	0.0813
8	0.1597	0.9470	0.8583	0.3548	0.1907	0.5021	0.3744	0.1674
10	0.1442	1.1488	0.4458	0.6210	0.3083	0.5336	0.3861	0.1727
15	0.2586	1.3435	1.1861	1.1843	0.4187	0.8782	0.5000	0.2236
20	0.5261	1.4899	1.2868	0.4524	0.9137	0.9338	0.4561	0.2040
30	1.7711	1.8412	1.4096	1.1324	1.4598	1.5228	0.2882	0.1289
45	3.0038	1.0610	1.1439	2.0004	1.5365	1.7491	0.7941	0.3551
60	1.3152	1.0171	0.9093	1.4739	1.3542	1.2139	0.2393	0.1070
90	0.5576	0.6383	0.9288	0.7097	0.6161	0.6901	0.1441	0.0645
120	0.4373	0.4502	0.3961	0.5873	0.4103	0.4562	0.0763	0.0341
240	0.2621	0.2413	0.3189	0.4254	0.3344	0.3164	0.0721	0.0322
480	0.1650	0.1743	0.3491	0.2905	0.1898	0.2338	0.0817	0.0365
720	died	0.1672	0.1455		died	0.1564	0.0154	0.0069
1440		0.0945	0.1198	0.1069		0.1070	0.0126	0.0057

TCA Conc (μg/mL)

Time	animal number							
2		0.3115	0.1637	0.1038	0.1757	0.1887	0.0877	0.0392
4	0.1038	0.5511	0.2556	0.1078	0.1557	0.2348	0.1871	0.0837
6	0.1118	0.3314	0.3195	0.1917	0.3234	0.2556	0.0990	0.0443
8	0.1438	0.9064	0.6070	0.1198	0.3075	0.4169	0.3356	0.1501
10	0.1358	1.2938	0.2755	0.1797	0.2755	0.4321	0.4856	0.2171
15	0.1086	1.2147	0.6677	0.2731	0.2388	0.5006	0.4505	0.2015
20	0.8280	1.4327	0.8250	0.6613	0.4041	0.8308	0.4371	0.1955
30	1.3225	2.0581	0.8857	1.0893	1.0039	1.2719	0.4677	0.2092
45	1.2419	0.9863	1.4136	1.3776	1.2874	1.2614	0.1684	0.0753
60	1.6476	2.5189	0.6860	0.8378	1.4647	1.4310	0.7312	0.3270
90	2.2266	2.8655	0.4776	0.8442	1.5477	1.5923	0.9782	0.4375
120	2.3296	3.0092	0.6956	1.0111	1.5909	1.6789	0.9162	0.4097
240	2.0876	2.1379	0.5950	1.0175	1.2802	1.4720	0.7402	0.3310
480	1.4407	1.0198	0.5199	0.7571	0.7683	0.9012	0.3496	0.1564
720	died	0.7667	0.4888		died	0.6277	0.1965	0.0879
1440		0.1382	0.1398	0.1334		0.1371	0.0033	0.0015

Appendix A-2b. Pharmacokinetic parameters of TCE and its metabolites after PZ induction & TCE (10 mg/kg, po): Feb. 13, 2005

TCE: two compartmental model

	animal number								
	A	B	C	J	L	Ave	STDEV	SEM	
AUC	0.72	0.99	0.62	0.68	0.69	0.74	0.14	0.06	ug*h/ml
Beta_HL	1.20	0.65	0.94	0.52	1.15	0.89	0.30	0.13	h
CL_F	3.87	2.81	4.48	4.07	4.01	3.85	0.62	0.28	ml/h/kg
V2_F	4.09	4.16	9.04*	4.67	4.26	4.30	0.26	0.12	L/kg
Tmax	2.00	4.00	2.00	4.00	2.00	2.80	1.10	0.49	min
Cmax	1.99	3.20	2.13	1.99	2.18	2.30	0.51	0.23	ug/ml

CH: non-compartmental model

	A	B	C	J	L	Ave	STDEV	SEM	
AUC	1.35	1.92	1.36	1.58	1.21	1.48	0.28	0.12	ug*h/ml
Beta_HL	1.70	2.16	2.65	2.26	2.20	2.19	0.34	0.15	h
Tmax	20	15	8	20	20	16.60	5.27	2.36	min
Cmax	1.61	1.41	1.56	0.97	0.64	1.24	0.42	0.19	ug/ml

TCOH: Non compartmental model

	A	B	C	J	L	Ave	STDEV	SEM	
AUC	3.67	5.68	6.47	7.65	3.60	5.41	1.77	0.79	ug*h/ml
Beta_HL	4.99	9.60	6.21	9.87	4.76	7.09	2.48	1.11	h
Tmax	45	30	30	45	45	39.0	8.22	3.35	min
Cmax	3.00	1.84	1.05	2.00	1.54	1.89	0.72	0.29	ug/ml

TCA: Non compartmental model

	A	B	C	J	L	Ave	STDEV	SEM	
AUC_24h	24.65	25.73	12.43	15.66	17.91	19.27	5.75	2.57	ug*h/ml
Beta_HL	5.20	5.16	6.14	6.58	6.02	5.82	0.62	0.28	h
Tmax	90	120	45	45	120	84.00	37.65	18.82	min
Cmax	2.33	3.01	1.41	1.38	1.59	1.94	0.71	0.35	ug/ml

Appendix A-3a. Blood PK monitoring of control & TCE (50 mg/kg, po): Feb. 11, 2005 and May 02, 2006

TCE Conc (μg/mL)

Time	animal number							
Min	A	B	C	M_A	M_D	Ave	STDEV	SEM
2	3.1742	6.9687	7.1001			5.7477	2.2296	1.2873
4	8.0013	6.8884	8.2238	11.3712	9.9223	8.2202	2.2618	0.9234
6	5.7939	7.8845	4.3965	10.1743	8.1111	6.8711	2.2261	0.9088
8	2.3679	6.1186	14.5978			7.6948	6.2655	3.6174

10	9.6613	7.9575	9.7124	11.4027	9.5600	8.6816	2.6304	1.0739
15	3.3056	6.9614	2.9371	9.9695	7.4968	5.5737	2.9964	1.2233
20	7.2351	4.4220	5.9216	6.5519	6.3629	5.3946	1.9637	0.8017
30	5.3561	4.2615	6.6476	6.1109	5.5596	5.5410	2.4457	0.9244
45	3.1779	1.7768	2.6087	3.4649	2.5672	3.0289	1.7466	0.6602
60	2.3898	2.1235	2.1964	2.5829	1.4805	2.3962	1.4187	0.5362
90	0.7917	1.1456	1.2223	1.3545	1.3545	1.0971	0.2796	0.1057
120	0.7399	0.8946	0.9924	0.8190	1.2285	0.8500	0.2197	0.0830
180	0.3225	0.3780	0.6494	0.4882	0.7324	0.4921	0.1495	0.0565
240	0.3006	0.4583	0.4203	0.3622	0.7087	0.4216	0.1368	0.0517
360	0.1547	0.1722	0.3327	0.2835	0.6064	0.3114	0.1484	0.0561
480	0.2087	0.1562	0.2860	0.2205	0.4646	0.2707	0.0985	0.0372
720	0.1241	0.1678	0.1970	0.1969	0.2835	0.2062	0.0603	0.0246
1440	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

CH Conc (μg/mL)

Min	A	B	C	M_A	M_D	Ave	STDEV	SEM
2	0.1102	0.2463	0.1811			0.1792	0.0681	0.0393
4	0.2660	0.3130	0.2456	0.5433	0.3158	0.3816	0.1532	0.0625
6	0.2435	0.5642	0.2014	0.8961	0.5348	0.4266	0.2931	0.1197
8	0.1109	0.5607	0.7081			0.4599	0.3111	0.1796
10	0.9215	0.8422	0.7158	0.8619	0.7794	0.8011	0.0905	0.0370
15	0.3839	1.0099	0.2870	0.9387	0.6599	0.7097	0.3169	0.1294
20	1.3510	0.6218	0.6990	0.5547	1.0923	0.7876	0.3591	0.1466
30	1.4141	0.8885	1.1130	0.9814	1.5816	1.1487	0.4254	0.1608
45	1.5390	0.5263	0.5362	1.7608	1.9457	1.1986	0.7012	0.2650
60	2.3980	1.3818	0.7418	0.9700	1.1066	1.1726	0.6766	0.2557
90	0.8618	0.7046	0.5509	0.6543	0.5974	0.6032	0.2358	0.0891
120	0.8997	0.3006	0.5892	0.4950	0.3869	0.4503	0.2519	0.0952
180	0.2917	0.3767	0.2804	0.3129	0.2830	0.2426	0.1200	0.0453
240	0.2327	0.3807	0.1917	0.2617	0.2276	0.1951	0.1257	0.0475
360	0.1811	0.0845	0.1609	0.1266	0.1380	0.0987	0.0738	0.0279
480	0.3206	0.1126	0.1277	0.0839	0.1124	0.1082	0.1076	0.0407
720	0.0727	0.0522	0.0825	0.0292	0.0313	0.0446	0.0306	0.0125
1440	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

TCOH Conc (μg/mL)

Min	A	B	C	M_A	M_D	Ave	STDEV	SEM
2	0.0390	0.1788	0.1322			0.1167	0.0712	0.0411
4	0.1370	0.2711	0.2473	1.0404	0.5512	0.5341	0.3861	0.1576
6	0.1408	0.5080	0.1979	1.2057	0.7923	0.5442	0.3996	0.1632
8	0.1189	0.5489	0.7667			0.4782	0.3296	0.1903
10	0.9027	0.8590	0.8333	1.1575	1.0748	0.9768	0.1307	0.0534
15	0.3881	1.1148	0.3263	1.2953	1.2126	0.9250	0.4439	0.1812
20	1.4554	0.6221	0.9969	0.7166	1.9774	1.0889	0.5280	0.2155
30	1.5353	1.1148	1.4592	1.5020	3.5208	1.8292	0.9161	0.3463
45	1.7198	0.7372	0.7819	3.1487	5.2571	2.3053	1.6567	0.6262
60	2.4922	2.9812	1.2994	2.4597	4.8574	2.4253	1.2977	0.4905
90	1.6190	1.6142	1.1795	2.5080	2.9007	1.8598	0.8115	0.3067
120	1.0578	1.4105	1.0654	1.7845	1.9292	1.3995	0.4488	0.1696
180	0.5323	0.7028	0.8801	1.1300	1.5434	0.7845	0.4512	0.1705
240	0.4319	0.5951	0.5395	1.0886	1.0542	0.6603	0.2910	0.1100
360	0.4026	0.3383	0.5331	0.7751	0.7786	0.4039	0.3229	0.1220
480	0.4581	0.3485	0.4794	0.5374	0.8991	0.3889	0.3161	0.1195
720	0.2526	0.2534	0.3938	0.1292	0.5701	0.2665	0.1993	0.0814
1440	0.0247	0.0978	0.0000	0.0000	0.0000	0.0175	0.0366	0.0149

TCA Conc (μg/mL)

Min	A	B	C	M_A	M_D	Ave	STDEV	SEM
2	0.1243	0.2720	0.2354			0.2106	0.0769	0.0344
4	0.2193	0.3495	0.3685	0.6505	0.5872	0.4350	0.1788	0.0800
6	0.4284	0.6214	0.2968	1.0434	0.5556	0.5891	0.2828	0.1265
8	0.1316	0.6536	1.0148			0.6000	0.4440	0.1986
10	1.1464	0.9636	1.1508	1.3190	0.7724	1.0704	0.2087	0.0933
15	1.0864	1.1888	0.9490	1.2829	1.6307	1.2275	0.2572	0.1150
20	1.8336	0.7808	1.1669	1.5223	1.8746	1.4356	0.4636	0.2073
30	2.3937	1.2794	1.8892	1.5900	3.6227	2.1550	0.9175	0.4103
45	2.8645	0.8671	1.0221	4.8830	5.9806	3.1234	2.2822	1.0206
60	3.4450	4.1044	2.5004	4.6526	7.9681	4.5341	2.0805	0.9304
90	5.2611	4.5958	4.9145	8.4515	9.0658	6.4577	2.1247	0.9502
120	4.6519	5.1561	5.0630	10.7235	11.9251	7.5039	3.5184	1.5735
180	5.7096	5.1386	5.1403	11.8325	8.8083	7.3259	2.9445	1.3168
240	6.5582	6.4212	6.0797	13.0142	12.8968	8.9940	3.6208	1.6193
360	6.1974	6.2232	5.8501	12.8240	9.9511	8.2092	3.0776	1.3764
480	5.8566	6.1541	5.5971	10.2402	12.3542	8.0404	3.0718	1.3738
720	6.1670	4.8610	4.9453	9.2871	11.6236	7.3768	2.9745	1.3302
1440	3.5954	2.6296	2.2773	3.5256	3.4973	3.1050	0.6088	0.2722

Appendix A-3b. Pharmacokinetic parameters of TCE and its metabolites after control & TCE (50 mg/kg, po): Feb. 11, 2005 and May 02, 2006

TCE: Two compartmental model

	A	B	C	M_A	M_D	Ave	STDEV	SEM	
AUC	9.28	10.22	10.25	13.42	15.02	11.64	2.46	1.10	ug*h/ml
Beta_HL	3.52	3.13	3.12	3.83	4.74	3.67	0.67	0.30	h
CL_F	1.50	1.36	1.13	1.03	0.92	1.19	0.23	0.10	ml/h/kg
Tmax	10.0	6.0	8.0	10	4	7.6	2.6077	1.1662	min
Cmax	9.66	7.88	14.60	11.40	9.92	10.69	2.51	1.12	ug/ml

CH: Non compartmental model

	A	B	C	M_A	M_D	Ave	STDEV	SEM	
AUC	4.04	2.98	2.56	3.26	3.41	3.25	0.55	0.25	ug*h/ml
HL	2.83	2.62	2.56	2.11	2.63	2.55	0.27	0.12	h
Tmax	60	60	30	45	45	48.0	12.55	5.61	min
Cmax	2.40	1.38	1.11	1.76	1.95	1.72	0.50	0.22	ug/ml

TCOH: Non compartmental model

	A	B	C	M_A	M_D	Ave	STDEV	SEM	
AUC	9.10	9.60	10.32	11.20	10.16	10.08	0.79	0.35	ug*h/ml
HL	6.72	6.82	4.97	4.14	5.50	5.63	1.15	0.51	h
Tmax	60.0	60.0	60.0	45.0	45.0	54.00	8.22	3.67	min
Cmax	2.49	2.98	1.46	3.15	5.26	3.07	1.39	0.62	ug/ml

TCA: Non compartmental model

	A	B	C	M_A	M_D	Ave	STDEV	SEM	
AUC	125.46	108.85	102.96	159.14	134.34	126.15	22.32	9.98	ug*h/ml
Beta_HL	ND	13.26	10.48	8.59	8.37	10.17	2.26	1.01	h
Tmax	240	240	240	240	240	240	0.0	0.0	min
Cmax	6.56	6.42	6.08	13.01	12.89	8.99	3.62	1.62	ug/ml

Appendix A-4a. Blood PK monitoring of PZ induction (200 mg/kg, ip, 3 days) TCE (50 mg/kg, po): Sep. 21, 2004

TCE: Conc (μg/mL)

Time	animal number						
min	A	B	C	E	Ave	STDEV	SEM
2	5.4843	3.0952	8.9459	14.1701	6.0555	4.7203	1.9271
4	3.6898	3.0156	9.0627	14.6373	5.9679	4.9400	2.0167
6	4.4225	3.8544	7.7327	11.5261	5.0149	3.9939	1.6305
8	2.4263	10.2201	6.1241	11.5819	6.3518	4.5340	1.8510
10	9.2143	11.4412	7.4859	6.8806	9.2432	2.3941	0.9774
15	1.6458	1.8635	2.4289	7.5974	3.5645	2.4825	1.0135
20	6.4240	2.9890	4.8897	9.1503	5.9802	2.2694	0.9265
30	4.2685	4.5473	1.3751	7.5336	3.7865	2.6133	1.0669
45	3.8571	3.7562	1.6485	5.3569	4.2892	1.9383	0.7913
60	3.1430	3.5677	1.2211	4.9348	3.1457	1.4873	0.6072
90	2.7315	2.4900	2.0467	2.9678	2.3392	0.5980	0.2441
120	1.2158	1.9378	1.4839	2.8245	1.8655	0.7054	0.2880
180	0.7210	0.7794	1.3443	1.3931	0.9062	0.4624	0.1888
240	0.2612	0.9217	0.5479	1.1223	0.7133	0.3840	0.1568
360	0.1678	0.6116	0.2909	0.6297	0.4250	0.2316	0.0945
480	died	0.4969	0.2984	0.3992	0.3982	0.0993	0.0405
720		0.2378	0.2450	0.2708	0.2543	0.0233	0.0095
1440		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

CH: Conc (μg/mL)

min	A	B	C	E	Ave	STDEV	SEM
2	0.2679	0.2333	0.4520	0.2794	0.3039	0.0853	0.0382
4	0.1685	0.2313	0.4761	0.7157	0.4296	0.2279	0.1019
6	0.2386	0.3097	0.3924	0.8256	0.3847	0.2614	0.1169
8	0.5201	0.9219	0.3997	1.1741	0.7072	0.3273	0.1464
10	0.2093	1.2431	0.6582	0.7649	0.6170	0.4325	0.1934
15	0.1915	0.2396	0.1580	0.6854	0.2794	0.2310	0.1033
20	2.3565	0.6467	1.2797	1.5801	1.2272	0.8139	0.3640
30	2.0959	2.0206	1.4116	1.4953	1.4666	0.7150	0.3198
45	1.8783	2.5501	2.5365	1.3593	1.7617	0.8701	0.3891
60	2.0959	2.3146	1.4733	0.6289	1.6133	0.6552	0.2930
90	1.9128	2.0154	1.0066	1.2274	1.5949	0.4490	0.2008
120	1.3823	1.6481	1.3488	0.8329	1.1399	0.4691	0.2098
150	0.3374	0.7383	0.6776	0.6337	0.5679	0.1673	0.0748
180	0.4378	1.1079	1.1297	0.3821	0.7253	0.3657	0.1635
240	0.5190	0.8706	0.5642	0.3817	0.4671	0.3164	0.1415
300	0.3817	0.3775	0.5843	0.3340	0.4194	0.1120	0.0501
360	0.2947	0.4562	0.4851	0.1662	0.3505	0.1488	0.0665
480	died	0.2729	0.3600	0.1385	0.2571	0.1115	0.0558
600		0.2298	0.1017	0.0866	0.1394	0.0787	0.0454
720		0.0347	0.0322	0.0276	0.0315	0.0036	0.0021
1440		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

TCOH: Conc (μg/mL)

Time	animal number						
min	A	B	C	E	Ave	STDEV	SEM
2	0.2273	0.1446	0.2546	0.2999	0.2152	0.0791	0.0323
4	0.1640	0.1863	0.2690	0.8142	0.3267	0.2664	0.1088
6	0.2115	0.2129	0.2539	0.9551	0.3227	0.3139	0.1281
8	0.4466	0.7221	0.2258	1.3910	0.5751	0.5151	0.2103
10	0.5099	0.9048	0.3855	0.8832	0.5099	0.3610	0.1474
15	0.2316	0.2884	0.1827	0.7861	0.2954	0.2472	0.1009
20	2.5360	0.4157	0.8278	1.5341	0.9561	0.9221	0.3764
30	1.8046	1.8866	0.2431	1.8959	1.0328	0.9104	0.3717
45	1.9067	2.0541	1.1148	1.7204	1.3156	0.6780	0.2768
60	2.1778	2.6144	1.0796	0.8904	1.4819	0.8555	0.3493
90	2.0275	2.4209	2.3684	1.8305	2.0600	0.3335	0.1492
120	1.4845	2.1038	1.6506	1.3349	1.4255	0.5662	0.2532
180	0.6375	1.2210	1.1781	0.7903	0.9339	0.2544	0.1138
240	0.6050	0.9517	0.9235	0.6416	0.6585	0.3151	0.1409
360	0.5901	0.6890	0.6004	0.5693	0.6122	0.0528	0.0236
480	died	0.7118	0.5728	0.5319	0.6055	0.0943	0.0471
720		0.4784	0.2673	0.3098	0.3518	0.1117	0.0645
1440		0.1545	0.2123	0.2356	0.2008	0.0418	0.0241

TCA: Conc (μg/mL)

min	A	B	C	E	Ave	STDEV	SEM
2	0.2695	0.3392	0.3624	0.2555	0.2900	0.0493	0.0221
4	0.5316	0.5390	0.3810	0.7806	0.5361	0.1903	0.0851
6	0.2462	0.2462	0.2555	1.0175	0.4175	0.3633	0.1625
8	0.5854	1.2731	0.2741	1.4450	0.8460	0.5257	0.2351
10	0.8905	1.4589	0.3903	0.9896	0.8950	0.5066	0.2266
15	0.2509	0.4089	0.4771	0.8363	0.4717	0.2865	0.1281
20	5.2548	0.6598	1.5193	1.7098	2.0373	1.6871	0.7545
30	3.7402	5.3524	3.9864	2.3556	3.3223	1.2438	0.5562
45	4.1816	6.2770	3.3174	2.0908	3.3135	1.7453	0.7805
60	6.4629	12.1684	2.6344	1.0593	5.5424	4.0915	1.8298
90	7.4293	20.2109	11.7363	7.1691	9.8285	4.7102	2.1064
120	5.9611	19.2631	13.4600	10.6165	10.0841	4.3708	1.9547
180	12.2790	9.0675	5.7297	6.9563	6.6598	1.9890	0.8895
240	9.3519	11.7307	4.8804	6.6664	5.9045	2.9617	1.3245
360	5.7185	7.5250	2.8639	4.5142	4.4080	1.3724	0.6138
480	died	5.5531	2.4922	3.8991	3.7658	1.4491	0.7246
720		3.4084	2.3751	4.1667	3.1371	0.8506	0.4911
1440		0.3996	0.0000	1.1876	0.7506	0.5270	0.3043

Appendix A-4b. Pharmacokinetic parameters of TCE and its metabolites after PZ induction & TCE (50 mg/kg, po): Sep. 21, 2004

TCE: two compartmental model

	A	B	C	E	Ave	STDEV	SEM	
AUC	7.89	9.62	7.62	8.86	8.50	0.92	0.41	ug*h/ml
Beta_HL	3.78	4.06	2.62	5.45	3.98	1.16	0.52	h
CL_F	1.76	0.85	2.62	0.81	1.51	0.86	0.39	ml/h/kg
Tmax	10	10	4	4	7.0	3.46	1.55	min
Cmax	9.66	7.88	14.60	12.34	11.12	2.95	1.32	ug/ml

CH: non compartmental model

	A	B	C	E	Ave	STDEV	SEM	
AUC	5.25	7.84	6.95	4.36	6.10	1.58	0.79	ug*h/ml
HL	2.39	2.18	2.25	2.22	2.26	0.09	0.05	h
Tmax	20	45	45	20	32.50	14.43	7.22	min
Cmax	2.36	2.55	2.54	1.58	2.26	0.46	0.23	ug/ml

TCOH: non compartmental model

	A	B	C	E	Ave	STDEV	SEM	
AUC	12.30	16.06	12.23	13.83	13.61	1.80	0.90	ug*h/ml
HL	5.76	7.35	7.28	5.74	6.53	0.90	0.45	h
Tmax	60	60	90	30	60.0	24.5	12.3	min
Cmax	2.54	2.61	2.37	2.90	2.60	0.22	0.11	ug/ml

TCA: non compartmental model

	A	B	C	E	Ave	STDEV	SEM	
AUC_24 h	84.18	105.39	61.49	87.49	84.64	18.03	9.01	ug*h/ml
HL	6.88	4.21	9.55	8.33	7.24	2.30	1.15	h
Tmax	180	90	120	120	127.5	37.75	18.87	min
Cmax	9.88	17.58	11.70	9.23	12.10	3.80	1.90	ug/ml

Appendix A-5a. Blood PK monitoring of control & TCE (200 mg/kg, po): Sep 28, 2004 and Dec 15, 2006

TCE Concentration (μg/mL)

min	A	B	C	E	Ave	STDEV	SEM
2	6.8815	13.3087	11.4118	11.0497	10.6629	2.7086	1.2113
4	13.2719	23.6892	14.5917	12.9404	16.1233	5.0941	2.2782
6	14.1681	34.3890	16.2921	18.2749	20.7810	9.2257	4.1258
8	17.9680	35.0397	25.8071	22.6763	25.3728	7.2051	3.2222
10	27.4768	33.7260	28.1705	10.8594	25.0582	9.8704	4.4142
15	33.5234	32.7070	17.5506	21.8108	23.0115	10.6953	4.7831
20	31.6511	24.2233	13.3946	21.2644	22.6334	7.5515	3.3771
30	32.1913	28.6923	19.3369	13.5604	23.4452	8.5365	3.8176
45	21.5652	20.6874	21.0932	19.7482	18.5235	4.3506	1.9456
60	8.4653	28.9378	13.9778	17.7347	17.2789	8.6548	3.8705
90	11.1049	17.3602	13.4867	16.3105	14.5656	2.8281	1.2648
120	13.7108	16.8446	12.3664	14.1282	14.2625	1.8784	0.8400
180	5.4757	3.7477	8.9717	8.0816	6.5692	2.3956	1.0713
240	1.6820	0.8778	2.6458	4.3063	2.3780	1.4748	0.6595
300	1.0126	0.5746	1.1614	1.3628	1.0279	0.3345	0.1496
360	0.4211	0.3118	1.1295	1.0117	0.7185	0.4118	0.1842
480		0.1056	0.5709	0.3254	0.3339	0.2328	0.1041
600		0.1166	0.4371	0.1559	0.2365	0.1748	0.0782
720		0.0700	0.2161		0.1430	0.1033	0.0462
1440		0.0000	0.0000		0.0000	0.0000	0.0000

CH Concentration (μg/mL)

min	A	B	C	D	E	F	G	Ave	STDEV	SEM
2	0.0000	0.0000	0.1304	0.1324	0.2375	0.2063	0.1363	0.1204	0.0918	0.0347
5	0.2647	0.0564	0.1479	0.3114	0.2920	0.1752	0.0798	0.1896	0.1022	0.0386
10	0.5820	0.4808	0.7027	0.5450	0.8779	1.0044	0.8137	0.7152	0.1922	0.0726
15	0.2569	0.2122	0.4419	1.1932	1.1562	1.3314	1.0414	0.8048	0.4815	0.1820
30	2.2969	0.5450	1.1660		1.3626	1.3606	0.4847	1.2026	0.6629	0.2506
60	2.7835	2.0886	2.4020	2.7446	1.5903	1.3451	0.6774	1.9474	0.7815	0.2954
90	1.7032	1.0278	1.2691	1.5981	2.1159	1.9271	1.3139	1.5650	0.3860	0.1459
120	1.1777	0.5450	0.9090	1.5280	1.5475	1.4774	1.2886	1.2105	0.3712	0.1403
180	0.7572	0.3640	0.4477	0.5295	0.8643	1.3820	0.7202	0.7236	0.3410	0.1289
240	0.4107	0.2998	0.2881	0.5762	0.4166	0.9713		0.4938	0.2560	0.0968
360	0.2239	0.1567	0.1762	0.3805	0.2560	0.5246	0.2083	0.2752	0.1321	0.0499
480	0.0720	0.0983	0.1275	0.1664	0.1168	0.2852	0.0983	0.1378	0.0713	0.0270
720	0.0555	0.0302	0.0243	0.0409	0.1071	0.0993	0.0311	0.0555	0.0341	0.0129

TCOH Concentration (μg/mL)

min	A	B	C	D	E	F	G	Ave	STDEV	SEM
2	0.6150	0.5891	0.7639	0.8740	0.2136	0.2913	0.9711	0.6169	0.2836	0.1072
5	0.9323	0.0000	0.7639	0.3561	0.2913	0.6992	0.5244	0.5096	0.3190	0.1206
10	1.4372	0.8546	1.0164	1.0229	1.8257	1.6185	1.2624	1.2911	0.3543	0.1339
15	0.7057	0.3172	0.9905	2.5896	3.1723	2.5314	2.0846	1.7702	1.0930	0.4131
30	5.3217	1.0617	2.0846		3.8585	2.4601	0.9905	2.6295	1.6869	0.6376
60	6.6618	4.5707	5.2375	6.2798	6.4352	3.6384	1.9681	4.9702	1.7195	0.6499
90	5.4317	2.6285	3.5348	5.0303	7.1344	4.9721	3.5607	4.6132	1.5022	0.5678
120	3.1140	2.5767	3.2953	5.4576	6.2604	4.3829	4.1175	4.1721	1.3225	0.4999
180	2.0005	1.5214	1.7804	1.8969	3.0234	4.6289	2.8486	2.5286	1.0810	0.4086
240	1.8127	1.0747	1.6120	1.7674	2.2853	3.4248		1.9962	0.8009	0.3027
360	1.6606	0.8837	1.1815	1.6347	1.8127	2.6382	1.5343	1.6208	0.5504	0.2080
480	0.5341	0.6927	0.8125	1.1977	1.1394	1.8710	0.8222	1.0100	0.4462	0.1687
720	0.3561	0.1295	0.0971	0.2266	0.9679	1.6056	0.3884	0.5387	0.5535	0.2092
1440			0.0000	0.0777	0.0518	0.0000	0.0453	0.0350	0.0341	0.0129

TCA Concentration (μg/mL)

min	A	B	C	D	E	F	G	Ave	STDEV	SEM
2	0.3005	0.9098	2.7005	2.2521	0.0850	0.3293	0.8651	1.0632	1.0193	0.3853
5	0.1166	0.7489	1.9216	1.0306	0.3609	0.9702	0.8450	0.8562	0.5748	0.2173
10	1.1858	1.3668	1.8468	1.4761	3.2149	1.1743	0.7776	1.5775	0.7921	0.2994
15	0.0907	1.2864	3.9938	1.9445	1.9963	1.8871	1.5249	1.8177	1.1647	0.4402
30	4.8130	1.9388	2.3584		2.5797	1.2001	1.1283	2.3364	1.3492	0.5100
60	7.5664	8.0608	8.9518	7.7159	5.9454	2.0969	1.5278	5.9807	2.9887	1.1296
90	10.8545	4.3818	7.1583	5.5200	11.6765	6.3736	6.9054	7.5529	2.7098	1.0242
120	15.2146	6.6697	11.2770	10.4837	14.6656	6.9628	8.8655	10.5913	3.4151	1.2908
180	15.8670	12.2657	19.9512	14.0965	20.6439	16.3326	17.6461	16.6861	3.0080	1.1369
240	9.7795	6.1753	6.4627	11.9294	9.9031	16.3068		10.0928	3.7588	1.4207
360	11.8366	9.5545	8.8116	11.7547	12.5839	15.3316	11.6599	11.6475	2.1182	0.8006
480	12.0148	10.1725	13.2866	12.1427	13.0193	15.2899	11.6282	12.5077	1.5923	0.6018
720	8.7483	11.4917	13.1530	10.9413	11.0060	14.7870	8.4997	11.2324	2.2422	0.8475
1440	5.1602	5.3780	4.8850	3.5902	4.3052	6.7310	1.3671	4.4881	1.6839	0.6364
2880	0.5682		0.6253	0.3904	1.5566	1.9909	0.4971	0.9381	0.6663	0.2519
4320	0.4744	0.3491	0.4730		0.7277	0.3059		0.4660	0.1643	0.0621

Appendix A-5b. Pharmacokinetic parameters of TCE and its metabolites after Control and TCE (200 mg/kg, po): Sep 28, 2004

TCE two compartmental model

	A	B	C	E	Ave	STDEV	SEM	
AUC	45.31	56.07	42.07	52.63	49.02	6.45	2.92	ug*h/ml
Beta_HL	3.87	3.83	4.81	5.43	4.49	0.78	0.39	h
CL_F	1.23	0.99	1.32	1.06	1.15	0.15	0.08	ml/h/kg
V2_F	18.78	13.54	26.94	29.28	22.13	7.29	3.64	L/kg
Tmax	15	8	10	8	10.25	3.3040	1.6520	min
Cmax	33.52	35.04	28.17	22.68	29.85	5.62	2.81	ug/ml

CH Non compartmental model

	A	B	C	D	E	F	G	Ave	STDEV	SEM
AUC (ug*hr/ml)	6.3971	3.8213	4.8300	7.0809	6.7485	8.8969	4.8060	6.0830	1.7210	0.6505
HL (hr)	1.8999	2.0275	1.8420	1.9623	2.3477	2.6131	2.0407	2.1047	0.2765	0.1045
Tmax (hr)	1.0000	1.0000	1.0000	1.0000	1.5000	1.5000	1.5000	1.2143	0.2673	0.1010
Cmax (ug/ml)	2.7835	2.0886	2.4020	2.7446	2.1159	1.9271	1.3139	2.1965	0.5094	0.1925

TCOH Non compartmental model

	A	B	C	D	E	F	G	Ave	STDEV	SEM
AUC (ug*hr/ml)	23.4622	14.3688	18.0126	25.9184	35.4609	42.7364	22.3624	26.0460	9.9026	3.7428
HL (hr)	3.2523	3.4635	3.1890	4.2039	3.6893	3.1600	3.5477	3.5008	0.3671	0.1388
Tmax (hr)	1.0000	1.0000	1.0000	1.0000	1.5000	1.5000	2.0000	1.2857	0.3934	0.1487
Cmax (ug/ml)	6.6618	4.5707	5.2375	6.2798	7.1344	4.9721	4.1175	5.5677	1.1343	0.4287

TCA Non compartmental model

	A	B	C	D	E	F	G	Ave	STDEV	SEM
AUC (ug*hr/ml)	410.55	469.75	439.78	433.05	468.20	559.97	460.76	463.15	47.73	18.04
HL (hr)	13.9423	13.0284	12.8358	14.1823	16.4873	12.2336	14.9935	13.9576	1.4501	0.5481
Tmax (hr)	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000	0.0000	0.0000
Cmax (ug/ml)	15.8670	12.2657	19.9512	14.0965	20.6439	16.3326	17.6461	16.6861	3.0080	1.1369

Appendix A-6a. Blood PK monitoring of PZ & TCE (200 mg/kg, po): Sep 06, 2004

TCE Conc (μg/mL)

Time	animal number							
min	A	B	C	J	L	Ave	STDEV	SEM
2		12.5396	11.9709	13.6410		12.7172	0.8491	0.3797
4	12.4674	12.6046	12.7400	6.9947	13.2005	11.6015	2.5899	1.1583
6	12.4439	14.0148	12.6877	12.2850	13.3088	12.9481	0.7123	0.3186
8	11.9366	12.3627	12.1803	12.1930	13.0217	12.3389	0.4108	0.1837
10	11.7669	12.3410	11.9907	12.1550	13.0506	12.2609	0.4895	0.2189
15	11.4238	11.9257	11.8192	10.9002	12.0467	11.6231	0.4669	0.2088
20	10.5427	11.3642	11.2721	11.5285	10.6998	11.0815	0.4336	0.1939
30	11.0970	11.4112	11.1114	11.4906	10.6366	11.1493	0.3362	0.1504
45	10.7702	10.8785	10.7142	10.8424	8.5674	10.3545	1.0011	0.4477
60	10.5373	9.8800	10.3603	9.7500	6.3141	9.3683	1.7383	0.7774
90	7.6177	5.3950	8.1485	4.2052	5.1332	6.0999	1.6972	0.7590
120	5.6911	3.5497	4.6999	4.1618	3.6689	4.3543	0.8747	0.3912
150	4.5428	3.9993	4.8371	3.1200	2.7228	3.8444	0.9055	0.4050
180	3.6653	3.0785	3.0370	2.6632	2.8203	3.0528	0.3814	0.1706
240	2.5621	2.2533	3.3818	3.1236	2.4664	2.7575	0.4746	0.2122
300	3.0099	1.4390	2.3165	2.7065	1.7604	2.2465	0.6495	0.2905
360	1.1772	0.8161	1.0147	1.3993	1.4210	1.1657	0.2573	0.1151
480	died	0.8351	0.9777	0.6211	0.4053	0.7098	0.2504	0.1252
600		0.5994	1.7523	died	0.8549	1.0689	0.6055	0.3496
720		1.0274	1.1881		0.2338	0.8164	0.5109	0.2950
1440		0.0000	0.0000		0.0000	0.0000	0.0000	0.0000

CH Conc (μg/mL)

min	A	B	C	J	L	Ave	STDEV	SEM
2		0.5614	0.7228	0.8048		0.6963	0.1238	0.0554
4	1.1688	1.1320	1.4429	0.2368	0.9123	0.9786	0.4555	0.2037
6	1.6259	1.5256	1.7223	1.2383	1.0986	1.4421	0.2640	0.1181
8	2.2982	1.8627	2.1139	1.7125	1.1635	1.8302	0.4356	0.1948
10	2.7763	2.2929	2.5225	2.2752	1.3157	2.2365	0.5536	0.2476
15	2.9764	2.3225	2.5645	1.1898	1.7958	2.1698	0.6946	0.3106
20	1.1261	2.8144	2.8524	2.5323	1.0232	2.0697	0.9174	0.4103
30	3.1259	2.9764	3.0623	2.7173	2.1795	2.8123	0.3864	0.1728
45	3.2636	2.9318	3.2066	2.9285	2.3690	2.9399	0.3543	0.1584
60	3.7044	2.9305	3.2072	2.3375	2.1992	2.8758	0.6219	0.2781
90	2.1591	2.7075	2.9410	2.1277	1.3728	2.2616	0.6080	0.2719
120	3.1167	1.5361	2.2470	1.4934	1.1248	1.9036	0.7905	0.3535
150	2.6609	1.0284	1.7387	0.9897	0.7470	1.4330	0.7797	0.3487
180	1.9807	0.7962	1.0271	0.8730	0.6769	1.0708	0.5243	0.2345
240	1.3203	0.4827	1.0133	1.1832	0.6972	0.9393	0.3454	0.1545
300	1.7184	0.4007	0.7602	0.6664	0.4093	0.7910	0.5419	0.2423
360	0.5457	0.1587	0.2427	0.3529	0.2315	0.3063	0.1507	0.0674
480	died	0.1774	0.2719	0.2043	0.1122	0.1914	0.0661	0.0331
600		0.0830	0.3611	died	0.1128	0.1856	0.1527	0.0881
720		0.0577	0.1820		0.0420	0.0939	0.0767	0.0443
1440		0.0344	0.0649		0.0000	0.0331	0.0325	0.0188

TCOH Conc (µg/mL)

Time	animal number							
min	A	B	C	J	L	Ave	STDEV	SEM
2		0.7419	0.5184	0.8211		0.6938	0.1570	0.0906
4	1.1128	1.7252	1.2085	0.2588	1.8813	1.2373	0.6377	0.2852
6	1.7590	1.6970	1.7519	1.5637	2.6247	1.8793	0.4240	0.1896
8	2.8106	3.1933	2.5643	2.5196	2.8043	2.7784	0.2677	0.1197
10	3.5618	4.3453	3.4325	3.7414	3.2050	3.6572	0.4314	0.1929
15	4.4276	4.5986	3.9657	2.0460	4.6872	3.9450	1.0974	0.4908
20	1.4672	6.0007	5.6949	4.3923	3.0207	4.1152	1.8932	0.8467
30	5.4565	6.1960	5.9678	5.1820	6.0854	5.7775	0.4370	0.1954
45	5.6988	6.2281	6.1638	5.9599	6.1740	6.0449	0.2187	0.0978
60	6.6516	6.2579	5.9670	4.6840	5.8384	5.8798	0.7379	0.3300
90	4.5413	5.7137	5.3679	3.8120	3.6559	4.6182	0.9143	0.4089
120	5.5208	3.8551	4.6550	3.0694	3.7085	4.1618	0.9464	0.4233
150	5.1757	3.0803	3.4474	2.2420	2.7494	3.3390	1.1184	0.5002
180	3.9837	2.3597	2.3354	1.8099	2.0664	2.5110	0.8532	0.3816
240	2.7251	1.6711	2.3518	2.4498	2.2256	2.2847	0.3891	0.1740
300	4.0794	1.4931	2.0899	1.5645	1.6445	2.1743	1.0901	0.4875
360	1.6829	0.6893	0.8171	0.9065	0.8540	0.9900	0.3956	0.1769
480	died	1.2167	2.2679	2.2781	2.2499	2.0031	0.5244	0.2622
600		0.5211	1.7692	died	1.2806	1.1903	0.6289	0.3631
720		0.4627	0.9469		0.1929	0.5342	0.3821	0.2206
1440		0.1321	0.0670		0.0643	0.0878	0.0384	0.0222

TCA Conc (µg/mL)

min	A	B	C	J	L	Ave	STDEV	SEM
2		0.6568	1.0357	1.0768		0.9231	0.2315	0.1337
4	0.8810	2.1157	1.9041	0.6284	1.7115	1.4481	0.6551	0.2930
6	1.3420	0.9284	2.9272	1.2094	2.5167	1.7848	0.8805	0.3938
8	2.1946	3.9945	4.1272	2.2357	2.7314	3.0567	0.9419	0.4212
10	2.3999	5.0271	5.0177	3.7230	2.7378	3.7811	1.2330	0.5514
15	4.0798	6.4355	5.7629	2.1978	5.9902	4.8932	1.7511	0.7831
20	1.2031	9.6059	9.6059	5.4661	4.3261	6.0414	3.6088	1.6139
30	7.1491	13.1015	12.5204	9.0817	12.8615	10.9428	2.6780	1.1976
45	8.7722	16.2592	16.2750	15.9592	18.2296	15.0991	3.6503	1.6325
60	16.5971	20.3864	18.5265	15.4666	18.6559	17.9265	1.9210	0.8591
90	7.4807	23.7399	20.4748	19.9380	8.8385	16.0944	7.4037	3.3110
120	22.4894	23.2441	22.7010	19.7296	22.0316	22.0391	1.3626	0.6094
150	24.6872	25.2525	19.6096	17.3834	23.0420	21.8604	3.2655	1.4604
180	24.0146	23.4178	15.8140	12.0247	20.3738	19.2635	5.2964	2.3686
240	20.6832	22.8368	14.0520	11.9521	19.2117	17.7471	4.5800	2.0483
300	23.2473	19.1801	13.6635	7.9607	18.3717	16.4847	5.8561	2.6189
360	27.0871	14.4593	11.5763	6.3881	14.8698	14.8761	7.6193	3.4075
480	died	10.3384	7.9291	7.6291	6.1260	8.0057	1.7438	0.8719
600		4.1903	5.8150	died	5.6950	5.2334	0.9053	0.5227
720		3.5067	3.1325		2.6604	3.0998	0.4241	0.2448
1440		0.6237	0.4373		0.2116	0.4242	0.2064	0.1191

Appendix A-6b. Pharmacokinetic parameters of TCE and its metabolites after PZ and TCE (200 mg/kg, po): Sep 06, 2004

TCE two compartmental model

	A	B	C	J	L	Ave	STDEV	SEM	
AUC	36.73	35.80	43.87	35.71	29.24	36.27	5.20	2.32	ug*h/ml
Beta_HL	3.89	4.88	3.37	4.42	3.48	4.01	0.64	0.29	h
CL_F	1.51	1.55	1.27	1.56	1.90	1.47	0.14	0.07	ml/h/kg
V2_F	7.40	14.44	7.90	14.97	11.20	11.18	4.08	2.04	L/kg
Tmax	4	6	4	2	6	4.4	1.67	0.75	min
Cmax	12.47	14.01	12.74	13.64	13.31	13.23	0.63	0.28	ug/ml

CH Non compartmental model

	A	B	C	J	L	Ave	STDEV	SEM	
AUC	12.36	8.79	12.86	8.40	6.56	9.79	2.71	1.21	ug*h/ml
HL	1.89	2.33	4.98	2.25	2.27	2.74	1.26	0.57	h
Tmax	60	30	60	45	45	48.0	12.55	5.61	min
Cmax	3.70	2.98	3.21	2.93	2.37	3.04	0.48	0.22	ug/ml

TCOH: non-compartmental model

	A	B	C	J	L	Ave	STDEV	SEM	
AUC	40.21	25.40	33.77	37.93	26.21	32.71	6.71	3.00	ug*h/ml
HL	4.03	5.82	4.24	4.82	6.54	5.09	1.07	0.48	h
Tmax	60	60	45	45	45	51.00	8.22	3.67	min
Cmax	6.65	6.26	6.16	5.96	6.17	6.24	0.25	0.11	ug/ml

TCA Non compartmental model

	A	B	C	J	L	Ave	STDEV	SEM	
AUC_24 h	197.52	191.47	156.30	152.01	167.10	172.88	20.60	9.21	ug*h/ml
HL	4.14	4.95	4.50	3.84	3.51	4.19	0.56	0.25	h
Tmax	180	150	120	90	150	138.0	34.21	15.30	min
Cmax	27.09	25.25	22.70	19.94	23.04	23.60	2.71	1.21	ug/ml

Appendix B. Data for Chapter 3

Appendix B-1a. Blood TCA TK monitoring after control & TCA (10 mg/kg, iv): Jan. 17, 2006

	TCA Conc (μg/mL)						
Time	animal number						
Min	A	B	C	D	Ave	STDEV	SEM
5	22.6603	17.7552	24.7970	20.1039	21.3291	3.0590	1.5295
10	21.4012	18.3699	22.9740	16.9242	19.9173	2.7628	1.3814
15	19.8877	20.2565	17.0684	16.6317	18.4610	1.8748	0.9374
30	17.0599	20.0191	21.3630	20.0403	19.6206	1.8192	0.9096
60	19.1267	18.1092	19.1627	18.6328	18.7578	0.4954	0.2477
120	18.8988	19.2419	19.0885	18.4324	18.9154	0.3513	0.1756
240	16.2594	15.1560	14.6901	14.6874	15.1982	0.7409	0.3705
360	11.2793	11.9947	11.3418	11.2697	11.4714	0.3503	0.1752
480	10.3075	11.1791	9.4761	died	10.3209	0.8516	0.4258
720	9.8476	9.4741	8.3201		9.2140	0.7963	0.3981
1440	7.5421	5.5961	3.1280		5.4220	2.2122	1.1061
2880	1.6891	1.4370	0.7449		1.2904	0.4889	0.2444

Appendix B-1b. Toxicokinetic parameters of TCA after control & TCA (10 mg/kg, iv) administration: Jan. 17, 2006

	A	B	C	Ave	STDEV	SEM	SEM
AUC	370.72	345.53	267.87	328.04	53.61	30.95	ug*h/ml
Beta_HL	14.41	13.50	11.00	12.97	1.77	1.02	h
Cmax	26.05	19.52	23.61	23.06	3.30	1.91	ug/ml
CL	0.45	0.48	0.62	0.52	0.09	0.05	ml/min/kg
Vss	0.56	0.55	0.54	0.55	0.01	0.00	L/kg

Appendix B-1c. Urine TCA TK monitoring after control & TCA (10 mg/kg, iv): Jan. 17, 2006

	TCA Conc (μg/mL)				Urine volume (mL)			
Time (hr)	A	B	C	E	A	B	C	E
2	120.2237	200.6979	86.3269	221.6700	1.1	0.4	1.3	0.5
4	106.3236	239.2280	135.8308	123.3939	1.0	0.5	0.8	1.2
8	123.6377	49.9916	149.9748	164.8503	0.9	1.6	0.7	0.6
12	90.4726	67.0619	100.7148	118.2728	1.1	0.9	0.8	1.5
24	103.1534	53.1618	63.6478	47.0652	1.8	6.3	1.8	3.7
36	108.0306	16.5826	28.5318	94.1305	0.7	4.3	3.2	0.8
48	23.1668	14.8755	14.3878	21.2159	3.6	11.1	11.5	9.3

TCA amount excreted in urine (μg)

Time (hr)	A	B	C	E	Ave	STDEV	SEM
2	132.25	80.28	112.23	110.84	108.90	21.44	10.72
4	106.32	119.61	108.66	148.07	120.67	19.17	9.58
8	111.27	79.99	104.98	98.91	98.79	13.51	6.76
12	99.52	60.36	80.57	177.41	104.46	51.19	25.60
24	185.68	334.92	114.57	174.14	202.33	93.73	46.86
36	75.62	71.31	91.30	75.30	78.38	8.83	4.42
48	83.40	165.12	165.46	197.31	152.82	48.68	24.34
Accum (μg)	794.06	911.58	777.77	981.98	866.35	97.45	48.72
b. w.	162	159	156	167			
Dose (μg)	1620	1590	1560	1670			
F_{Elim} (48 hr)	0.4902	0.5733	0.4986	0.5880	0.5375	0.0503	0.0252
F_{Elim} (2 hr)	0.0816	0.0505	0.0719	0.0664	0.0676	0.0130	0.0065

	Accumulated TCA amount in urine							Cumulative Excretion (%)		
Time (hr)	A	B	C	E	Ave	STDEV	SEM	Ave	STDEV	SEM
2	132.25	80.28	112.23	110.84	108.90	21.44	10.72	6.76	1.30	0.65
4	238.57	199.89	220.89	258.91	229.57	25.15	12.58	14.24	1.24	0.62
8	349.84	279.88	325.87	357.82	328.35	35.05	17.53	20.38	1.87	0.94
12	449.36	340.24	406.44	535.23	432.82	81.71	40.85	26.81	4.40	2.20
24	635.04	675.15	521.01	709.37	635.14	81.93	40.96	39.38	4.28	2.14
36	710.66	746.46	612.31	784.67	713.53	73.93	36.97	44.26	3.65	1.82
48	794.06	911.58	777.77	981.98	866.35	97.45	48.72	53.75	5.03	2.52

t(mid) hr	dt	Xu	Xu/dt	ln(Xu/dt)
1	2	108.90	54.4482	3.9972
3	2	120.67	60.3344	4.0999
6	4	98.79	24.6971	3.2067
10	4	104.46	26.1160	3.2625
18	12	202.33	16.8605	2.8250
36	24	231.20	9.6335	2.2653

Appendix B-2a. Blood TCA TK monitoring after PZ induction (200 mg/kg, ip, 3 days) & TCA (10 mg/kg, iv): Feb. 13, 2006

	TCA Conc (μg/mL)							
Time	animal number							
Min	A'	D'	E'	F'	H'	Ave	STDEV	SEM
5	32.5437	31.3631	33.0027	33.9373	28.0258	31.7745	2.2913	1.0247
10	23.3779	22.1696	22.7474	28.8027	25.3880	24.2744	3.0590	1.3680
15	26.9613	22.1392	23.1567	28.6811	22.7364	24.7349	2.9048	1.2991
30	17.1981	22.0368	20.1898	23.3557	23.2479	21.2057	2.5776	1.1527
60	23.7705	23.5714	18.5530	23.2700	24.8046	22.7939	2.4399	1.0912
120	16.9216	26.2894	14.3392	16.0728	22.2415	19.1729	4.9524	2.2148
240	12.9746	14.9613	7.7447	10.7640	15.0359	12.2961	3.0876	1.3808
360	6.8212	11.6862	5.7746	8.6599	9.9000	8.5684	2.3643	1.0573
480	6.2399	6.0664	5.7850	5.0793	6.5295	5.9400	0.5519	0.2468
720		2.8811	2.3945		5.3599	3.5452	1.5903	0.7112
1440	0.7144	0.3056	0.6943		0.5895	0.5759	0.1884	0.0842
2880	0.0374	0.0255	0.0358	0.1227	0.0452	0.0533	0.0394	0.0176

Appendix B-2b. Toxicokinetic parameters of TCA after PZ induction (200 mg/kg, ip, 3 days) & TCA (10 mg/kg, iv): Feb. 13, 2006

	A'	D'	E'	F'	H'	Ave	STDEV	SEM	
AUC	146.47	116.54	149.66	119.37	157.68	137.94	18.72	7.08	ug*h/ml
Beta_HL	5.25	5.40	4.08	5.77	7.53	5.60	1.25	0.47	h
Cmax	39.37	24.32	38.22	26.66	30.55	31.82	6.75	2.55	ug/ml
CL	1.14	1.43	1.11	1.40	1.06	1.23	0.17	0.07	ml/min/kg
Vss	0.51	0.45	0.50	0.59	0.53	0.52	0.05	0.02	L/kg

Appendix B-2c. Urine TCA TK monitoring after PZ induction (200 mg/kg, ip, 3 days) & TCA (10 mg/kg, iv): Feb. 13, 2006

	TCA Conc (μg/mL)					Urine volume (mL)				
Time	A'	D'	E'	F'	H'	A'	D'	E'	F'	H'
2 hr	284.11	337.10	605.83	582.58	516.25	1.4	1.3	0.6	0.5	0.9
4 hr	297.44	454.03	407.53	399.67	477.62	0.9	0.5	0.6	0.4	0.4
8 hr	235.56	328.56	291.29	294.71	268.38	1.1	0.7	0.6	0.4	0.7
12 hr	86.50	199.66	165.82	187.01	151.80	2.2	0.9	0.9	0.7	0.8
24 hr	156.93	96.75	83.42	69.40	64.62	2.0	3.6	2.4	5.9	5.1
48 hr	40.68	27.69	26.67	23.25	29.40	5.1	4.2	9.3	10.2	6.1

TCA amount excreted in urine (μg)

Time (hr)	A'	D'	E'	F'	H'	Ave	STDEV	SEM
2	397.75	438.23	363.50	291.29	464.63	391.08	67.82	30.33
4	267.70	227.01	244.52	159.87	191.05	218.03	42.90	19.18
8	259.12	229.99	174.77	117.88	187.87	193.93	54.17	24.22
12	190.30	179.70	149.23	130.91	121.44	154.31	29.97	13.40
24	313.85	348.32	200.21	409.48	329.55	320.28	76.30	34.12
48	207.49	116.31	248.01	237.13	179.35	197.66	52.78	23.60
Accum (μg)	1636.21	1539.56	1380.24	1346.56	1473.88	1475.29	117.93	52.74
b. w.	172	163	161	162	158			
Dose (μg)	1720	1630	1610	1620	1580			
F_{Elim} (48 hr)	0.9513	0.9445	0.8573	0.8312	0.9328	0.8961	0.0608	0.0272
F_{Elim} (2 hr)	0.2313	0.2689	0.2258	0.1798	0.2941	0.2400	0.0438	0.0196

	Accumulated TCA amount in urine								Cumulative Excretion (%)		
Time (hr)	A'	D'	E'	F'	H'	Ave	STDEV	SEM	Ave	STDEV	SEM
2	397.75	438.23	363.50	291.29	464.63	391.08	67.82	30.33	24.00	4.38	1.96
4	665.45	665.25	608.01	451.16	655.67	609.11	91.44	40.89	37.32	5.51	2.46
8	924.57	895.24	782.79	569.04	843.54	803.04	141.49	63.28	49.16	8.21	3.67
12	1114.87	1074.93	932.02	699.95	964.98	957.35	162.47	72.66	58.59	9.17	4.10
24	1428.72	1423.25	1132.23	1109.43	1294.53	1277.63	153.09	68.47	78.22	8.32	3.72
48	1636.21	1539.56	1380.24	1346.56	1473.88	1475.29	117.93	52.74	90.34	5.52	2.47

Time (hr)	t(mid) hr	dt	Xu	Xu/dt	ln(Xu/dt)
2	1	2	391.08	195.5399	5.2758
4	3	2	218.03	109.0146	4.6915
8	6	4	193.93	48.4815	3.8812
12	10	4	154.31	38.5787	3.6527
24	18	12	320.28	26.6901	3.2843
48	36	24	197.66	8.2358	2.1085

Appendix B-3a. Blood TCA TK monitoring after Control & TCA (50 mg/kg, iv): Oct. 14, 2004

	TCA Conc (μg/mL)							
min	A	B	C	D	F	Ave	STDEV	SEM
2		115.7205	118.0259	118.0509	145.5903	124.3469	14.2044	6.3524
5		112.6884	115.3697	105.5467	136.4941	117.5247	13.3084	5.9517
10		115.5200	115.1191	102.3016	118.4644	112.8513	7.1895	3.2153
15		110.5334	114.0541	105.3337	117.2616	111.7957	5.1097	2.2851
20	110.9218	111.9492	106.1105	108.5913	117.9382	111.1022	4.4357	1.9837
30	102.1513	109.4183	110.7213	103.0283	119.0533	108.8745	6.8318	3.0553
45	111.2601	108.2280	105.6094	86.5022	109.3932	104.1986	10.1024	4.5179
60	106.1231	102.3893	109.1426	92.5664	111.7612	104.3965	7.4787	3.3446
90	66.0437	62.2578	60.1616	63.0582	67.4666	63.7976	2.9430	1.3161
120	64.8178	66.6535	67.1617	60.9048	69.3913	65.7858	3.1783	1.4214
180	65.7960	65.9612	64.3922	60.5554	66.5773	64.6564	2.4279	1.0858
240	64.1190	61.4892	49.1914	54.4383	67.4539	59.3384	7.4215	3.3190
360	62.0736	56.9156	60.1489	44.7321	65.8214	57.9383	8.0557	3.6026
480	38.5840	27.0192	37.6624	31.4984	32.6892	33.4906	4.7391	2.1194
600	29.7841	40.6817	37.7472	26.6394	32.6375	33.4980	5.7279	2.5616
720	28.7821	30.2420	30.6431	20.4857	27.5286	27.5363	4.1302	1.8471
1320	24.7475	24.8802	26.8975	died		25.5084	1.2048	0.6024
1620	22.9838	24.2284	26.8385			24.6836	1.9673	1.1358
1850	21.0756	23.3849	22.0636			22.1747	1.1586	0.6689
2100	19.6511	22.5414	20.0286			20.7404	1.5711	0.9071
2800	10.4700	16.3214	17.4598			14.7504	3.7504	2.1653
3500	died	2.2310	4.0814			3.1562	1.3084	0.7554
5760		1.1313	0.5069		0.1964	0.6115	0.4761	0.2749

Appendix B-3b. Toxicokinetic parameters of TCA after Control & TCA (50 mg/kg, iv): Oct. 14, 2004

	A	B	C	F	Ave	STDEV	SEM	
AUC	1772.07	1301.44	1452.33	1408.04	1483.47	202.55	90.58	ug*h/ml
Beta_HL	10.68	7.41	7.90	7.11	8.28	1.64	0.73	h
Cmax	114.98	121.68	127.48	137.19	125.33	9.41	4.21	ug/ml
CL	0.47	0.64	0.57	0.59	0.57	0.07	0.03	ml/min/kg
Vss	0.92	0.75	0.68	0.70	0.76	0.11	0.05	L/kg
CLD2	1.3167	1.8339	3.0197	2.0843	2.0636	0.7130	0.3189	ml/min/kg

Appendix B-3c. Urine TCA TK monitoring after Control & TCA (50 mg/kg, iv): Oct. 28, 2005

Time	TCA Conc (μg/mL)				Urine volume (mL)							
hr	A	C	D	E	A	C	D	E	t(mid) hr	Xu	Xu/dt	ln(Xu/dt)
2	975.38	847.30	407.23	798.04	0.9	0.6	1.2	0.8	1	628.33	314.1667	5.7499
4	600.99	1615.78	1044.35	988.52	0.8	0.4	0.8	1.0	3	737.78	368.8883	5.9105
8	857.15	1697.89	1198.70	945.83	0.8	0.3	0.6	0.8	6	667.74	166.9357	5.1176
12	666.68	492.62	512.32	403.95	1.5	1.1	1.4	1.7	10	736.46	184.1157	5.2156
24	295.57	331.70	279.15	177.34	3.1	4.6	3.4	4.3	18	1038.44	86.5364	4.4606
36	387.53	495.90	292.29	111.66	2.5	1.9	2.6	5.9	30	832.44	69.3701	4.2395
48	118.23	128.08	174.06	210.18	6.8	6.4	4.8	4.9	42	872.26	72.6884	4.2862
72	187.19	177.34	200.33	197.05	12.6	11.2	12.6	10.7	60	2244.36	93.5152	4.5381

	TCA amount excreted in urine (μg)						
Time (hr)	A	C	D	E	Ave	STDEV	SEM
2	877.84	508.38	488.68	638.43	628.33	179.12	89.56
4	480.79	646.31	835.48	988.52	737.78	221.23	110.61
8	685.72	509.37	719.22	756.66	667.74	109.49	54.74
12	1000.01	541.88	717.25	686.71	736.46	191.63	95.82
24	916.27	1525.80	949.11	762.57	1038.44	334.93	167.46
36	968.81	942.21	759.94	658.79	832.44	148.39	74.19
48	803.95	819.72	835.48	1029.90	872.26	105.88	52.94
72	2358.65	1986.23	2524.17	2108.40	2244.36	242.54	121.27
Accum (μg, 72 hr)	8092.06	7479.90	7829.33	7629.99	7757.82	264.83	132.42
b. w.	172	174	167	171			
Dose (μg)	8600	8700	8350	8550			
F_{Elim} (72 hr)	0.9409	0.8598	0.9376	0.8924	0.9077	0.0389	0.0194
Accum (48 hr)	5733.41	5493.67	5305.16	5521.58	5513.46	175.33	87.67
F_{Elim} (48 hr)	0.6667	0.6315	0.6353	0.6458	0.6448	0.0158	0.0079
F_{Elim} (2 hr)	0.1021	0.0584	0.0585	0.0747	0.0734	0.0206	0.0103

Time		TCA accumulated amount in urine (μg)							Cumulative Excretion (%)		
hr	dt	A	C	D	E	Ave	STDEV	SEM	Ave	STDEV	SEM
2	2	877.84	508.38	488.68	638.43	628.33	179.12	89.56	7.34	2.06	1.03
4	2	1358.64	1154.69	1324.16	1626.95	1366.11	195.41	97.70	15.99	2.36	1.18
8	4	2044.36	1664.06	2043.38	2383.61	2033.85	293.98	146.99	23.81	3.60	1.80
12	4	3044.37	2205.94	2760.63	3070.32	2770.32	401.55	200.77	32.43	4.88	2.44
24	12	3960.64	3731.74	3709.74	3832.89	3808.75	114.58	57.29	44.55	1.30	0.65
36	12	4929.46	4673.95	4469.68	4491.68	4641.19	212.87	106.43	54.28	2.09	1.05
48	12	5733.41	5493.67	5305.16	5521.58	5513.46	175.33	87.67	64.48	1.58	0.79
72	24	8092.06	7479.90	7829.33	7629.99	7757.82	264.83	132.42	90.77	3.89	1.94

Appendix B-4a. Blood TCA TK monitoring after PZ induction (200 mg/kg, ip, 3 days) & TCA (50 mg/kg, iv): Oct, 23, 2004

	TCA Conc (μg/mL)					
Time	animal number					
Min	B	C	D	Ave	STDEV	SEM
1	116.6392	126.4278	120.0221	121.0297	4.9714	2.8703
5	95.4672	95.9045	98.5928	96.6548	1.6925	0.7569
10	102.1558	101.7442	99.3389	101.0796	1.5215	0.6804
15	100.0206	113.9123	91.0167	101.6499	11.5345	5.1584
20	101.5512	100.5737	90.8109	97.6453	5.9389	2.6560
30	95.3643	97.9754	89.1516	94.1638	4.5328	2.0271
45	96.2518	101.4355	92.9975	96.8949	4.2556	1.9032
60	87.9682	102.0143	79.2601	89.7475	11.4810	5.1344
90	84.3666	89.3831	73.5491	82.4329	8.0922	3.6189
120	51.2001	54.4801	34.2277	46.6360	10.8703	4.8613
150	51.3223	39.4436	28.4266	39.7308	11.4505	5.1208
240	46.4602	46.6917	48.2481	47.1333	0.9723	0.4348
360	15.7022	21.4840	27.1789	21.4550	5.7384	2.5663
520	13.0878	18.9468	18.4805	16.8384	3.2564	1.4563
800	7.6212	6.6565	7.1967	7.1581	0.4835	0.2162
1440	4.8428	4.9618	4.9811	4.9286	0.0749	0.0335
2880	0.6130	0.2288	0.1680	0.3366	0.2413	0.1079

Appendix B-4b. Toxicokinetic parameters of TCA after PZ induction (200 mg/kg, ip, 3 days) & TCA (50 mg/kg, iv): Oct, 23, 2004

	B	C	D	Ave	STDEV	SEM	
AUC	495.11	494.67	444.12	477.96	29.31	16.92	ug*h/ml
Beta_HL	3.00	2.96	2.64	2.87	0.20	0.11	h
Cmax	114.58	115.68	116.73	115.66	1.08	0.62	ug/ml
CL	1.68	1.68	1.88	1.75	0.11	0.06	ml/min/kg
Vss	0.90	0.71	0.85	0.82	0.10	0.06	L/kg

Appendix B-4c. Urine TCA TK monitoring after PZ induction (200 mg/kg, ip, 3 days) & TCA (50 mg/kg, iv): Oct. 31, 2005

	TCA Conc (μg/mL)						Urine volume (mL)					
Time (hr)	A'	B'	D'	G'	C'	E'	A'	B'	D'	G'	C'	E'
2	2,960.41	1,991.75	3,308.69	1,610.81	462.56	963.22	0.5	0.8	0.4	0.5	1.6	1.1
4	723.78	1,491.09	1,953.65	1,594.48	1,001.31	2,802.59	0.8	0.9	0.3	0.5	0.9	0.5
8	1,752.30	359.17	468.01	1,975.42	614.94	990.43	0.7	2.5	1.5	0.4	1.6	0.5
12	805.41	429.91	359.17	952.34	587.73	293.86	0.6	2.8	2.1	0.6	1.9	2.5
24	386.38	299.31	239.44	729.22	359.17	255.77	5.5	3.1	6.6	2.0	3.7	5.7
48	310.19	255.77	201.35	195.91	277.54	201.35	4.7	5.6	11.4	7.3	11.7	11.1

TCA Amount Excreted in Urine (μg)

Time (hr)	A'	B'	D'	G'	C'	E'	Ave	STDEV	SEM
2	1480.20	1593.40	1323.48	805.41	740.10	1059.54	1167.02	354.66	144.79
4	579.02	1341.98	586.10	797.24	901.18	1401.30	934.47	361.01	147.38
8	1226.61	897.92	702.01	790.17	983.90	495.22	849.30	250.36	102.21
12	483.24	1203.75	754.25	571.40	1116.68	734.66	810.67	290.37	118.54
24	2125.07	927.85	1580.34	1458.44	1328.92	1457.89	1479.75	388.55	158.62
48	1457.89	1432.32	2295.41	1430.14	3247.20	2235.00	2016.33	726.26	296.50
Accm (μg)	7352.04	7397.21	7241.57	5852.79	8317.99	7383.61	7257.54	792.77	323.65
b. w.	176	174	164	158	196	179			
Dose (μg)	8800	8700	8200	7900	9800	8950			
F_{Elim} (48 hr)	0.8355	0.8503	0.8831	0.7409	0.8488	0.8250	0.8306	0.0481	0.0196
F_{Elim} (2 hr)	0.1682	0.1831	0.1614	0.1020	0.0755	0.1184	0.1348	0.0425	0.0173

Time	TCA Amount Excreted in Urine accumulated (μg)									Cumulative Excretion, %		
hr	A'	B'	D'	G'	C'	E'	Ave	STDEV	SEM	Ave	STDEV	SEM
2	1480.20	1593.40	1323.48	805.41	740.10	1059.54	1167.02	354.66	144.79	13.48	4.25	1.73
4	2059.23	2935.38	1909.57	1602.65	1641.29	2460.84	2101.49	514.54	210.06	25.18	5.89	2.94
8	3285.84	3833.29	2611.58	2392.82	2625.19	2956.06	2950.79	533.58	217.83	35.88	6.23	3.12
12	3769.08	5037.05	3365.83	2964.22	3741.87	3690.72	3761.46	696.37	284.29	44.82	8.99	4.50
24	5894.15	5964.90	4946.17	4422.65	5070.79	5148.61	5241.21	590.72	241.16	62.96	5.86	2.93
48	7352.04	7397.21	7241.57	5852.79	8317.99	7383.61	7257.54	792.77	323.65	82.74	6.10	3.05

t_mid(hr)	dt	Xu	Xu/dt	ln(Xu/dt)
1	2	1167.02	583.5107	6.3691
3	2	934.47	467.2349	6.1468
6	4	849.30	212.3259	5.3581
10	4	810.67	202.6665	5.3116
18	12	1479.75	123.3126	4.8147
36	24	2016.33	84.01354	4.4310

Appendix B-5. Blood TCA monitoring after control & TCE (10 mg/kg, po): Feb. 19, 2005

	TCA Conc (μg/mL)							
	animal number							
min	B	C	D	E	F	Ave	STDEV	SEM
2	0.2283	0.0601	0.0968	0.1287	0.1268	0.1281	0.0625	0.0255
4	0.1315	0.1851	0.1963	0.3457	0.1221	0.1962	0.0897	0.0366
6	0.4500	0.3833	0.3805	0.4275	0.1466	0.3576	0.1216	0.0496
8	0.6106	0.3232	0.2593	0.4040	0.2142	0.3623	0.1561	0.0637
10	0.5402	0.2208	0.5477	0.6088	0.3495	0.4534	0.1624	0.0663
15	0.8729	0.1195	0.3863	0.6200	0.4577	0.4913	0.2796	0.1141
20	0.6944	0.7861	0.2161	0.5862	0.3856	0.5337	0.2319	0.0947
30	0.7933	0.4209	0.2942	0.8279	0.4539	0.5580	0.2384	0.0973
45	1.5013	0.6798	1.4208	0.4487	0.3562	0.8814	0.5429	0.2216
60	1.2311	1.1334	1.6012	0.7831	0.3551	1.0208	0.4726	0.1929
90	1.3769	1.9578	1.9548	0.5212	0.8666	1.3355	0.6433	0.2626
120	2.0304	0.7658	1.2863	1.0563	0.8448	1.1967	0.5080	0.2074
180	2.6003	1.8725	1.0890	1.1334	0.7264	1.4843	0.7500	0.3354
240	2.4182	1.8556	2.0187	1.6602	1.0116	1.7929	0.5182	0.2317
360	2.4993	2.0709	1.8068	1.5219	0.8218	1.7441	0.6287	0.2812
480	2.0713	1.7857	1.5054	1.6670	0.9353	1.5929	0.4218	0.1886
720	2.0886	1.5952	1.4588	1.1182	0.8233	1.4168	0.4812	0.2152
1440	0.7955	0.6241	0.4785	0.5547	0.3494	0.5605	0.1663	0.0679

Appendix B-5. Toxicokinetic parameters of TCA after control and TCE (10 mg/kg, po) administration: Feb. 19, 2005

	TCA Non compartmental model								
	B	C	D	E	F	Ave	STDEV	SEM	
AUC_INF	54.73	42.09	38.14	39.55	28.34	40.57	9.48	4.24	ug*h/ml
HL	10.59	10.04	8.98	10.03	10.86	10.10	0.72	0.32	h
Tmax	180	360	240	480	240	300.0	120.0	53.67	min
Cmax	2.60	2.07	2.02	1.67	1.01	1.87	0.59	0.26	ug/ml

Appendix B-6. Blood TCA monitoring after PZ induction (200 mg/kg, ip, 3 days) TCE (10 mg/kg, po): Feb. 13, 2005

	TCA Conc (μg/mL)							
Time	animal number							
	A	B	C	J	L	Ave	STDEV	SEM
2		0.3115	0.1637	0.1038	0.1757	0.1887	0.0877	0.0392
4	0.1038	0.5511	0.2556	0.1078	0.1557	0.2348	0.1871	0.0837
6	0.1118	0.3314	0.3195	0.1917	0.3234	0.2556	0.0990	0.0443
8	0.1438	0.9064	0.6070	0.1198	0.3075	0.4169	0.3356	0.1501
10	0.1358	1.2938	0.2755	0.1797	0.2755	0.4321	0.4856	0.2171
15	0.1086	1.2147	0.6677	0.2731	0.2388	0.5006	0.4505	0.2015
20	0.8280	1.4327	0.8250	0.6613	0.4041	0.8308	0.4371	0.1955
30	1.3225	2.0581	0.8857	1.0893	1.0039	1.2719	0.4677	0.2092
45	1.2419	0.9863	1.4136	1.3776	1.2874	1.2614	0.1684	0.0753
60	1.6476	2.5189	0.6860	0.8378	1.4647	1.4310	0.7312	0.3270
90	2.2266	2.8655	0.4776	0.8442	1.5477	1.5923	0.9782	0.4375
120	2.3296	3.0092	0.6956	1.0111	1.5909	1.6789	0.9162	0.4097
240	2.0876	2.1379	0.5950	1.0175	1.2802	1.4720	0.7402	0.3310
480	1.4407	1.0198	0.5199	0.7571	0.7683	0.9012	0.3496	0.1564
720	died	0.7667	0.4888		died	0.6277	0.1965	0.0879
1440		0.1382	0.1398	0.1334		0.1371	0.0033	0.0015

Appendix B-6. Toxicokinetic parameters of TCA after PZ induction & TCE (10 mg/kg, po): Feb. 13, 2005

	TCA: Non compartmental model								
	A	B	C	J	L	Ave	STDEV	SEM	
AUC_INF	24.65	25.73	12.43	15.66	17.91	19.27	5.75	2.57	ug*h/ml
HL	5.20	5.16	6.14	6.58	6.02	5.82	0.62	0.28	h
Tmax	90	120	45	45	120	84.00	37.65	18.82	min
Cmax	2.33	3.01	1.41	1.38	1.59	1.94	0.71	0.35	ug/ml

Appendix B-7a. Blood TCA monitoring after control & TCE (50 mg/kg, po): Feb. 11, 2005 and May 02, 2006

	TCA Conc (μg/mL)							
Min	A	B	C	M_A	M_D	Ave	STDEV	SEM
2	0.1243	0.2720	0.2354			0.2106	0.0769	0.0344
4	0.2193	0.3495	0.3685	0.6505	0.5872	0.4350	0.1788	0.0800
6	0.4284	0.6214	0.2968	1.0434	0.5556	0.5891	0.2828	0.1265
8	0.1316	0.6536	1.0148			0.6000	0.4440	0.1986
10	1.1464	0.9636	1.1508	1.3190	0.7724	1.0704	0.2087	0.0933
15	1.0864	1.1888	0.9490	1.2829	1.6307	1.2275	0.2572	0.1150
20	1.8336	0.7808	1.1669	1.5223	1.8746	1.4356	0.4636	0.2073
30	2.3937	1.2794	1.8892	1.5900	3.6227	2.1550	0.9175	0.4103
45	2.8645	0.8671	1.0221	4.8830	5.9806	3.1234	2.2822	1.0206
60	3.4450	4.1044	2.5004	4.6526	7.9681	4.5341	2.0805	0.9304
90	5.2611	4.5958	4.9145	8.4515	9.0658	6.4577	2.1247	0.9502
120	4.6519	5.1561	5.0630	10.7235	11.9251	7.5039	3.5184	1.5735
180	5.7096	5.1386	5.1403	11.8325	8.8083	7.3259	2.9445	1.3168
240	6.5582	6.4212	6.0797	13.0142	12.8968	8.9940	3.6208	1.6193
360	6.1974	6.2232	5.8501	12.8240	9.9511	8.2092	3.0776	1.3764
480	5.8566	6.1541	5.5971	10.2402	12.3542	8.0404	3.0718	1.3738
720	6.1670	4.8610	4.9453	9.2871	11.6236	7.3768	2.9745	1.3302
1440	3.5954	2.6296	2.2773	3.5256	3.4973	3.1050	0.6088	0.2722

Appendix B-7b. Toxicokinetic parameters of TCA after control & TCE (50 mg/kg, po): Feb. 11, 2005 and May 02, 2006

	TCA: Non compartmental model								
	A	B	C	M_A	M_D	Ave	STDEV	SEM	
AUC_INF	229.10	159.14	134.34	267.01	264.01	210.72	60.91	27.24	ug*h/ml
Beta_HL	ND	13.26	10.48	8.59	8.37	10.17	2.26	1.01	h
Tmax	240	240	240	240	240	240	0.0	0.0	min
Cmax	6.56	6.42	6.08	13.01	12.89	8.99	3.62	1.62	ug/ml

Appendix B-8a. Blood TCA monitoring after PZ induction (200 mg/kg, ip, 3 days) and TCE (50 mg/kg, po): Sep. 21, 2004

	TCA: Conc (μg/mL)						
min	A	B	C	E	Ave	STDEV	SEM
2	0.2695	0.3392	0.3624	0.2555	0.2900	0.0493	0.0221
4	0.5316	0.5390	0.3810	0.7806	0.5361	0.1903	0.0851
6	0.2462	0.2462	0.2555	1.0175	0.4175	0.3633	0.1625
8	0.5854	1.2731	0.2741	1.4450	0.8460	0.5257	0.2351
10	0.8905	1.4589	0.3903	0.9896	0.8950	0.5066	0.2266
15	0.2509	0.4089	0.4771	0.8363	0.4717	0.2865	0.1281
20	5.2548	0.6598	1.5193	1.7098	2.0373	1.6871	0.7545
30	3.7402	5.3524	3.9864	2.3556	3.3223	1.2438	0.5562
45	4.1816	6.2770	3.3174	2.0908	3.3135	1.7453	0.7805
60	6.4629	12.1684	2.6344	1.0593	5.5424	4.0915	1.8298
90	7.4293	20.2109	11.7363	7.1691	9.8285	4.7102	2.1064
120	5.9611	19.2631	13.4600	10.6165	10.0841	4.3708	1.9547
180	12.2790	9.0675	5.7297	6.9563	6.6598	1.9890	0.8895
240	9.3519	11.7307	4.8804	6.6664	5.9045	2.9617	1.3245
360	5.7185	7.5250	2.8639	4.5142	4.4080	1.3724	0.6138
480	died	5.5531	2.4922	3.8991	3.7658	1.4491	0.7246
720		3.4084	2.3751	4.1667	3.1371	0.8506	0.4911
1440		0.3996	0.0000	1.1876	0.7506	0.5270	0.3043

Appendix B-8b. Toxicokinetic parameters of TCA after PZ induction & TCE (50 mg/kg, po): Sep. 21, 2004

	TCA: non compartmental model							
	A	B	C	E	Ave	STDEV	SEM	
AUC	91.63	106.90	71.82	100.25	92.65	15.23	7.61	ug*h/ml
HL	6.88	4.21	9.55	8.33	7.24	2.30	1.15	h
Tmax	180	90	120	120	127.5	37.75	18.87	min
Cmax	9.88	17.58	11.70	9.23	12.10	3.80	1.90	ug/ml

Appendix B-9a. Blood TCA monitoring after Control and TCOH (50 mg/kg, iv): Oct. 21, 2004

	TCA Conc (μg/mL)						
	A	B	E	F	Ave	STDEV	SEM
5							
10							
20							
30							
45	1.5532	0.9376	0.5114	1.9794	1.2454	0.6499	0.3249
60	1.6858	2.5477	2.4056	2.7560	2.3488	0.4648	0.2324
90	5.4079	6.6949	3.1112	4.0441	5.4044	2.1609	1.0805
120	2.9739	6.1135	7.0985	5.5547	5.4351	1.7606	0.8803
180	2.9265	6.8486	4.9770	6.5160	5.2670	1.7350	0.8675
240	2.0457	2.9313	3.5232	4.5413	3.2604	1.0478	0.5239
300	7.5673	2.8081	5.1380	8.9643	5.5796	2.5404	1.2702
360	3.9849	2.6377	4.2903	4.6124	3.8813	0.8678	0.4339
480	5.2090	2.3038	8.3818	7.5199	5.8536	2.7194	1.3597
720	3.9091	2.1144	6.4166	4.6313	4.2678	1.7809	0.8905
1440	1.5367	1.6456	1.5580	1.4869	1.5568	0.0663	0.0331
2880	0.8926	0.4783	0.7387	0.1894	0.5748	0.3086	0.1543
4320	0.4783	0.0000	0.1894	0.0000	0.1669	0.2260	0.1130

Appendix B-9b. Toxicokinetic parameters of TCA after Control & TCOH (50 mg/kg, iv): Oct. 21, 2004

	TCA: non compartmental model							
	A	B	E	F	Ave	STDEV	SEM	
AUC_INF	97.68	95.86	156.21	125.39	118.79	28.38	14.19	ug*h/ml
HL	14.52	16.10	13.57	13.80	14.50	1.14	0.57	h
Tmax	360	180	480	300	330.0	124.90	62.45	min
Cmax	7.57	6.85	8.38	8.96	7.95	0.91	0.45	ug/ml

Appendix B-9c. Calculation of F_M (fraction metabolized) after control and 50 mg TCOH/kg i.v. Oct. 21, 2004

	A	B	E	F	Ave	STDEV	SEM	Unit
TCA AUC	5860.5791	5751.8682	9372.8896	7523.2819	7127.1547	1702.5343	851.2671	ug*min/ml
TCA AUC	35.8665	35.2012	57.3616	46.0421	43.6178	10.4194	5.2097	min*umole/ml
Ave bw	182.5	172.5	163.2	162.4				g
TCOH dose	9125	8625	8160	8120	50 mg/kg			ug
TCOH dose	61.0776	57.7309	54.6185	54.3507	56.9444	3.1538	1.5769	umole
TCA CL	0.5472	0.5472	0.5472	0.5472				ml/min/kg
TCA AUC X CL	19.6257	19.2617	31.3877	25.1937				umole
Fm (TCA)	0.3213	0.3336	0.5747	0.4635	0.4233	0.1197	0.0598	
TCOH CL	10.9840	10.9840	10.9840	10.9840				ml/min/kg
CL_F	3.5294	3.6648	6.3122	5.0915	4.6495	1.3146	0.6573	ml/min/kg

Appendix B-10a. Blood TCA monitoring after PZ induction and TCOH (50 mg/kg, iv): Sep. 17, 2005

	TCA Conc (μg/mL)						
Time	animal number						
Min	A'	C'	D'	F'	Ave	STDEV	SEM
5							
15							
30							
60	0.2113	0.2451	0.2473	0.1067	0.1997	0.0805	0.0403
120	0.2213	0.4945	0.2452	0.2335	0.2986	0.1309	0.0655
240	0.0000	0.3445	0.1817	0.2282	0.1886	0.1432	0.0716
450	0.0000	0.2082	0.1268	0.1574	0.1231	0.0887	0.0443
720	0.0338	0.0518	0.0972	0.0534	0.0590	0.0269	0.0135
1440	0.0000	0.0249	0.0328	0.0101	0.0170	0.0147	0.0074

Appendix B-10b. Toxicokinetic parameters of TCA after PZ induction & TCOH (50 mg/kg, iv): Sep. 17, 2005

	TCA: non compartmental model							
	A'	C'	D'	F'	Ave	STDEV	SEM	
AUC	2.21	2.57	2.94	2.16	2.47	0.36	0.15	ug*h/ml
HL	3.71	5.27	5.25	4.18	4.74	0.89	0.32	h
Tmax	120	120	60	120	105.0	30.00	15.00	min
Cmax	0.22	0.49	0.25	0.23	0.30	0.13	0.07	ug/ml

Appendix B-10c. Calculation of F_M (fraction metabolized) after PZ and 50 mg TCOH/kg i.v. Sep 17, 2005

	A'	C'	D'	F'	Ave	STDEV	SEM	Unit
TCA AUC	132.4679	154.1075	176.4236	129.3989	148.0995	21.8511	10.9255	ug*min/ml
TCA AUC	0.8107	0.9431	1.0797	0.7919	0.9064	0.1337	0.0669	min*umole/ml
Ave bw	169	178	161	167				g
TCOH dose	8450	8900	8050	8350	50 mg/kg			ug
TCOH dose	56.5596	59.5716	53.8822	55.8902	56.4759	2.3566	1.1783	umole
TCA CL	1.3941	1.3941	1.3941	1.3941				ml/min/kg
TCA AUC X CL	1.1302	1.3148	1.5052	1.1040				umole
Fm (TCA)	0.0200	0.0221	0.0279	0.0198	0.0224	0.0038	0.0019	
TCOH CL	19.4265	19.4265	19.4265	19.4265				ml/min/kg
CL_F	0.3882	0.4288	0.5427	0.3837	0.4358	0.0741	0.0370	ml/min/kg