TOXICOKINETICS OF VOLATILE ORGANIC COMPOUNDS AND METABOLITES KINETICS

by

CAREY C. HINES

(Under the Direction of CATHERINE WHITE AND JAMES BRUCKNER)

ABSTRACT

Volatile Organic Chemicals (VOCs) represent a significant threat to the health of both humans and animals. Widespread use of volatile organic chemicals (VOCs) has resulted in their dissemination throughout the environment. Many people living in the U.S. are exposed daily to these chemicals, primarily via indoor air and drinking water. Home use of volatile organic chemical (VOC)-contaminated tap water commonly results in exposure by multiple routes. Previously, toxicity/carcinogenicity risk assessments focused primarily on the amount of chemical ingested in the water an individual consumed. It is now recognized that inhalation exposures during showering and other water-use activities can also contribute significantly to the total systemically absorbed dose. The goal of this research is to understand the pharmacokinetics and pharmacodynamics of DCE, TCE, TRI and their metabolites when exposure occurs via different routes, as well as to contribute to the development of a physiological based pharmacokinetic model for DCE, TCE, and TRI. Many of the previous toxicokinetic studies with volatile organic compounds have been done by inhalation exposure. This is due to the fact that this is the primary route of exposure, but also due to the expense and time associated with oral gavage and gastric infusion. Compounds entering the body through the lung are only affected by one elimination organ which is the lung

before reaching systemic circulation, where as, when a compound is ingested it must pass through three elimination organs: Gut (efflux transporters), liver, and the lungs before reaching systemic circulation. The most important contribution of the findings in these studies are that they illustrate the importance of designing experimental protocols that are relevant to actual human exposures.

INDEX WORDS: Toxicokinetics, volatile organic compounds.Trichloroethylene, Dichloroethylene, and Trichloroethane,

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DEDICATION

This is dedicated to my friend and beloved Naomi. Thanks for believing in me and for your many encouraging words. You spent many nights helping me focus on the end of the tunnel when all I could see was the beginning.

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First I would like to acknowledge Jesus, for without him this would not have been possible. I would like to give a special thanks to Dr. White, Dr. Bruckner, Summer, Danielle, and Lonnie for all their help and encouragement.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW OF DICHLOROETHYLENE, 1,1,1-TRICHLOROETHYLENE, and 1,1,1-TRICHLOROETHANE

Volatile Organic Chemicals (VOCs) represent a significant threat to the health of both humans and animals and are widely present in the environment. This is due to the massive production of these compounds and the ubiquitous use over the past thirty years. Between 1969 and 1993 over 1.84×10^9 kilograms were produced in the Unites States alone. Many of these compounds make up a huge percentage of the chemicals found at hazardous waste sites around the United States and the world. The Environmental Protection Agency (EPA) defines VOCs as organic chemical compounds with vapour pressures under normal conditions to significantly vaporize and enter the atmosphere (CFR).. These compounds consist of a wide range of carbon-based molecules, such as aldehydes, ketones and hydrocarbons. VOCs are used in many different manufacturing industries such as food processing, textiles, wood products, furniture and fixtures, paper, printing and publishing, chemicals, petroleum, rubber, leather, stone and clay, primary metals, fabricated metals, industrial machinery, electronics, and transportation equipment. They are primarily used as solvents, carriers, or extractants; in dry cleaning of textiles; in metal cleaning and degreasing; in textile manufacturing; as insulation fluids/coolants; and as chemical intermediates (Doherty 2000).

The ability of VOCs to cause health effects varies greatly from those that are highly toxic, to those with no known health effects. As with other toxicants, the extent and nature of health effects will depend on the agent or its metabolites reaching the appropriate sites in the body at a concentration and for a length of time sufficient to produce a toxic response. Chemical and physical properties of the agent, the exposure situation, how the agent is metabolized by the system, and the over all susceptibility of

the biological system or subject, all contribute to the toxicity manifested from exposure to a VOC. Route of administration may play a significant role in the extent and location of manifested toxicity. Ingestion of VOCs can cause greater damage to the liver since a large percentage of an ingested compound will be introduced into the liver. Inhalation of these compounds may cause more damage to the Clara cells of the lung and other extrahepatic tissues due to the large percentage of the inhaled compound going directly into systemic circulation.

Although inhalation is by far the dominant route of exposure for most of the volatile chemicals, exposure to VOCs like trichloroethylene can also occur through the ingestion of contaminated foods and drinking water and through dermal contact (ATSDR 1995). Humans may be at an increased risk of exposure, due to the fact the tap water is contaminated with VOCs. VOCs vaporize at a much faster rate than water, causing serious health threats when contaminated water is used for showering or cooking. There are several populations, which are at an increased risk. These are the populations who live near waste sites, in urban and industrial areas, populations living near military bases, and those in an occupational setting where there is a continuous exposure to elevated levels of these compounds. According to EPA's website, its role is to protect plants, animals, humans, wildlife, aquatic life, and the environment from the negative effects that pollutants and toxic substances can have on their health. Under a broad range of federal statutes, EPA gathers health/safety and exposure data, requires the necessary testing, and controls human and environmental exposures for numerous chemical substances and mixtures. Under these laws, EPA regulates the production and distribution of commercial and industrial chemicals in order to ensure that

chemicals made available for sale and use in the United States do not harm human health or the environment. Along with the regulation of these substances, the Agency has created databases and documents that further the knowledge of the American people about the effects and prevalence of such substances.

Although, there is a generous database of information on the various VOCs from which to draw from there is still a paucity of data on systemic disposition and the toxicity that follows the absorption of VOCs from various portals and/or the influence of first-hepatic and respiratory elimination. The characterization of the effects of route of administration on toxicokinetics is imperative to the field of risk assessment. Volatile organic compounds due to the physical and chemical properties are present in various environmental media and are used in a number of different applications. In this thesis the focus is on route and rate of exposure in rats to determine if there is a dramatic difference in disposition, toxicities or degrees of toxicity associated with each route and rate of exposure.

The Environmental Protection Agency (EPA) has identified 1,350 hazardous waste sites as the most serious in the nation. These sites make up the National Priorities List (NPL) and are targeted for long-term federal cleanup. 1, 1-dichloroethylene (DCE), 1, 1- Trichloroethylene (TCE), and 1,1- Trichloroethane (TRI) has been identified at over 70 percent of these sites. Exposure to DCE, TCE, or TRI may cause numerous adverse health effects, such as lung, liver, as well as kidney toxicity.

The goal of this research is to understand the pharmacokinetics and pharmacodynamics of DCE, TCE, TRI and their metabolites when exposure occurs via

different routes, as well as to contribute to the development of a physiological based pharmacokinetic model for DCE, TCE, and TRI. The objectives of these studies are to define the effect that the route of administration and rate of oral administration has on the metabolic and toxicity profile of DCE, TCE, and TRI in rats. It is our belief that the disposition and the metabolic/toxicity profile of DCE, TCE, and TRI will vary as a function of the route of administration. Information derived from these studies should provide a more comprehensive understanding of DCE's, TCE's, and TRI's toxicokinetics and would allow for better risk assessments in the human population.

1,1-Dichloroethylene (DCE)

DCE does not occur naturally, but is produced commercially by the dehydrochlorination of 1,1,2-trichloroethane in the presence of excess base. DCE is primarily used for the production of polyvinylidene chloride polymers (PVDC). PVDC is used primarily by the food packing industry as cast and extruded film (Saran and Velon wraps) and as a barrier coating for paper, cellulose, polypropylene, and other plastics. DCE enters the environment through release during its manufacture and use, from the breakdown of PVDC products, and from the biotic or abiotic breakdown of 1,1,1-trichloroethane, tetrachloroethylene, 1,1,2-trichloroethene, and 1,1-dichloroethane (ATSDR, 1994; and EPA, 1985 a,b).

DCE is rapidly absorbed and distributed to all tissues following inhalation and oral exposures in mature animals (Jones & Hathway, 1978a,b; Putcha et al., 1986). DCE is rapidly oxidized by cytochrome P-450 2E1 to DCE epoxide, 2-chloroacetyl-chloride and 2,2-dichloroacetylaldehyde (fig.1.1). It is not known if the metabolism of DCE is the same in humans, although *in vitro* microsomal preparations from human liver and lung

tissues indicate the formation of the same primary metabolites (Benson, 2003 and EPA, 2002). The liver is the target organ following acute, long term or chronic exposure at less than an acutely toxic exposure in rats (EPA iris). Studies in other species such as mice indicate that the kidney is the target organ following inhalation exposure. Liver, kidneys, and the clara cells of the lungs are suspected to be target organs based on their expression of cytoctome p-450 2E1 (EPA, 2002).

DCE has been shown to be hepatotoxic after both oral and inhalation administration in with Sprague-Dawley rats (Jenkins and Andersen 1978; and Reynolds et al. 1984). In these studies, the toxicity was characterized by significant increases in sorbitol dehyrogenase (approximate 2-3 hundred-fold increase). In a 14-day study, DCE exhibited hepatotoxicity at high doses (NTP, 1982). In several chronic studies of DCE given by oral gavage, there were no significant biological changes (NTP, 1982; Quast et al., 1983; Maltoni et al. 1985). DCE cytotoxicity has been attributed to the ability of cytochrome P-450 catalyzed metabolic activation of DCE to its reactive intermediates that bind covalently to cellular macromolecules (EPA, 2002). Forkert and Moussa (1991,1992) observed that the extent of binding is inversely related to loss of glutathione (GSH), so that the severity of tissue damage parallel the decline in GSH.

There are several factors that may affect the levels of GSH. Two of these factors are age and fasting. In a human study conducted by Erden-Inal (2002) there were significantly lower levels of GSH in healthy individuals of age groups 0.2-1 year old and 41-69 years old as compared to other age groups. In an animal study, similar results were observed. Immature rats (24 ± 5 days of age) had significantly lower GSH levels when compared to their adult rat counterparts (3-4 months of age). It was also

observed in this study that in the younger animals, GSH stores were depleted faster than the adults after a single dose of acetaminophen. One hour after adult rat treatment, the level of hepatic GSH remained within the normal range, whereas during the same time frame, GSH depletion was about 20% in young rat tissue (Allameh, 1997). GSH levels are depleted when animals are fasted (Tateishi et al., 1974).

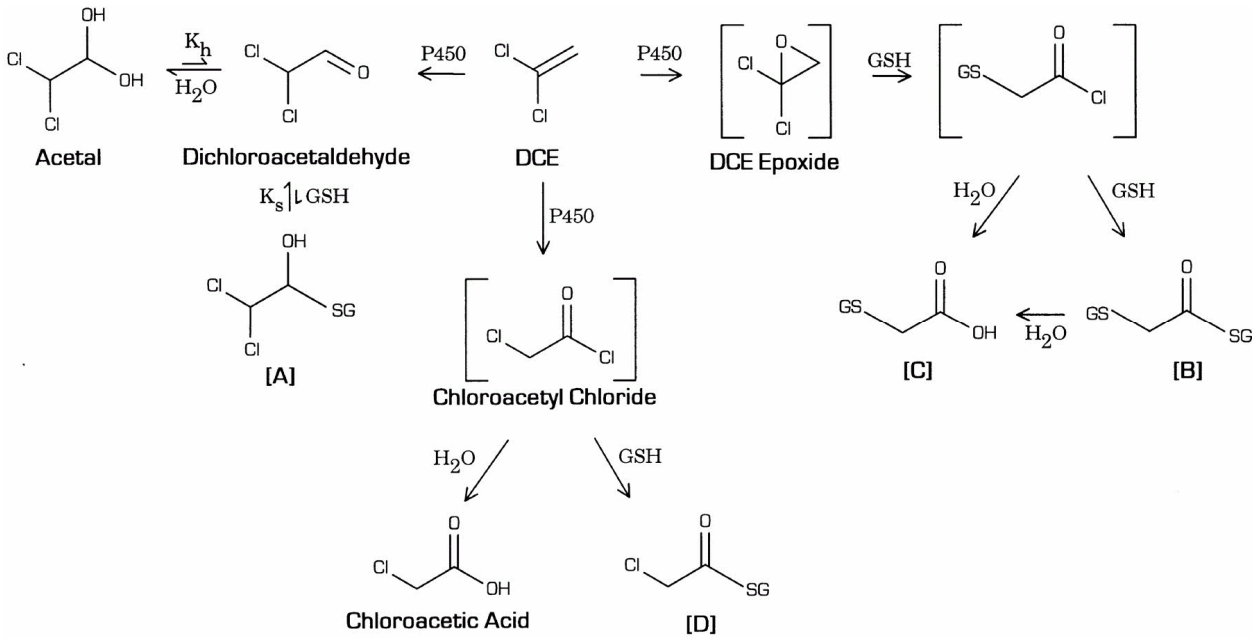

Figure 1.1: Proposed schematic DCE metabolism (Forkert, 1999)

1,1,1 Trichloroethylene (TCE)

TCE, also known by a variety of chemical and trade names; trichloroethene, ethinyl trichloride, and ethyl trichloroethylene to name a few, achieved public notoriety for its role in contaminating drinking water wells in Woburn, Massachusetts in the 1960's (Doherty 2000). As of 1997, TCE was reported as being present in 852 of 1430 national

priority list sites (NPL), making it one of the most commonly found contaminants at superfund sites (ATSDR, 1997). Over 12 billion pounds of TCE were produced in the United States from 1940 to 1990, with a peak production of approximately 600 million pounds coming in 1970 (Doherty, 2000). TCE has no know natural sources of production, but due to its effectiveness, noncorrosivity, nonflammability and ease of recycling it has been produced heavily in the Unites States and around the world. Few solvents match the performance of TCE in cleaning and degreasing (Doherty, 2000).

TCE was first produced in 1864 by the reductive dehalogenation of hexachloroethane (Doherty, 2000). Over the course of its history, TCE has been used in various entities, including the electronics, defense, chemical, medical, rail, automotive, boat, shoe, food processing, textile, and dry-cleaning industries. Many consumer products contain TCE including shoe polish, drain and pipe cleaners, household cleaners, spot removers, disinfectants, paint removers, wig cleaners, upholstery cleaners, deodorizers, typewriter correction fluid, adhesives, mildew preventives, and septic tank cleaners (Aviado et al., 1976; Schaumburg, 1990; National Safety Council, 1997). Vapor degreasing has been the predominat use of TCE. From 1940 to 1952, about 92% of TCE was consumed in vapor degreasing (Chem. Week 1953). In the early 1970s, approximately 87% of TCE produced in the United States was used in vapor degreasing (Lowenheim and Moran, 1975) and in 1991 that number rose to 90% of production (Chem. Mktg. Rep., 1992).

Several factors have led to the decrease in TCE production and use. Although TCE was termed the “ideal cleansing liquid, “ evidence of its toxicity was mounting in the early- and mid 1930s (Thomas, 1934). In 1970, the clean air act (CAA) controlled

TCE as a VOC due to its suspected contribution to ozone and smog formation. Later in March 1975 the National Cancer Institute (NCI) found that TCE causes cancerous tumor growths in mice livers (NCI, 1976).

Lee et al. (2000) and Templin et al. (1995), indicate the rate of systemic absorption and metabolism of TCE is rapid. In these studies, rats were dosed by oral gavage. TCE peak blood concentrations were observed within 2 to 26 minutes, and the metabolites, trichloroethanol (TCOH) and trichloroacetic acid (TCA), occurred at 2.5 and 12 hours, respectively. TCA concentration did not peak until well after the TCE concentration in the blood was below detectable levels (Templin et al. 1995). D'Souza et al. (1985) reported that oral and intravenous bioavailability of TCE was 60-90 % in nonfasted rats and greater than 90% in fasted rats. Peak blood levels occurred between 6-10 min and blood concentrations were 2-3 times higher in the fasted rats compared with the nonfasted rats.

Metabolism of TCE occurs by two pathways; oxidative and glutathione (GSH) dependent. An understanding of these pathways is important in determing susceptibility, target organ specificity, and extrapolation of animal data to humans. Research has focused on the liver, when observing the oxidative pathway, because it poses the highest activities of any tissue of various isoforms of Cytochrome P 450 (P450). P450 isoforms are present in most tissues in varying forms and amounts. Four different P450 isoforms have been identified as playing a role in TCE metabolism: CYP 1A1/2, CYP 2B1/2, CYP 2C11/6, and CYP 2E1 (Koop et al. 1985; Nakajima et al. 1988; Nakajima, et al. 1990; Guengerich et al. 1991; Guengerich et al. 1991; Nakajima et al. 1992; Nakajima et al. 1992; Nakajima et al. 1993). Of the four, CYP 2E1 appears to be

the major isoform with the highest affinity for TCE (Nakajima et al. 1990; Guengerich et al. 1991). The oxidation of TCE yields several metabolites. The initial step is thought to convert TCE to an epoxide, but there are some notable studies by Miller and Guengerich (1982; 1983), that suggest TCE epoxide is not an essential intermediate in the formation of chloral hydrate (CH). They proposed that the conversion takes place through an oxygenated transition state or chlorine migration. Others have provided substantial evidence that DCE, which is very similar to TCE, is metabolized by P450 to an epoxide intermediate(Forkert et al. 1991; Dowsley et al. 1996; Forkert 1999; Forkert et al. 1999). EPA's Health Assessment Document for TCE (EPA 1985) concludes that an epoxide intermediate is involved in the intial step of the oxidative metabolism of TCE. After the formation of the epoxide intermediate, CH (in equilibrium with chloral) is the major metabolite produced. It is rapidly reduced by CYP 2E1 to trichlroethanol (TCOH) and oxidized to trichloroacetic acid (TCA) by an aldehyde oxidase. TCA is produced by the oxidation of TCOH by CYP 2E1 (Ni et al. 1996). DCA is derived from TCA and TCOH (Lash 2000a). A scheme summarizing the major metabolic pathways for TCE is shown (figure1.2).

Only a few of the major metabolites have been characterized pharmacokinetically in rodents and humans. The plasma half-life of TCA in humans ranges from 86 to 99 hr after inhalation of either 50 or 100 ppm TCE, 6 hr/day for either 5 or 10 days (Fisher et al., 1998). Oral administration of either TCOH (10 mg/kg) or CH (15 mg/kg) in humans resulted in a 63- to 65-hr plasma half-life for TCA. Administration of TCA alone (3 mg/kg) resulted in a plasma half-life for TCA of 51 hr (Fisher et al., 1998). There are species differences with respect to half-life values. The plasma half-life for TCA is much

shorter in rodents than in humans. After intravenous administration of TCA (5-6 mg/kg), the TCA plasma half-lives were 12 and 7 hr in males and females, respectively (Fisher et al., 1991). In mice exposed to TCE vapors (42-889 ppm) for 4 hr, the estimated plasma half-life values for TCA were 16 and 7 hr for males and females, respectively (Fisher et al., 1991). In male and female rats exposed to TCE vapors (500-600 ppm), the estimated half-life value for TCA in plasma was 15 hr (Fisher et al., 1989).

TCOH has a half-life in the blood of about 12 hr in humans exposed to 50 ppm TCE vapors 6 hr per day for 5 days (Muller et al., 1972) and is 3 hr in mice administered 1,200 mg/kg TCE via oral bolus intubation (Abbas et al., 1997). CH and conjugated TCOH have half-life values of 3 and 5 hr, respectively (Abbas et al., 1997).

The toxicity of TCE is ascribed to its bioactivation, although the specific metabolites implicated in particular outcomes have not been fully resolved (Bull et al., 2000; Lash et al., 2000a; Odum et al., 1992). However, it has been proposed that chloral hydrate (CH), Dichloroacetic acid (DCA), and TCA are TCE metabolites associated with liver and lung toxicity, where as S-(1,2- dichlorovinyl)-L-cysteine is implicated in kidney toxicity (Lash et al., 2000b).

It has been well documented that TCE is a hepatotoxin and animal carcinogen. TCE affects different target organs in different species and strains of experimental animals. TCE may cause neurotoxic, hepatotoxic and or nephrotoxic effects (Kaneko et al., 1997; Bruning et al, 2000). Studies associate hepatic tumor induction with CH, TCA, and DCA in B6C3F_1, mice, while only DCA has been shown capable of inducing hepatic tumors in species other than the B6C3F_1 mice (Katz et al.,1981). DCA is an effective inducer of hepatic tumors in both mice and rats (Katz et al., 1981; Yount et al.,

1982; Herren-Freund et al., 1987; Bull et al., 1990; DeAngelo et al., 1991; Daniel et al., 1992; Richmond et al., 1995; DeAngelo et al., 1996; Pereira,1996; Stauber and Bull,. 1997; DeAngelo et al., 1999). There has been speculation on whether or not CH contributes directly to the carcinogenic effects of TCE, due to the fact that two of its metabolites are sufficiently potent as hepatic carcinogens in $B6C3F_1$ mice to account for the response to CH. TCA has been shown to induce hepatocellular carcinomas when administered in drinking water to male and female $B6C3F_1$ mice (Herren-Freund et al., 1987; Bull et al., 1990; Pereira, 1996). It has not induced hepatic tumors in F344 rats (DeAngelo et al., 1997).

At least four modes of action have been proposed for liver tumor induction after TCE exposure: somatic mutation, modification of cell signaling pathways, cell death and reparative hyperplasia, and hepatomegaly/cytomegaly (Bull 2000). According to Bull et al. (2000) the induction of liver tumor by TCE results from a modification of cell-signaling systems that control rates of cell division and death.

Urinary data collected by Dekant et al (1986) from rodents clearly identified the major metabolites of TCE as TCOH (free), TCA, and TCOH (conjugated). These account for approximately 92 - 94% of the total urinary metabolite recovery in rodents, with TCOH (conjugated) accounting for 70 – 86%, TCA accounting for 8 – 20%, and TCOH (free) accounting for 0.1 – 4% (Dekant et al., 1986).

Several studies have observed that TCE's first metabolic step is saturable in humans and laboratory animals. Saturation of metabolism can significantly affect its pharmacokinetics and toxic/carcinogenic potential (Watanabe et al., 1977). Rats exposed by inhalation to TCE concentrations of 50 or 500 ppm for 2 hours showed

metabolic saturation at 500 ppm (Dallas et al., 1991). This was indicated by the fact that TCE blood levels of the 500 ppm animals progressively increased over the 2-hour period, rather than approaching equilibrium after 25 minutes, as was the case at 50 ppm. Rodents that received radiolabled TCE by oral gavage showed saturation at 200 mg/kg, which was manifested in the increase in the proportion of exhaled unchanged TCE. Rodents that received 2 or 20 mg/kg exhaled approximately 3 – 4% of the dose as unchanged TCE, whereas rodents that received 200 mg/kg exhaled approximately 50 – 52% of the dose as unchanged TCE (Dekant et al., 1986). Ikeda et al. (1972) reported that urinary TCOH levels were proportional to inhaled TCE concentration of up to 200 ppm in human subjects. Urinary TCA levels, however, did not increase in proportion to dose at concentrations higher that 50 ppm.

Following inhalation exposure to TCE in humans, the unmetabolized parent compound is exhaled but its metabolites are primarily eliminated in the urine, and to a lesser degree through the feces. Exhaled air contained notable concentrations of TCE 18 hours after exposure because of the relatively long half- life for elimination of trichloroethylene from the adipose tissue (ie, 33.5-5 hours) compared to other tissues (Fernandez et al., 1977; Monster et al., 1979).

1,1,1-Trichloroethane

TRI, also known by a variety of chemical and trade names, became a widely used replacement for carbon tetrachloride (CTC), TCE and perchloroethylene (PCE) in metal degreasing and cleaning applications. The appeal of TRI was that it was less toxic than the chemicals it replaced, while offering excellent solvency for greases, oils, tars, waxes and many other organic materials (Doherty, 2000). In 1994, a chemical

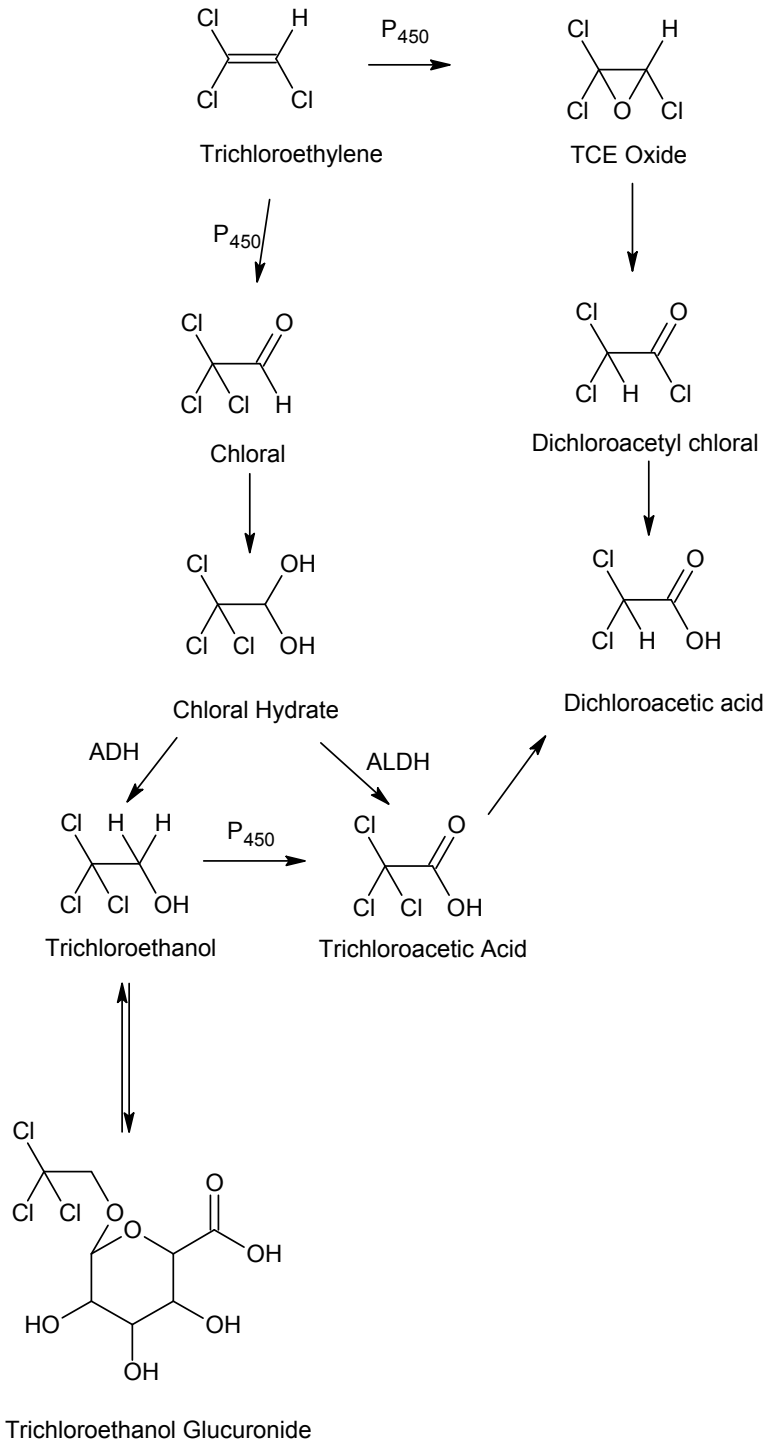

Figure 1.2: Proposed Schematic for TCE Metabolism

industry spokesman called TRI “by far the most used, easiest, and efficient cleaning solvent” (Kirchner, 1994). Between the years of 1966 until 1997 over 14 billion pounds were produce in the United states. TRI has no known natural sources of production.

TRI was first produced in 1931 by two methods: chlorination of 1, 1-dichloroethane (Doherty, 2000) and hydrochlorination, involving the addition of hydrochloric acid to 1,1-dichlrooethylene in the presence of a $FeCl_3$ catalyst (Doherty, 2000). TRI’s first commercial application was as a cold cleaning solvent, where it served as a replacement for CTC (Barber, 1957). As of 1994, 60% of TRI was used as a chemical intermediate, 25% in cold cleaning and degreasing, 5% in adhesives and 10% in miscellaneous uses (Chem. Mktg. Rep., 1995).

Problems with TRI began to surface in the mid-to-late 1970’s. Deaths resulting from the abuse of aerosol decongestants containing TRI caused the Food and Drug Administration (FDA) to recall the decongestants and require the registration of all drugs containing TRI intended for human use (USFDA, 1973). In the 1990’s regulatory pressure played a huge role in the demise of TRI: Federal excise tax due to its ozone depleting potential; Toxic Release Inventory “33-50” program, and CAA amendments were all contributors in the decrease usage of TRI.

TRI has been found in the ambient air around chemical manufacturing areas, in remote and rural areas, and around suburban sites. Other sources for small emissions of TRI to the atmosphere include coal fired plants, incineration of medical waste, incineration of industrial waste containing waste solvents and certain plastics, and municipal wastewater sludge (ATSDR, 1995). When contained in consumer products, TRI can be released to the atmosphere during application, drying, or curing of the

consumer products. TRI has been reported in groundwater, surface water, and drinking water in the United States. It has also reported in seawater. Levels of TRI have been reported in raw, processed, and prepared foods. Additionally, it has been detected in soils and sediments (ATSDR, 1995).

The general population may be exposed to TRI through inhalation of ambient air and inhalation of indoor air contaminated by use of household products containing this chemical. The general population may be potentially exposed to TRI through emissions from hazardous waste sites. TRI has been identified at 696 of 1,408 NPL hazardous waste sites. Inhalation is expected to be the predominant exposure route; however, exposure can also occur through ingestion of contaminated foods and drinking water and through dermal contact. Therefore, available data suggest that because of its ubiquitous occurrence in the environment and its use in many consumer products, much of the general population is exposed to low levels of TRI (ATSDR, 1995).

Exposure of the general population from the commercial use of products may potentially be more significant than exposure resulting from industrial release. ATSDR reported maximum exposure levels to this chemical during a variety of personal activities: visiting the dry cleaners (185 ppb); working in chemistry lab (18.5 ppb) and as lab technician (12 ppb); using pesticides (20 ppb); and using paint (20 ppb) (ATSDR, 1995).

The average daily intake (ADI) for air is assumed to be: in rural areas (0.110 ppb)-12.2 µg; urban/suburban areas (0.420 ppb)- 46.5 µg; for residents in source dominated areas assume (1.2 ppb)- 133.0 µg. The ADI for water is assumed in HSDB (1996) to be: surface water source (0.4 ppb)- 0.8 µg; groundwater source (2.1 ppb)- 4.2

μg. ATSDR (1995) assumed an average urban air concentration of TRI of 1 ppb and the average rural concentration of 0.1 ppb and calculated daily nonoccupational intakes of 108 and 10.8 μg/day, respectively. The estimate is based on an average human air intake of 20 m^3/day. ATSDR (1995) noted that Wallace et al. (1985) has determined the mean daily air exposures for 12 subjects at 2 urban areas at 37 mg and the mean daily intake from all sources (air, food, water) between 50 and 1,000 mg/day.

TRI is rapidly and completely absorbed from the gastrointestinal tract (ATSDR, 1995) following oral administration and rapidly absorbed following inhalation and reaches steady state for lung retention of 25 to 30% following 4 to 6 hours of continuous exposure (Monster et al. 1979, Nolan et al. 1984). During a whole body exposure to vapors, there is minimal exposure through the skin, with the respiratory tract expected to receive the highest level of exposure (ATSDR 1995). TRI is distributed through out the body of animals exposed by inhalation with measurable amounts seen in the fat, liver, and to a lesser extent , the kidney and brain and was rapidly cleared from the tissues when exposure ceased (Holmberg et al. 1977, Schumann et al. 1982, Takahara 1986). The pattern of distribution for oral route of administration is similar with the primary site being the adipose tissue with smaller quantities of TRI or its metabolites distributed to skeletal muscle, liver, and skin (RTI, 1987).

TRI is metabolized by P-450. Analysis of urine following human and animal exposure to TRI identified TCOH, TCOH-glucuronide, and TCA as major metabolites in the urine, while CO_2 was identified in exhaled breath (Kawai et al., 1991; Mitoma et al., 1985; Monster et al.,1979; Nolan et al., 1984; Reitz et al., 1988; Schumann et al., 1982a). The metabolic scheme of TRI is illustrated in figure 1.3. Evidence of P-450

involvement in the metabolism is limited. An in vitro study with rat hepatic microsomes and nicotinamide adenine dinucleotide phosphate (reduced form)(NADPH) oxidized TRI to TCOH (Ivanetich and Van den Honert 1981). In another study TRI metabolism was significantly increased, when a known inducer of P-450 (phenobarbital) was used to pretreat animals (Koizumi et al. 1983). TCOH to TCA is thought to involve alcohol and aldehyde dehydrogenases or P-450 isozymes (Casciola and Ivanetich 1984; Ivanetich and Van den Honert 1981). An in vitro production of chloral hydrate from TRI was demonstrated in reaction mixtures containing rat nuclei P-450 preparations (Casciola and Ivantich 1984).

Metabolism is responsible for less than 10% of the absorbed dose regardless of the exposure route (Reitz et al. 1988). Metabolites have been measured in both the urine and the blood (Nolan et al. 1984). In humans, exposed to 70 or 145 ppm TRI in air for 4 hours, an estimated 60-80% of the absorbed compound was excreted unchanged in exhaled breath (Monster et al. 1979). Small fractions of TRI metabolites, trichloroethanol (2%) and trichloroacetic acid (0.5%), were collected 70 hours after the initial absorption of TRI. Studies have indicated species differences. Comparison of metabolic disposition in mice and rats indicated that mice metabolized 2-3 times more TRI on a body weight basis than rate; however, in both species, metabolism was a dose-dependent, saturable process that represented a minor route of elimination (Schumann et al. 1982a, 1982b).

Due to the lipophilic nature of TRI, It is very likely transported across cellular membranes by passive diffusion. The majority of TRI is exhaled unchanged from the lungs which accounts for greater than 90 % of the absorbed dose, regardless of the

route of exposure (Nolan et al. 1984; Mitoma et al. 1985; Reitz et al. 1988; WHO 1992; US EPA 1984).

Wang et al. (1996) examined comparative effects of exposure to four organic solvents on selected cytochrome P450 isoenzymes in male wistar rats. Rats were exposed to 4000 ppm of benzene, toluene, TCE, or TRI or 6 hours in a dynamic inhalation exposure chamber. Following termination, liver microsomes were tested for enzyme activity. TRI exposure resulted in little difference from controls for these enzyme activities. Similarily, TRI exposure resulted in no significant induction of CYP2E1 and CYP2B1/2 (Wang et al. 1996). TRI apparently has a comparatively low effect on hepactic cytochrome P450 enzymes.

Nolan et al. (1984) observed the kinetics and metabolism of TRI in 6 male humans following the inhalation of either 35 or 350 ppm for a single dose over six hours. Approximately 25% of the inhaled dose was absorbed; and then eliminated in three phases with half-lives of 44 minutes, 5.7 hours and 53 hours. Less than 1 % remained after nine days (Nolan et al. 1984).

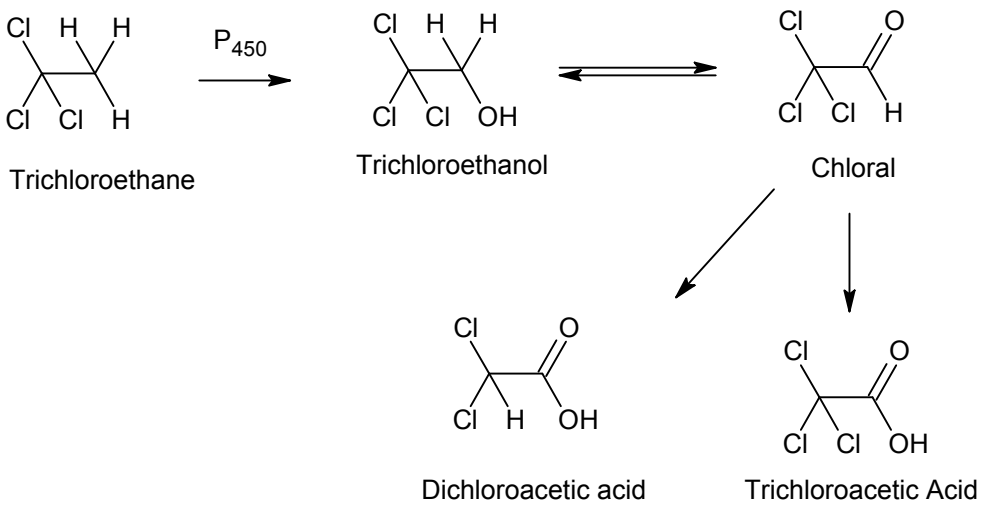

Figure 1.3: Proposed schematic of TRI metabolism

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CHAPTER 2

EFFECT OF RATE OF ORAL ADMINISTRATION AND ROUTE OF ADMINISTRATION ON THE TOXICOKINETICS OF DICHLOROETHYLENE IN RATS[12]

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ABSTRACT

The purpose of these experiments was to characterize the effects of route of exposure and oral dosage regimen on the bioavailability and toxicity of 1,1-dichloroethylene (DCE) in rats. Fasted male Sprague-Dawley rats received either 10 or 30 mg/kg of DCE by i.v. bolus, gavage (p.o.) or gastric infusion (g.i.) over 2 hours. Other rats inhaling 100 or 300 ppm DCE for 2 hours retained 10 and 30 mg/kg DCE, respectively. DCE was quantitated by gas chromatography. Toxicity was contrasted in the 30 mg/kg (p.o., g.i.) and 300-ppm inhalation groups by measuring plasma sorbitol dehydrogenase (SDH) and urinary N-acetyl-B-glucosaminidase (NAG) and gamma-glutamyltranspeptidase (GGT) activities. DCE's half-life (140 vs. 141 min) and clearance (0.065 vs. 0.06 L/min-kg) were comparable in the 10 and 30 mg/kg i.v. groups, reflecting linear kinetics in this dosage range. Clearance was not significantly different among routes of administration. However, the bioavailability of DCE increased approximately 2-fold when the oral dose increased from 10 to 30 mg/kg (gavage and g.i.), indicating saturation of first-pass metabolism. Additionally, bioavailability of DCE was consistently higher when given as an oral bolus than by g.i. Rats inhaling 100 or 300 ppm DCE had significantly greater bioavailability than their respective oral groups. SDH levels, an index of liver damage, were slightly elevated in the g.i. and inhalation groups and moderately elevated in the gavage group 24 h post exposure. GGT and NAG excretion in the urine, indices of kidney injury, were markedly elevated in rats that inhaled 300 ppm DCE. Slight or no differences in GGT and NAG from controls were observed in the rats dosed orally (p.o. and g.i.) with 30 mg/kg DCE. These findings

demonstrate that both the route of exposure and rate of oral administration substantially influence the toxicokinetics and systemic toxicity of DCE.

INTRODUCTION

Home use of volatile organic chemical (VOC)-contaminated tap water commonly results in exposure by multiple routes. Previously, toxicity/carcinogenicity risk assessments focused primarily on the amount of chemical ingested in the water an individual consumed. It is now recognized that inhalation exposures during showering and other water-use activities can also contribute significantly to the total systemically absorbed dose (Maxwell et al., 1991; Weisel and Jo, 1996). There is little information, however, on the systemic disposition and ensuing toxicities of VOC's absorbed from different portals or on the influence of first-pass hepatic and respiratory elimination. Quantitative risk assessments must often be conducted on the basis of toxicity data available for a single route of exposure, with direct extrapolation to other routes (Gerrity and Henry, 1990). Many oral cancer and non-cancer studies of VOC's have employed gavage dosing. The relevance of these bolus data to human risk assessment is questionable, as people typically consume VOC-contaminated water in smaller, divided doses over the course of the day.

1.1-Dichloroethylene (DCE), also known as 1.1-dichloroethene and vinylidene chloride, was selected for the current study. It is used primarily as an intermediate for the synthesis for other chemicals and in the production of polyvinylidene copolymers . These copolymers are utilized to produce flexible films (e.g., Saranwrap^{R,} , Velon^{R}) for food packaging and for barrier coatings for paper, cellulose and plastics. Environmental releases occur primarily by evaporation, though some DCE is released to soil, groundwater and surface waters. DCE is also formed by biotic and abiotic degradation

of common VOC's including 1,1,1-trichloethane (TRI), trichloroethylene (TCE) and perchloroethylene (PERC) (ATSDR, 1994). These are the most frequently found organic chemicals in groundwater in the proximity of hazardous waste sites (Fay and Mumtaz, 1996; Fay, 2005). The halocarbons appear to be major source of DCE detected in well water and in indoor air of homes and businesses located in VOC-contaminated areas.

Exposure to low levels of DCE in environmental media is of concern largely because of the VOC's cytotoxic and carcinogenic potential. The primary target organs of DCE are the liver, lungs, and kidneys (ATSDR, 1994; IRIS, 2001). DCE is usually not observed to be carcinogenic when given orally to mice and rats, but there is one report (Ponomarkov and Tomatis, 1980) of a modest increase in liver adenomas and carcinomas in rats gavaged with the chemical. The majority of rodent inhalation cancer bioassay results are also negative. Maltoni et al. (1985), however, describe statistically significant increases in kidney adenocarcinomas (males, high dose only), mammary carcinomas (females) and pulmonary adenomas (both sexes combined) in Swiss mice inhaling 25 ppm DCE for one year. It appears from these limited positive data that DCE may be toxic or carcinogenic to extrahepatic tissues of rodents upon inhalation but not ingestion.

Toxicokinetic studies can provide insight into the influence of exposure route and dosage regimen on DCE's toxic/carcinogenic potential. VOCs absorbed from the gastrointestinal (GI) tract are subject to first-pass elimination by the liver and lungs. In contrast, inhaled VOCs directly enter the arterial circulation and should be delivered to extrahepatic organs in relatively large amounts. Lee et al. (1996) demonstrated that the

liver and lungs, acting in concert, removed much of low doses of TCE before it reached the arterial blood of male Sprague-Dawley rats. DCE should also be efficiently removed, as it is also extensively metabolized and is more volatile than TCE. Although gavage dosing has commonly been utilized in toxicity and cancer bioassays of VOCs, this may result in such a rapid rate of delivery to the liver that much of the dose avoids metabolism and is distributed to/and acts upon other target organs.

The overall objective of the current investigation was to characterize the effects of exposure route and oral dosage regimen on the bioavailability and toxicity of DCE in rats. Experiments were designed to test the following hypotheses: a) Administration of equivalent oral and inhaled doses of DCE will result in significantly different internal doses, with more pronounced differences at lower dosage-levels; b) Inhalation will result in greater renal and pulmonary injury than ingestion, with the converse true for the liver; c) Administration of DCE as an oral bolus will result in higher peak blood concentrations, as well as more severe liver and kidney damage, than will prolonged ingestion of the same dose.

METHODS

Chemicals. 1,1-Dichloroethylene (DCE) of 99% purity was purchased from Aldrich Chemical Company (Milwaukee, WI). The purity was verified by gas chromatography. All other chemicals and biochemicals were obtained form Sigma Chemical Company (St. Louis, MO). Acepromazine hydrochloride and ketamine hydrochloride were purchased from Fort Dodge Laboratories (Fort Dodge, IA). Bayer Corporation (Shawnee Mission, KS) supplied xylazine hydrochloride.

Animals. Male Sprague-Dawley (S-D) rats were obtained from Charles River Breeding Laboratories (Raleigh, NC) and maintained on a 12-h light/dark cycle, with light from 7AM to 7PM. They were housed in groups of 3 in stainless steel wire mesh cages in a negative air flow animal rack. The rack was kept in an AAALAC- approved facility. The rats were maintained in a temperature (25ºC)- and humidity (40%)- controlled room for at least 1-week prior to use. Tap water and food (Purina Rat Chow 5001^{R}, Purina, St. Louis, MO) were available *ad libitum* during this period. Body weights for toxicokinetic and toxicity experiments were 325-375 and 140-150 g, respectively. Food was withheld for 24 h prior to DCE exposure. The experimental protocols were reviewed and approved by the University of Georgia animal care committee.

Animal Preparation. All rats were surgically cannulated for the toxicokinetic experiments. Each animal was anesthetized by i.m. injection of 0.1 ml/100 g body weight of a “cocktail” consisting of ketamine hydrochloride (100 mg/ml), acepromazine maleate (10 mg/ml), and xylazine hydrochloride (20 mg/ml) 3:2:1, v;v;v). An indwelling cannula (PE-50 polyehylene tubing) was implanted into the right carotid artery of each rat. The cannula was inserted into the artery toward the heart until the tip rested just above the aortic arch. The tubing was then securely ligated to the vessel. A second cannula was placed in the right jugular vein of some rats for i.v. dosing. For gastric infusion experiments, a ventral incision was made in the abdominal wall, and a flare-tipped cannula implanted into the fundus of the stomach. All cannulas were passed under the skin and exteriorized at the nape of the neck, so the recovered animals could move about freely. Water was provided but food was withheld during the 24-h postsurgical recovery period before dosing. Although fasting alone results in increased

liver cytochrome P450IIE1 (CYP2E1) activity, fasted S-D rats on which cannulation surgery is performend exhibit CYP2E1 activity similar to that of non-fasted controls (data now shown).

Inhalation Exposures. Exposures to DCE were conducted as described by Dallas et al. (1989). Each cannulated rat was placed into a restraining tube (Bartelle-Geneva, Switzerland) of the type used for nose-only exposures. A face mask with a miniaturized one-way breathing valve (Hans Rudolph Inc., St.Louis, MO) was secured to the animal's head with elastic straps. The mask was designed to fit 300-to 400-g rats, so that the valve entry port was immediately adjacent to the animal's nares and valve dead space was minimized. The valve included sampling ports, so the concentration of DCE in the separate inhaled and exhaled breath streams could be monitored. Desired concentrations of DCE were generated in a 70-L gas sampling bag (Calibrated Instruments, Ardsley, NY). The bag was connected in series by Teflon tubing to a pneumotachograph, the breathing valve, and an empty 70-L gas collection bag. Each rat was acclimated to the mask and restraining tube for 1 h until stable breathing patterns were established. Two- h exposures to 100 or 300 ppm DCE were initiated between 10:00 AM and 12:00 PM. Serial breath and micro blood samples were taken during and up to 3 h post exposure for DCE analysis.

Respiratory Measurements. The airflow created by an animal's inspiration was recorded, so the minute volume (V_E), respiratory rate (f) and tidal volume (V_T) could be determined during and following the inhalation exposures. An average value for these parameters was obtained for each rat by averaging measurements made at 10-min intervals. Mean values (+/- SD) (n= 6) for the 2-h, 100-ppm inhalation sessions were:

V_E = 230+/- 18 ml/min; f=136+/- 6 breaths/min; and V_T = 1.7 +/- 0.2 ml. Mean values (+/- SD) (n=6) for 2-h, 300-ppm sessions were V_E = 216+/- 37ml/min; f= 135+/- 23 breaths/min; V_T = 1.6+/- 0.2 ml.

Alveolar levels and cumulative uptake of DCE could be estimated from the exhaled breath data, f, V_E and dead space volume. A certain amount of the inhaled air resides in the valvular and physiological dead space and is exhaled without participating in alveolar gas exchange. The alveolar concentration was calculated by correcting for the contribution of DCE in this dead space to the exhaled breath concentration. Subtraction of the concentration of DCE in the alveolar air from that in the inhaled air yielded an estimation of the amount of compound that was taken up into the body each sampling period (Dallas et al., 1989). Thereby, it was possible to monitor percentage systematic uptake and cumulative uptake during the 2-h exposures.

Oral and Intravenous Dosage Regimens. Ten and 30 mg DCE/kg, the approximate doses absorbed during the 2-h 100- and 300-ppm inhalation sessions, were administered to unanesthetized, freely-moving rats i.v., oral gavage, and gastric infusion (g.i) Aqueous emulsions were prepared just prior to dosing using 5% Alkamuls^R (Rhone Poulene, Cranbury, NJ). The concentration of DCE in each emulsion was verified by headspace GC analysis. A total volume of 5 ml/kg of DCE emulsion was administered orally. A glass syringe and curved, blunt-tipped intubation needle served to give the oral bolus. A microprocessor-controlled P22 syringe infusion pump (Harvard Apparatus, Southnatick, MA) was used to infuse DCE via an indwelling gastric cannula. Serial blood samples were taken from the arterial cannula during and post exposure and analyzed for their DCE content.

Analytical Procedure. DCE concentrations in inhaled and exhaled air were measured with a Tracor MT560 gas chromatograph (GC) (Tracor Instruments, Austin, TX) equipped with an electron capture detector (ECD). Air samples were taken with a gas-tight syringe and injected directly onto a 6-ft x 1/8-in stainless steel column packed with 0.1% AT 1000 on GraphPak (Alltech Associates, Deerfield, IL). Operating temperatures were: 150º C, injection port; 360º C, ECD; and 70º C, column oven. The nitrogen (carrier gas) flow rate was 40 ml/min, with an additional make-up gas flow rate of 30 ml/min to the detector.

Blood DCE levels were quantified by GC headspace analysis. The volume of blood taken at a given sampling time depended upon the anticipated DCE concentration. Ten- to 100-µl samples were transferred to chilled headspace vials, which were immediately capped with a polytetrafluroethylene-lined septa and tightly crimped. The vials were placed into the HS-6 headspace autosampler of a Sigma 300GC (Perkin-Elmer, Norwalk, CT), equipped with an electron capture detector and a 6-ft x 1/8-in stainless steel column packed with FFAP Chromasorb W-AW (80-100 mesh) (Alltech Associates, Deerfield, IL). Operating temperatures were: 200º C, injection port, 360º C, ECD; and 70º C, column oven. The carrier gas was 5% argon-methane at a flow rate of 40ml/min, with a make-up gas flow rate of 20ml/min to the ECD.

Toxicity Experiments. The objective of these experiments was to elucidate the target organ toxicity of inhaled and ingested DCE. The higher dosage-levels (i.e., 300 ppm and 30 mg/kg) utilized in the toxicokinetic studies were employed here. Fasted male S-D rats of 140 to 150 g were utilized in the current study, since Anderson and Jenkins

(1977) found; heavier rats were much less susceptible to DCE-induced hepatotoxicity; and fasting markedly increased the severity of liver injury. Martin et al. (2003)As these investigations note that serum enzyme activities were maximal 24 h post gavage dosing, we chose this period for blood collection and tissue sampling. The time in which it takes DCE to have a toxic effect in the kidney is unclear, though most studies have been carried out 24 h post exposure. Twelve and 24 h were selected for monitoring urinary indices of nephrotoxicity.

Groups of 140- to150-g male S-D rats were subjected to; a) 300 ppm DCE inhalation for 2 h; b) 30 mg DCE/kg by gavage; or c) 30 mg DCE/kg by constant g.i. over 2 h. For inhalation exposures the animals were maintained individually in a 26.5-liter glass chamber equipped with a fan. The chamber was had a port for introduction of DCE and for withdrawal of air samples. These samples were taken every 10 min during the 2-h exposures and their DCE content measured by a GC with a flame ionization detector. The chamber concentration (+/-S.E., n=8) decreased gradually from 306 +/-11 ppm at the beginning of the session to 238 +/- 13 ppm at the end of the 2 h. As in the toxicokinetic experiments, orally-administered DCE was incorporated into a 5% aqueous Alkamuls® emulsion and given in a total volume of 5 ml/kg as a bolus or by g.i. Blood was taken by cardiac puncture 24 h post dosing for measurement of sorbitol dehydrogenase (SDH) activity by a standard spectrophotometric procedure.

The second subset of rats was kept for evaluation of urinary enzyme excretion over a 24-h period after initiation of the DCE exposures. These animals were maintained in Nalgene® plastic metabolism cages for collection of 12- and 24- h urine over ice. Total N-acetyl-glucosaminidase (NAG) and gamma-glutamyltranspeptidase

(GGT) activities were measured colormetrically in urine voided during each collection period.

Toxicokinetic Analyses. Blood DCE concentration time profiles were analyzed with Winnonlin (Pharsight, Apex, NC). The i.v. data were fitted to a two-compartment model. Data from animals receiving DCE orally were analyzed by noncompartmental analysis. A two- compartment model with a constant input function was fitted to the concentration versus time data for inhalation area under the blood concentration time curve (AUC), apparent clearance (CL), terminal half-life ($t_{1/2}$), maximum concentration (C_{max}), time to reach C_{max} (T_{max}) was obtained using Winnonlin. Bioavailability was calculated as follows:

$$F = \frac{AUC_{oral}\ Dose_{iv}}{AUC_{iv}\ Dose_{oral}}$$

The model that was the "best fit" for the DCE blood concentration data was determined by model selection criteria including weighted sum of residuals, Akaike's Information Criteria (AIC), F test, and Schwarz criteria (SC).

Statistical anaylses. Most results are expressed as mean ± S.E. The data were analyzed by one- and two-way analysis of variance followed by Tukey's multiple comparison test ($p < 0.05$).

RESULTS

Toxicokinetics. The target chamber concentrations for the DCE inhalation exposures in the kinetics experiments were 100 and 300 ppm. The actual concentrations inhaled by the animals were determined by analyses of air samples taken from sampling ports immediately adjacent to the animal's miniaturized breathing valve. The inhaled DCE

concentrations for the 6 rats in each group at the initiation of exposures were 101.6 ± 0.8 and 310.0 ± 3.5 ppm (± S.E.) for the 100 and 300 ppm groups, respectively.

Blood concentrations versus time profiles revealed that inhaled DCE was rapidly absorbed into and eliminated from the body (Fig. 2.1). DCE was very quickly absorbed from the lungs and readily available for distribution to body tissues, in that arterial blood concentrations were > 50% of near steady-state at the first sampling time (i.e., 2 min). Blood concentrations in the 100- and 300-ppm groups reached near equilibrium within 10 to 15 min, and increased asymptotically for the duration of the exposures (Figs. 2.1A and B). The C_{max} and AUC values increased 30-fold as the inhaled concentration increased from 100 to 300 ppm (Table 2.1). DCE concentrations in the exhaled breath exceeded alveolar concentrations during the inhalation exposures, due to the contribution of unabsorbed DCE in the respiratory dead space (Fig. 2.1). The converse was true post-exposure. Exhaled breath levels were relatively low, due to dilution with fresh air residing in the dead space. Alveolar and exhaled breath levels of DCE exhibited a biexponential decline post exposure, with initial precipitous drops. The terminal elimination half-lives for the 100- and 300-ppm groups were 50.4 and 50.3 min, respectively (Table 2.1).

DCE was injected i.v., in order to assess the dose-dependency of its disposition and to serve as a reference for determination of its oral bioavailability. Blood DCE concentration versus time profiles are shown in Fig.2.2. An extremely rapid initial drop in DCE levels is followed by a relatively slow, prolonged decline. $T_{1/2\alpha}$ and $T_{1/2ß}$, as well as CL values, are independent of dose, reflecting linear kinetics in the 10 to 30 mg/kg dosage range (Table 2.2). There is a~3-fold increase in AUC with the 3-fold increase in

administered dose. The elimination rate constants K_{12} and K_{21} indicate a rapid transfer of DCE from blood to tissues, followed by a slower release from the tissues back into the blood.

Systemically-absorbed doses of DCE were determined from the inhalation data, Subtracting of the quantity of DCE exhaled from that inhaled, with allowance for dead space, yielded the amount and percentage of chemical taken up each sampling time. Percentage systemic uptake diminished slightly during the first 20 – 30 min, but remained relatively constant at 61-66% at near steady-state. Uptake appeared to be independent of exposure level until the last 30 min of the 2-h sessions when uptake in the 300-ppm group progressively diminished (Fig 2.3). As described in the methods, cumulative uptake over the 2-h exposures could be determined by summation of uptake for each time-period and by monitoring respiratory minute volume. Cumulative systemic uptake is plotted against duration of exposure in Fig. 2.4. The mean (±S.E.) total systemically absorbed doses at 2 h in the 100- and 300- ppm groups were 9.7±0.9 and 29.7±1.7 mg/kg, respectively. Thus, systemic uptake was directly proportional to the inhaled concentration.

Ten and 30 mg DCE/kg were administered over 2 h by g.i. and the arterial blood time-courses contrasted with their corresponding inhalation profiles, in order to determine the influence of route of exposure an internal dosimetry. It is obvious in Figs. 2.5A and B that the pattern of systemic uptake and elimination for each g.i. group was quite different from that for its corresponding inhalation group. Blood DCE levels progressively increased during the 2-h infusions and then declined at a relatively slow rate post exposure. As a result, AUC values for the 300-ppm inhalation and 30-mg/kg

g.i. groups did not differ substantially, though the observed C_{max} was ~2-fold higher than that for the inhalation group (Table 2.1). At the lower exposure level there was a ~2-fold higher AUC and a ~3-fold higher Cmax in the inhalation group. This may be attributed to the more efficient first-pass hepatic elimination of the 10 mg/kg g.i. dose. Interestingly, clearance was independent of the route of exposure.

The influence of oral dosage regimen on the toxicokinetics of DCE was evaluated by comparing arterial blood profiles of the p.o. and g.i. groups (Fig. 2.6A and B). DCE was rapidly absorbed after it was given as an oral bolus. Blood levels were maximal within 5 to 6 min. With the 3-fold increase in bolus dose, there were 4- and 4.6-fold increases in C_{max} and AUC, respectively (Table 2.1). The 3-fold increase in g.i. dose resulted in 6.5-fold increases in C_{max} and AUC. Peak blood levels in these animals were markedly lower than those observed in the corresponding bolus animals. Prolonged elevation of blood levels in the g.i. groups, however, resulted in AUC and F values that appeared to be only modestly, but not significantly, lower than in the corresponding p.o. groups.

The extent of liver damage by DCE was significantly influenced by the oral dosage regimen but not by the exposure route. All three modes of administration produced significant increases in serum SDH activity over controls 24 h after dosing (Fig. 2.7). The increase was most pronounced in the gavage group. SDH levels in the g.i. and inhalation animals were not significantly different from one another.

The pattern of kidney injury by DCE was quite different from that in the liver. Urinary GGT excretion was markedly elevated in the rats that inhaled 300 ppm DCE (Fig. 2.8). The majority of GGT was eliminated during the first 12 h post exposure.

Urinary GGT activity was not different from controls at 12 or 24 h in animals dosed by gavage or g.i. Exposure route- and time-dependent changes in urinary NAG activity largely paralleled the aforementioned changes in GGT (Fig. 2.9). There was however a slight, but statistically-significant increase over controls in the 12-h NAG in the inhalation group.

DISCUSSION

There is a paucity of toxicokinetic data relevant to route-to-route extrapolations for VOCs. Dekant (1996) postulated that hepatic first-pass metabolism of orally-administered DCE would prevent the pronounced nephrotoxicity manifest in mice inhaling the chemical. A few study protocols have entailed exposure of rodents to a VOC orally and by inhalation (Reitz et al., 1988). In these cases the test chemical was often administered over different time-frames (e.g., by inhalation for several hours and gavage)(Reitz et al., 1988) . The utility of such data for route extrapolations is quite limited. Systemically-absorbed doses have rarely been determined for inhalation exposures, so it has usually not been possible to administer equal doses by different routes. Our experimental approach involved administration of equivalent doses of the chemical over the same time-frame by inhalation and g.i.. This seemed to be a logical approach to a rather complex problem, namely comparing the kinetics of a chemical entering the systemic circulation from different portals at different rates.

Findings in the present study demonstrate that route of exposure has a significant effect on the toxicokinetics of DCE. The lungs are an optimal site for absorption of DCE and other VOCs, due to the lungs' relatively large surface area, high

blood perfusion rate, and intimate alveolar-capillary interfaces with diffusion of chemical directly into the arterial circulation. The rapid, extensive absorption of DCE into the arterial circulation was anticipated for the small, unchanged lipophillic molecule. Substantially higher concentrations of DCE are obviously reaching the lungs,kidneys and other extrahepatic tissues of the animals exposed by inhalation. DCE is also well absorbed from the GI tract, but is subject to first-pass hepatic and pulmonary elimination. The pronounced influence of presystemic elimination on gastrically-infused DCE is evidenced by the relatively low Cmax, AUC_0^{∞} and bioavailability values of the g.i. groups. (Table 2.1) The influence of presystemic elimination is most pronounced at the lower dosage. These route-dependent differences are less evident at the higher dose. The appreciable amounts of the inhaled chemical are also likely reaching the liver as a result of the relatively high arterial concentrations (Fig. 2.5B) and the receipt of 10% of the cardiac output via the hepatic artery. The actual hepatic doses of DCE in the inhalation and g.i groups were not determined, but the comparable degree of hepatotoxicity suggests they were not too different.

Judging from DCE's volatility and extensive metabolism, the chemical should be quickly eliminated from the systemic circulation. This proved to be the case. Determination of the distribution rate constants K12 and k21 revealed there was rapid uptake by tissues from blood, followed by a slower release back into the bloodstream. There were no significant changes in clearance as a function of route of exposure or dose. The terminal elimination half-life for both i.v. doses was 140 min. D'Souza and Anderson (1988) found DCE's i.v. half-life in rats to be 120 min. These investigators' PBPK models predicted that metabolic saturation at relatively high DCE doses would

not result in reduction in clearance, due to rapid exhalation of the VOC. In the current study, first-pass hepatic and pulmonary elimination significantly diminished the bioavailability of orally-administered DCE. The first-pass effect was somewhat more pronounced in the g.i. than the p.o. animals, as expected with the slower delivery of the compound to the liver and its more efficient metabolism in the former group. Bioavailability increased significantly with dose in each oral group, indicative of saturation of first-pass hepatic metabolism. High dose studies have revealed that DCE oxidative metabolites can inhibit CYP2E1 activity in the liver and kidney of rodents (Hanioka et al., 1997; Forkert, 2001). It is not clear whether the dose used in the present study could significant inhibit CYP2E1 within 2h..

DCE is biotransformed to reactive, cytotoxic metabolites in each of three primary target organs: liver, kidney and lungs. DCE is largely metabolized by CYP2E1 in rodents to three reactive metabolites: DCE epoxide; 2-chloroacetyl chloride; and 2,2-dichloroacetaldehyde. Forkert (2001) concluded from studies of mouse liver and lung that DCE epoxide was the major cytotoxic metabolite. Bioactivation of DCE in mouse lung is catalyzed both CPY2E1 and CYP 2F2 (Simmonds et al., 2004a). Dowsley et al. (1999) also find CYP2E1-mediated DCE epoxide formation in human liver and lung microsomes. Simmonds et al. (2004b) report that recombinant rat CYP2E1 exhibits greater affinity and catalytic efficiency for DCE metabolism than recombinant human CYP2E1 or mouse CYP2F2. The electrophilic metabolites can damage cells by binding covalently to proteins and nucleic acid (Jones and Liebler, 2000). DCE covalent binding and cytotoxicity are greatest in murine cells with the highest CYP2E1 content (i.e., centrilobular hepatocytes, renal proximal tubular cells, bronchiolar Clara cells)

(Speerschneider and Dekant, 1995; Forkert, 2001). The strain of mice with the highest hepatic CYP2E1 content exhibits the most pronounced DCE-induced hepatic covalent binding, DCE epoxide-derived conjugate levels and centrilobular necrosis (Forkert and Boyd, 2001). Each of the three aforementioned electrophilic metabolites is detoxified by hydrolysis and/or conjugation with glutathione (GSH). Forkert (2001) and Brittebo et al. (1993) have demonstrated that GSH affords hepatocytes and proximal tubular cells protection from DCE until GSH levels are markedly depleted.

Route of administration had a pronounced influence on kidney injury by the higher DCE dosage used in the current study, as would be anticipated from the exposure routes' significant effects on peak blood levels and bioavailability. It is worthy to note that the exposure route had an even greater effect on the lower dosage, where nephrotoxicity was not evaluated. The kidneys were apparently subjected for a prolonged time to relatively large quantities of the chemical during inhalation of 300 ppm, due to the elevated arterial concentration (fig 2.5) and the high rate of renal blood flow. CYP2E1-catalyzed metabolic activation *in situ* has been reported to be responsible for DCE nephrotoxicity in mice (Brittebo et al., 1993; Speerschneider and Dekant, 1995). CYP2E1 is a constitutive enzyme in the kidneys of male S-D rats, but protein content and activities are significantly lower than in the liver (Goasduff et al., 1996). Cummings et al. (2001) reported higher CYP2E1 activity and TCE metabolism in proximal than in distal tubular cells of male Fischer-344 rats. Thus, it is probable that CYP2E1- mediated bioactivation *in situ* was responsible for the pronounced proximal tubular damage we saw in male S-D rats. Although the hepatic GSH conjugation/renal ß-lyase pathway plays a major role in the nephrotocxicity of halocarbons such as TCE

and PERC, this is not the case for DCE (Dekant, 1996). 2, 2-Dichloroacetaldehyde, a minor metabolite of DCE in mouse liver microsomal preparations that does undergo hepatic GSH conjugation and subsequent metabolism by renal ß -lyase, has not been detected *in vivo* (Forkert, 2001). Thus, the amount of parent compound reaching the kidneys, its subsequent bioactivation to DCE epoxide, and possible GSH depletion would appear to be the primary determinants of the extent of injury observed in the 300 ppm inhalation group current investigation.

Oral dosage regimens had only a modest effect on the magnitude of DCE nephrotoxicity, but substantially influenced the extent of hepatoxicity. With bolus dosing the rate of delivery of chemical to the liver exceeded the liver's metabolic capacity. The relatively high arterial levels in these animals would be expected to result in greater deposition and possible adverse effects in other tissues (e.g., kidneys, lungs) where it can be metabolically activated. Urinary GGT excretion was indeed somewhat higher in the p.o. than in the g.i. animals, though the converse was true for NAG. Liver injury, as reflected by serum SDH levels, was much more extensive in the bolus group. It would appear that so much DCE arrives and is metabolically activated in the liver of these animals, despite the onset of metabolic saturation, that the capacities of hepatocellular detoxification (e.g., GSH conjugation) and repair systems are exceeded.

Other investigators have observed that dosage regimen can significantly influence the outcome of oral studies. There have been a number of studies in which adverse effects of VOCs ingested in water are very different from those produced by simple oral bolus doses. Chloroform ($CHCl_3$), 1,1- and 1,2-dichloroethane have produced a high incidence of hepatocellular carcinoma when administered to B6C3F1

mice gavage. In contrast, Jorgenson et al. (1985) and Klaunig et al. (1986) found no evidence of hepatic tumors when comparable doses were supplied in the animals' drinking water. Larson et al. (1994) saw dose-dependent increases in hepatocellular necrosis and proliferation in female B6C3F1 mice in response to repeated daily bolus dosing with $CHCl_3$. Similarly, Coffin et al. (2000) found $CHCl_3$ enhanced cell proliferation and decreased methylation of the promoter region of the *c-myc* gene in mouse liver when given by gavage. Relatively minor changes occurred in mice ingesting equivalent daily doses of the chemical in water *ad libitum*. La et al. (1996) measured greater DNA binding and cellular proliferation in the liver, kidney and forestomach of male B6C3F1 mice that were gavaged with 1, 2,3-trichloropropane than in mice receiving the halocarbon via drinking water. It is not possible to relate these dissimilar findings to differences in internal dosimetry, since none of the investigators conducted toxicokinetic studies. Sanzgiri et al. (1995) did report that carbon tetrachloride is more hepatotxic to rats and reaches higher arterial levels when given p.o. rather than g.i. In the current study, we have demonstrated that external exposure route and dosage regimen can each significantly affect the magnitude of internal exposure and target organ toxicity of DCE, a common environmental contaminant. Such reports illustrate the importance of designing experimental protocols that are relevant to actual human exposures.

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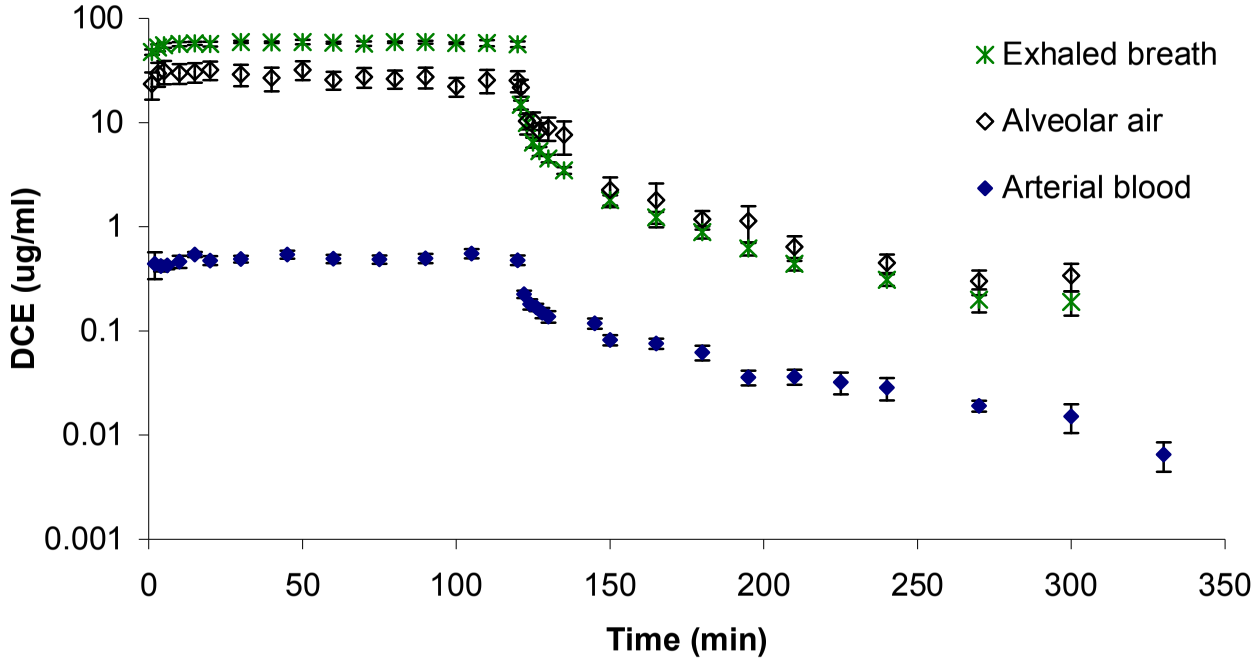

Figure 2.1 A: Dichloroethylene concentrations upon inhalation of 100 ppm of the chemical. Each point represents the mean ± SE for 6 rats

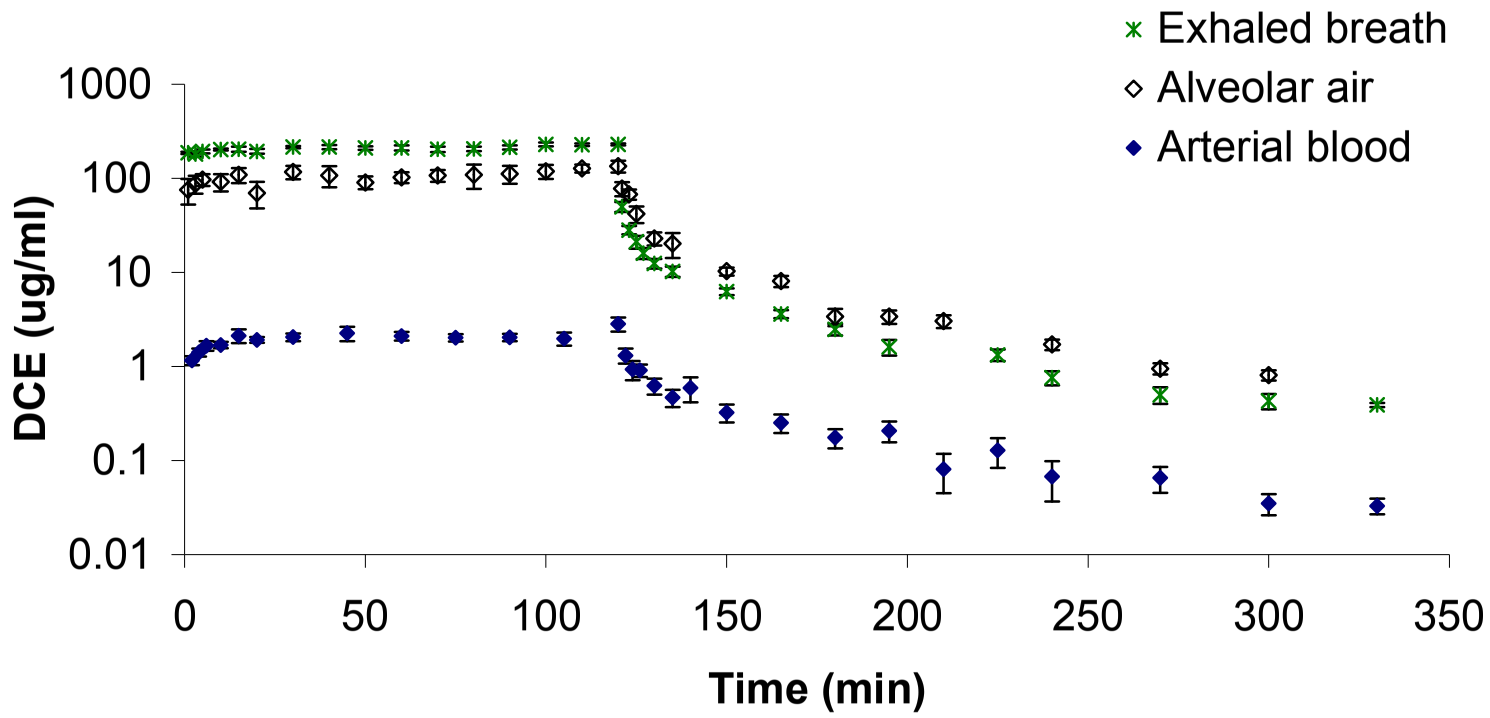

Figure 2.1B: Dichloroethylene concentrations upon inhalation of 300 ppm of the chemical. Each point represents the mean ± SE for 6 rats

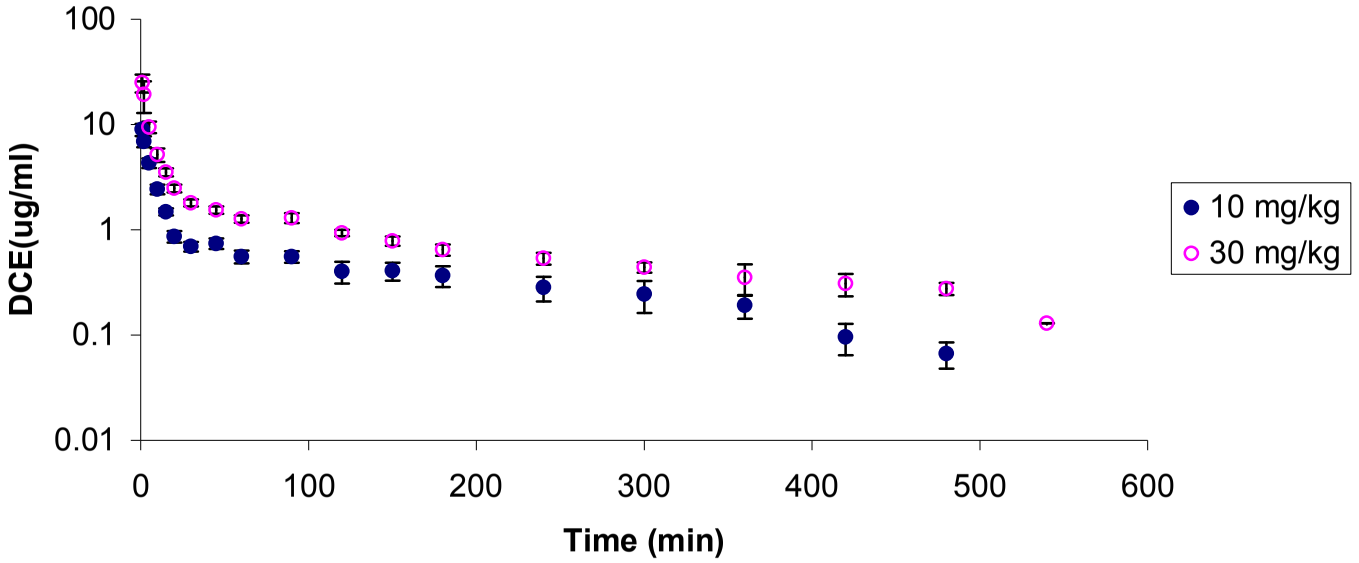

Figure 2.2: Concentrations of DCE in the arterial blood after IV administration of 10 or 30 mg DCE/kg. Brackets encase mean ± SE for groups of 6 rats.

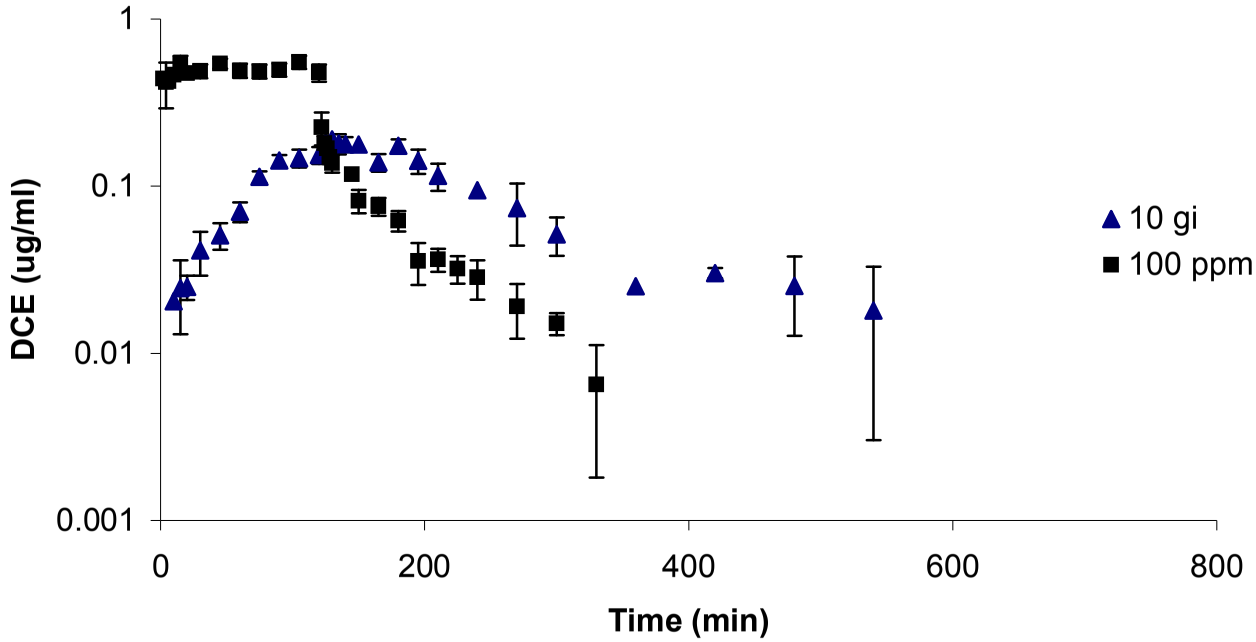

Figure 2.3: Percentage systemic uptake of DCE during inhalation of 100 or 300 ppm DCE for 2 h. Each point represents the mean % uptake ± S.E. for 6 rats.

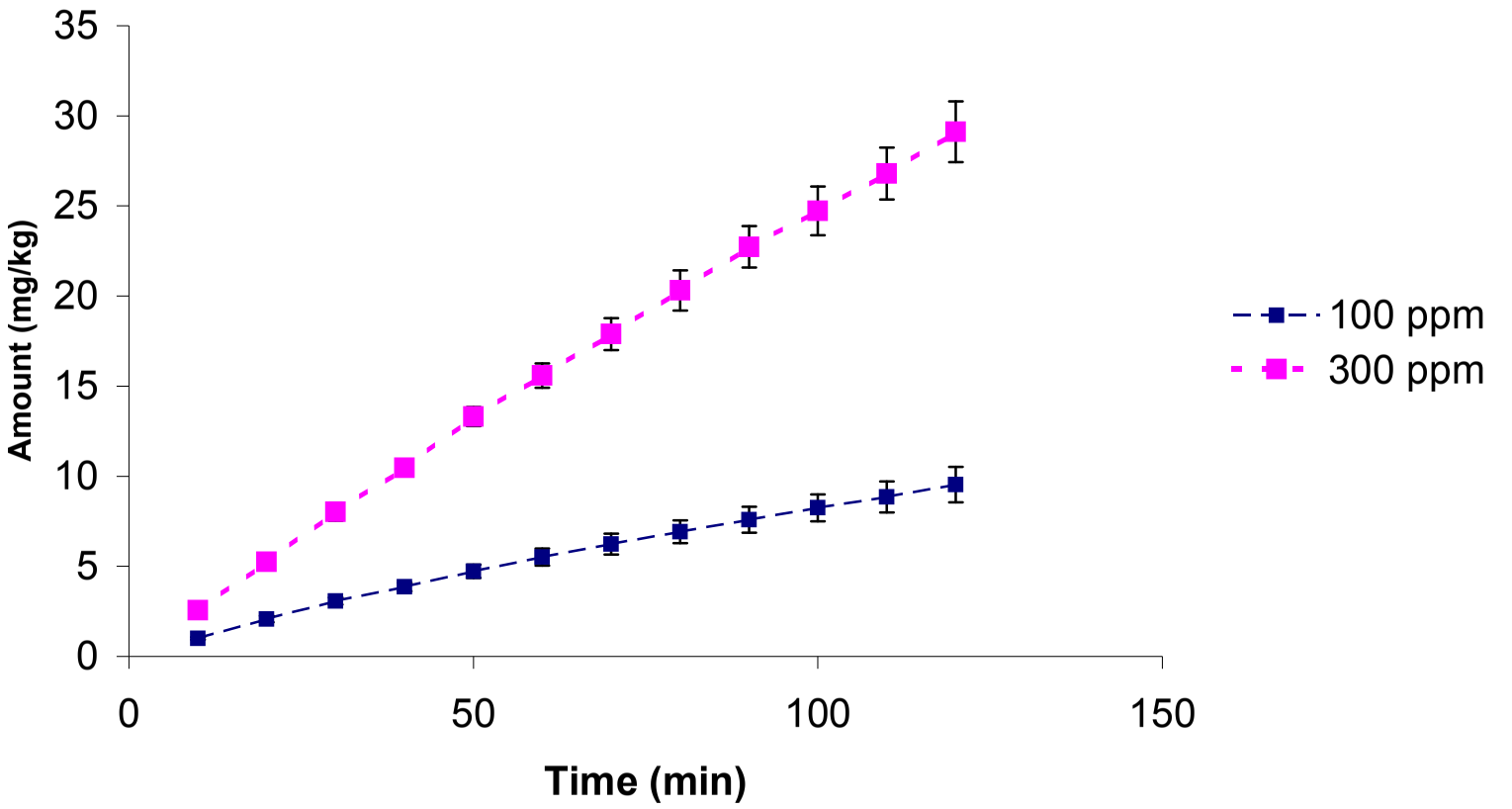

Figure 2.4: Cumulative uptake of DCE during inhalation of 100 or 300 ppm DCE for 2 h. The quantity of DCE retained during successive 10- min intervals was calculated on the basis of the measured minutes volume and the difference between inhaled and exhaled DCE concentrations. Each point represents the mean ± SE for 6 rats.

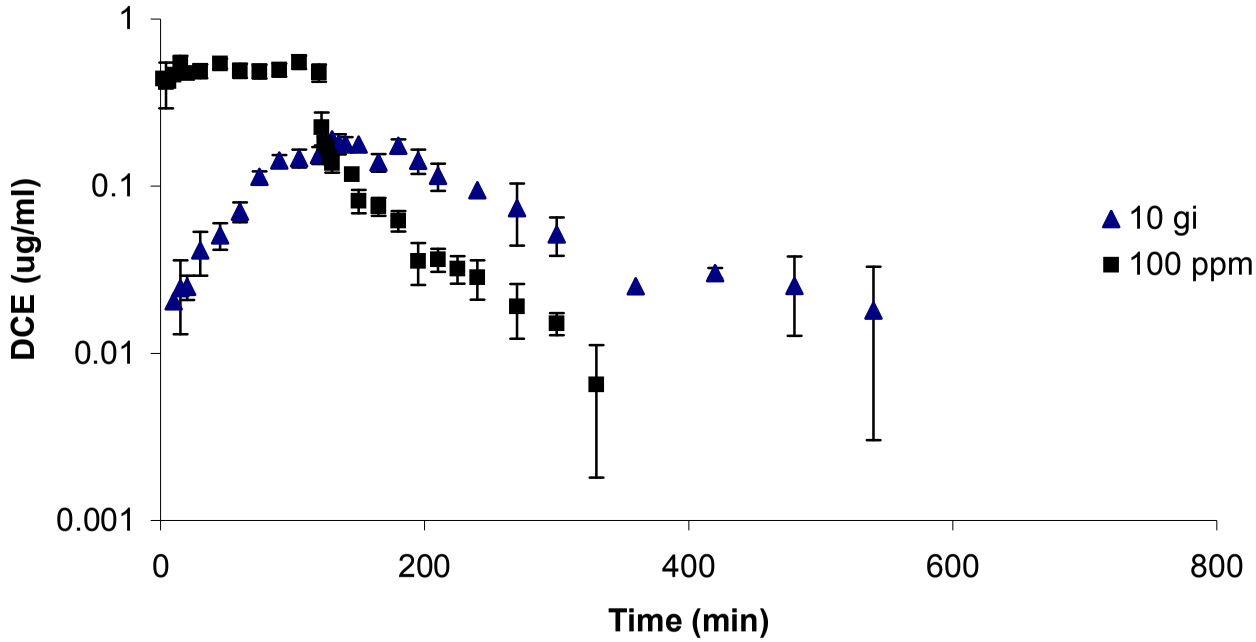

Figure 2.5A: Effect of route of exposure on arterial blood DCE concentration-versus time profiles. Rats inhaled 100 ppm DCE for 120 min. An equivalent dose of 10 mg/kg was given by gastric infusion (g.i.) over 120 min. Brackets encase mean ± SE for groups of 6 rats

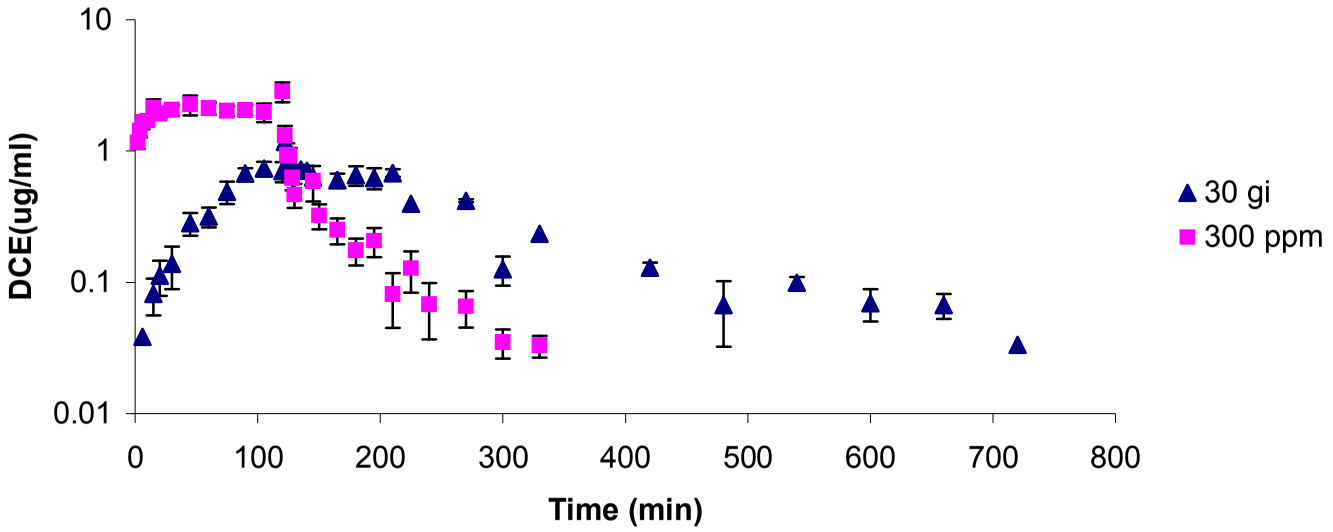

Figure 2.5B: Effect of route of exposure on arterial blood DCE concentration-versus time profiles. Rats inhaled 300 ppm DCE for 120 min. An equivalent dose of 30 mg/kg was given by gastric infusion (g.i.) over 120 min. Brackets encase mean ± SE for groups of 6 rats.

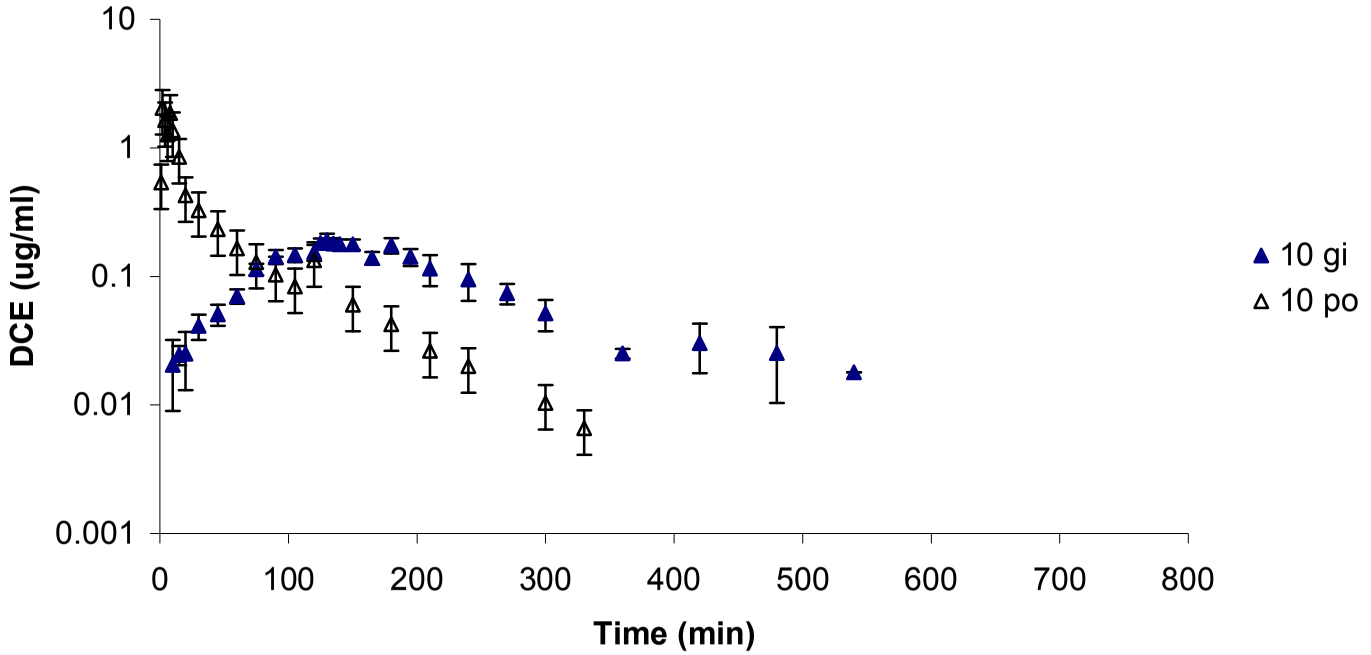

Figure 2.6A: Effect of pattern of ingestion on arterial blood DCE concentration-versus-time profiles. Rats were administered 10 mg DCE/kg in an aqueous emulsion either by gavage or by constant gastric infusion (g.i.) over 120 min. Values are means ± SE for groups of 6 rats.

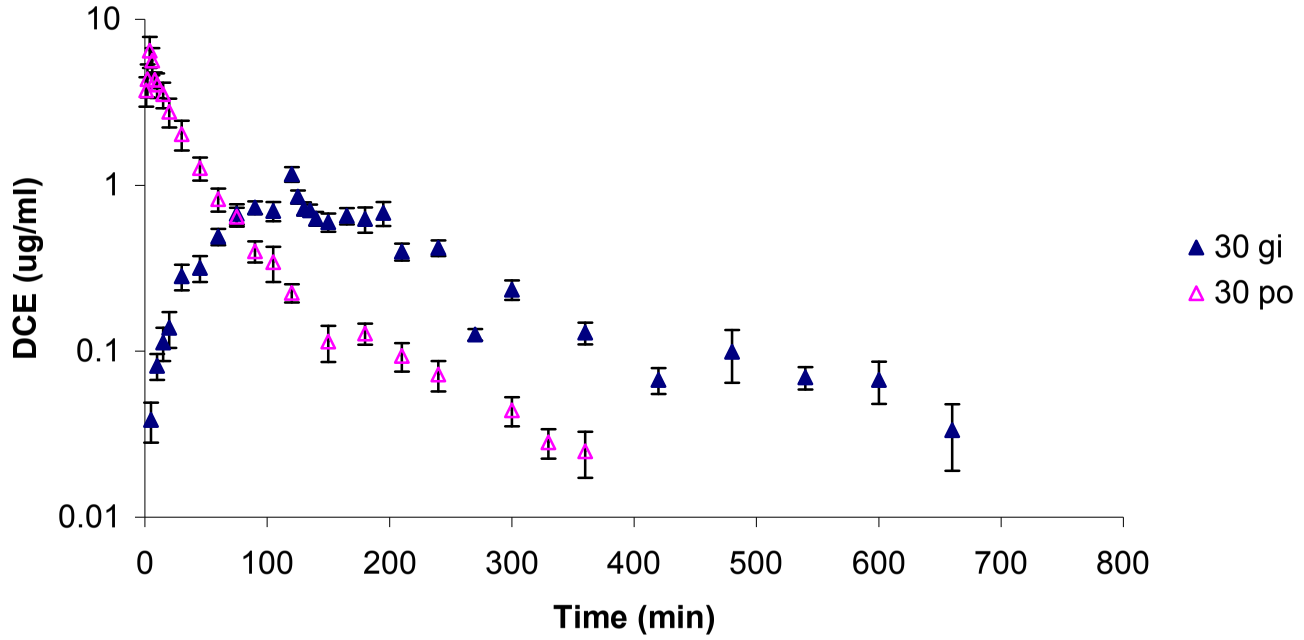

Figure 2.6B: Effect of pattern of ingestion on arterial blood DCE concentration-versus-time profiles. Rats were administered 30 mg DCE/kg in an aqueous emulsion either by gavage or by constant gastric infusion (g.i.) over 120 min. Values are means ± SE for groups of 6 rats.

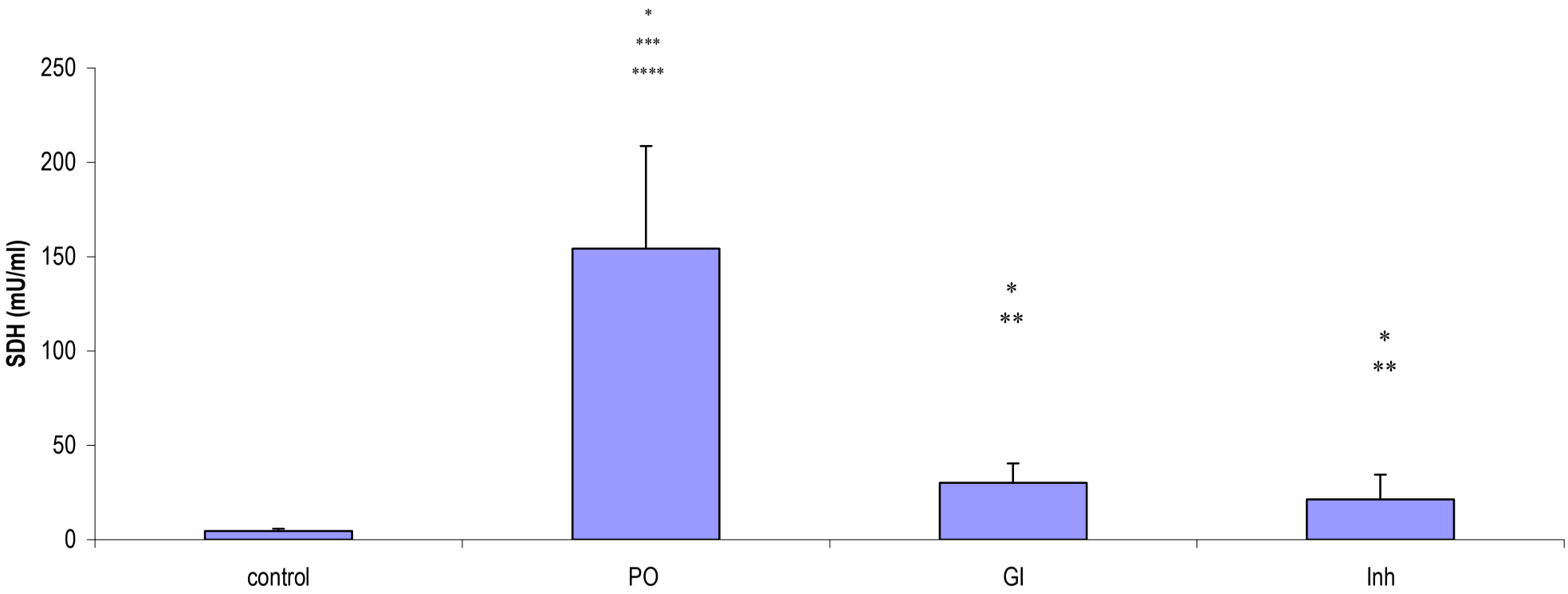

Figure 2.7: Serum dehydrogenase (SDH) levels 24 hours after rats were exposed to DCE by various routes and rates of administration. Each point represents the mean ± SE for 6 rats.

* Significantly different from control at p< 0.05

** Significantly different from oral gavage group at p< 0.05

*** Significantly different from GI at p< 0.05

**** Significantly different from Inhalation at p< 0.05

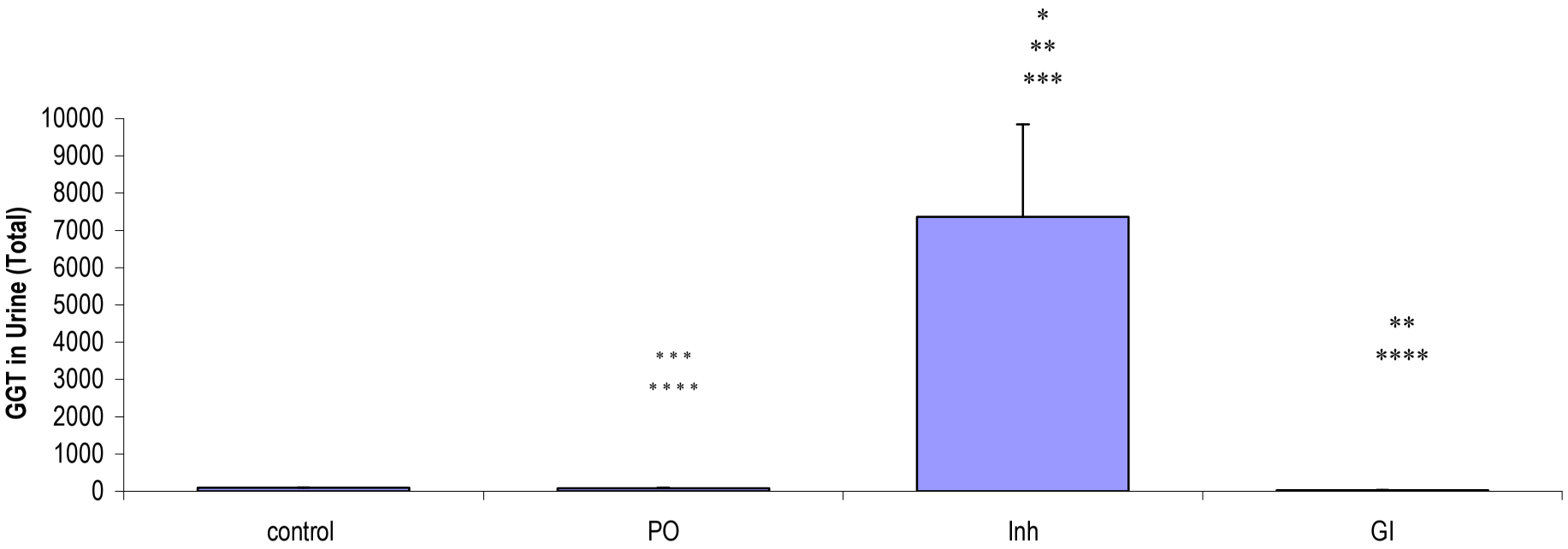

Figure 2.8: Urinary gamma-glutamyl transferase (GGT) levels 24 hours after rats were exposed to DCE by various routes and rates of administration. Each point represents the mean ± SE for 6 rats.

* Significantly different from control at p< 0.05

** Significantly different from oral gavage group at p< 0.05

*** Significantly different from GI at p< 0.05

**** Significantly different from Inhalation at p< 0.05

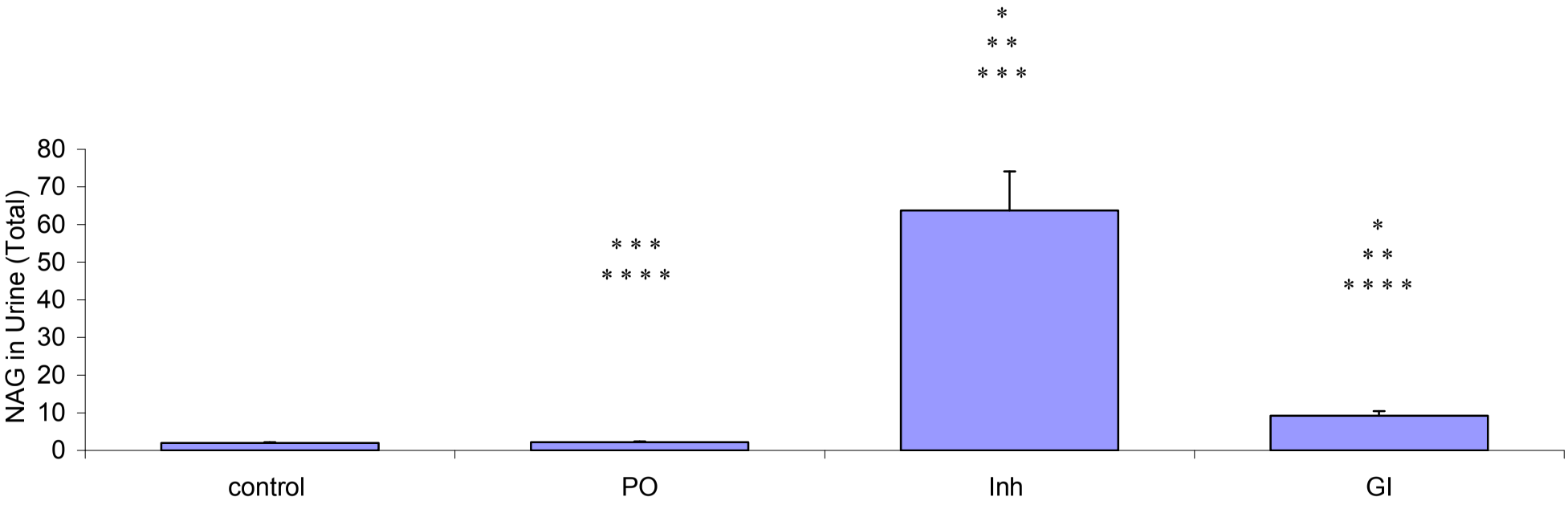

Figure 2.9: Urinary N- acetyl-glucosaminidase (NAG) levels 24 hours after rats were exposed to DCE by various routes and rates of administration. Each point represents the mean ± SE for 6 rats.

* Significantly different from control at p< 0.05

** Significantly different from oral gavage group at p< 0.05

*** Significantly different from GI at p< 0.05

**** Significantly different from Inhalation at p< 0.05

Table 2.1
Influence of dose, route, and pattern of exposure on DCE pharmacokinetic parameter estimates.

Dose (mg/kg) or (ppm) inh	Cmax (mg/L)	Tmax (min)	T1/2 (min)	Cl (ml/min*kg)	AUC (mg*min/L)	F (%)
10 p.o.	**2.2 ± 0.8[a]**	**7 ± 5[a]**	**47 ± 9[a, b]**	**58 ± 10[a]**	**51 ± 16[a]**	**24.4**
10 g.i.	**0.2 ± 0.1[b]**	**146 ± 34[b]**	**78 ± 16[a]**	**53 ± 18[a]**	**33 ± 10[a]**	**17.1**
100 inhal	**0.6 ± 0.1[b]**	**55 ± 18[c]**	**50 ± 24[b]**	**53 ± 10[a]**	**72 ± 10[a]**	**39.4**
30 p.o.	**8.9 ± 4.1[c]**	**5 ± 3[a]**	**88 ± 29**	**71 ± 20[a]**	**233 ± 88[b]**	**46.5**
30 g.i.	**1.3 ± 0.6[d]**	**123 ± 28[b]**	**88 ± 36**	**64 ± 39[a]**	**214 ± 117[b]**	**40.1**
300 inhal	**2.8 ± 0.7[d]**	**90 ± 35[c]**	**50 ± 20**	**64 ± 21[a]**	**279 ± 73[b]**	**55.7**

Rats were administered 10 or 30 mg DCE/kg as an oral bolus (p.o.) or over a 2-h period by constant gastric infusion (g.i.) Other groups of rats inhaled 100 or 300 ppm DCE for 2h. Serial arterial samples were taken for DCE analysis during and after exposures to characterize blood profiles. Values are means ± S.D. for groups of 6 rats. Values that are significantly different ($p<0.05$) from one another are designated by different superscripts.

Table 2.2
Pharmacokinetic parameter of DCE after IV administration

Dose (mg/kg)	$T_{1/2\,\beta}$ (min)	$T1/2_{\alpha}$ (min)	CL (L/min-kg)	AUC (mg*min/L)	K12 $(min)^{-1}$	K21 $(min)^{-1}$
10	140 ± 40 [a]	3.9 ± 0.7 [a]	60 ± 30 [a]	193 ± 73 [a]	0.12 ± 0.04 [a]	0.02 ± 0.003 [a]
30	141 ± 52 [a]	3.9 ± 1.8 [a]	63 ± 15 [a]	501 ± 110 [b]	0.14 ± 0.07 [a]	0.02 ± 0.01 [a]

Serial blood samples were taken from rats for DCE analysis following i.v. injection of 10 or 30 mg DCE/kg. Values are means ± S.D. for groups of 6 rats. Values that are significantly different ($p<0.05$) from one another are designated by different superscripts.

CHAPTER 3

PHARMACOKINETICS OF INHALED 1,1,1-TRICHLOROETHANE IN RATS[34]

[3] C.C. Hines, S. Muralidhara, C. Dallas, J. V. Bruckner, C. White
[4]Toxicology and Applied Pharmacology

ABSTRACT

The purpose of these experiments was to characterize the toxicokinetics of trichloroethane (TRI) administered via inhalation in rats Fasted male Sprague-Dawley rats received either 50 or 500 ppm TRI by inhalation over 2 hours. Serial blood samples were obtained to determine plasma kinetics. Another set of rats received 50 ppm TRI and were sacrificed at various time points to determine tissue distribution. TRI was quantitated by gas chromatography. The toxicokinetics of TRI were dose independent in this dose range. No significant differences in clearance and half-life were noted between the 50 and 500ppm doses. A proportional increase in Cmax and AUC were noted. All tissues evaluated had higher TRI concentrations as compared to the blood levels during the elimination phase. TRI was sequestered in the kidney and fat tissue yielding significant increases in AUC.

INTRODUCTION

1,1,1-Trichloroethane (TRI) is a halogenated, short-chain aliphatic hydrocarbon (i.e., halocarbon) that is widely used as an organic solvent (ATSDR, 2006). Its popularity as a metal degreaser, general purpose solvent, spot cleaner and component of aerosols and a variety of household products increased substantially with the decline in use of other halocarbons [e.g., methylene chloride, trichloroethylene (TCE), perchloroethylene (PCE)] which were found to be high-dose rodent and potential human carcinogens. TRI has not been shown to be carcinogenic in mice, rats or humans (ATSDR, 2006). NIOSH (1990) estimated that some 2.5 million workers were exposed to relatively high vapor levels of TRI between 1981 and 1983. Its use has steadily declined during the 1990s (Doherty, 2000) due to its ozone depleting properties. TRI was to be phased out by 2002, under the revised Montreal Protocol, but it now appears that TRI's production and use in the U.S. will continue well into the 21st century.

Many persons are exposed daily to relatively low levels of TRI, primarily via indoor air and drinking water. Ashley et al. (1994) found TRI and several other volatile organic chemicals (VOCs) in the blood of > 75% of 600 + nonoccupationally-exposed persons. TCE and TRI were also frequently detected in a subset of 982 adults examined in the NHANES III survey (Churchill et al., 2001), and more recently in 951 other members of the general population (Blount et al., 2006). TRI, TCE and PCE are the most common trinary chemical mixture in groundwater at hazardous waste sites in the U.S. (Fay, 2006). VOC levels in surface waters usually diminish rapidly due to dilution and evaporation. TRI and other VOCs in groundwater, however, can be trapped

for years and serve as a major source of exposure of large populations. Household activities that involve use of TRI-containing products and heating of tap water can result in significant inhalation of the halocarbon's vapors (Bruckner and Warren, 2006; Weisel and Jo, 1996). It has not been established whether cumulative exposures to TRI and other VOCs in drinking water are adequate to cause adverse effects (e.g., impair CNS functions).

The principal manifestation of acute or chronic inhalation of TRI is central nervous system (CNS) depression, ranging in severity from slight dizziness or headache to anesthesia and death (ATSDR, 2006). The halocarbon has a quite limited cytotoxic potential, ostensibly due to its limited biotransformation to relatively non-toxic metabolites (Eben and Kimmerle, 1974; Schumann et al., 1982a). Quast et al. (1988) observed minimal histological changes in periportal and centrilobular hepatocytes of male and female F-344 rats inhaling 1,500 ppm TRI 6 h/day, 5 days/week for up to 2 years. McNutt et al. (1975) reported increases in smooth endoplasmic reticulum, microbodies and lipid vacuoles in centrilobular hepatocytes of male CF-1 mice inhaling 1,000 ppm TRI continuously for up to 14 weeks. Necrosis of individual hepatocytes was occasionally observed. Very high inhaled concentrations of TRI, particularly when accompanied by hypoxia, can sensitize the myocardium to catecholamines, producing cardiac arrhythmias (Reinhardt et al., 1973). Deliberate abuse of TRI and other solvents, perhaps the most extreme form of VOC exposure, frequently results in deaths from anesthesia and/or cardiac fibrillation (Reinhardt et al., 1971). Thus, TRI has several target organs, depending primarily upon its dose.

Information on the systemic disposition of TRI in laboratory animals and humans is quite limited. The pharmacokinetics of inhaled TRI has been studied in rodents and in humans, but relatively little information is available on the time-course of the parent compound in target organs (e.g., brain, liver, heart), storage sites (e.g., fat) or other major tissues. Inhalation experiments with human volunteers have of necessity been limited to monitoring blood and exhaled breath levels during and following exposures (Astrand et al., 1973; Nolan et al., 1984; Stewart et al., 1961). Most kinetic experiments with rodents have also been limited to serial blood sampling (Dallas et al., 1989; Schumann et al., 1982a), due to the necessity to sacrifice large numbers of animals to obtain organs and to the difficulty of analyzing VOCs in tissues. Schumann et al. (1982a,b) measured radioactivity in the liver, kidney and fat at the termination of 6-h exposures of male B6C3F1 mice and F-344 rats to ^{14}C-TRI vapor. Holmberg et al. (1977) have quantified TRI in the blood, kidney, liver and brain of male NMRI mice subjected to a series of TRI vapor levels for different durations. The most complete time-course data were provided for the mice inhaling 100 ppm. More recently, Warren and his co-workers monitored blood and brain TRI concentrations for up to 30 min in male Swiss-Webster mice (Warren et al., 2000) and 100 min in male Sprague-Dawley rats (Warren et al., 1998), in an effort to relate TRI's CNS effects to its internal dosimetry.

Kinetic data play important roles in understanding a chemical's mode of action and in developing physiologically-based pharmacokinetic (PBPK) models. Correlation of the accumulation of TRI in the brain with CNS effects (Warren et al., 1998, 2000) supports the hypothesis that modification of neuronal membrane structure by such

lipophilic compounds alters the conformation of membrane-bound proteins (e.g., ion channels) (DeJong et al., 1998; Sikkema et al., 1995). Systemic disposition data are essential for development and validation of physiologically based pharmacokinetic (PBPK) models needed to predict target organ dosimetry for different exposure scenarios. The EPA is currently evaluating available PBPK models for TRI to determine which is(are) most suitable for use in deriving reference doses (RfDs) and reference concentrations (RfCs) for its Integrated Risk Information System (IRIS). Most of the published PBPK models consist of a limited number of tissue compartments and are primarily used to simulate blood TRI time-courses (ATSDR, 2006). A brain compartment is not included in models under current consideration due to a lack of experimental dosimetry data for this target organ. Therefore, such model-assisted risk assessments must assume that blood TRI concentrations accurately reflect brain or other target organ concentrations over time for different inhaled concentrations and durations of exposure.

The overall objective of the current investigation was to provide a comprehensive description and pharmacokinetic analyses of the systemic uptake, disposition and elimination of inhaled TRI in the adult rat. Two related applications of these data would be their use in development of target organ-inclusive PBPK models and in verification of the accuracy of the models' predictions of target organ dosimetry. The disposition data will be utilized to derive a variety of tissue:blood partition coefficients required for modeling, including a brain:blood partition coefficient. Related aims are to compare our *in vivo* partition coefficient with published *in vitro* values, and to determine whether the

in vivo brain:blood partition coefficient varies with TRI exposure concentration or duration.

MATERIALS AND METHODS

Chemicals. 1,1,1-Trichloroethane (TRI) of 99% purity was obtained from Aldrich Chemical (Milwaukee, WI). The chemical's purity was verified by gas chromatographic analysis. All other chemicals were purchased from Sigma-Aldrich (Kansas City, KS).

Animals. Male Sprague-Dawley (S-D) rats weighing 225 – 275 g were purchased from Charles River Breeding Laboratories (Raleigh, NC). They were housed in groups of 3 in stainless steel wire mesh cages in a negative airflow animal rack in an AAALAC-approved animal care facility. The rats were maintained for 7 – 10 days prior to use in a temperature (25°C)- and humidity (40%)–controlled room with light from 0700 – 1900 h. Tap water and Purina Rat Chow 5001® (Purina, St. Louis, MO) were available *ad libitum* during this period. The experimental protocol was reviewed and approved by the University of Georgia Animal Care Committee. The animals' body weight at the start of experiments ranged from 325 – 375 g. TRI exposures were initiated at 0900 h each morning.

Inhalation Apparatus. TRI exposures of freely moving rats were conducted in 1-m^3 Rochester-type dynamic flow chambers. The chambers were operated at flow rates of 0.2 – 0.4 m^3/min (i.e., ¼ to ½ change of the entire volume/min). These flow rates were utilized to rapidly establish an equilibrium of vapor throughout the chamber without producing unacceptable drafts. A negative pressure of 20 – 50 mm was maintained.

TRI vapors were generated by bubbling nitrogen through TRI in a 5-L Erlenmeyer flask with a sidearm to carry the TRI vapor to the chamber via a Teflon® tube. TRI concentrations within the chamber were monitored continuously with a Miran Model 1B2 infrared gas analyzer from Foxboro Analytical (Foxboro, MA). TRI concentrations were maintained at 50 and 500 ppm during the 2-h sessions.

Animal Treatments. Groups of S-D rats were exposed to 50 ppm TRI for up to 2 h. Serial sacrifices of sets of 6 rats were conducted after 5, 10, 20, 30, 45, 60, 90 and 120 min of TRI inhalation. Additional sets of 6 rats were killed at selected intervals for up to 2160 min after the initiation of the exposures. Blood was withdrawn into a gas-tight syringe by cardiac puncture after cervical dislocation. Each rat was then decapitated and 0.3- to 1.5-g specimens of the following tissues rapidly dissected into a 20-ml vial containing 5 ml of ice-cold isooctane saline (4:1, v/v) and tightly capped until analysis: liver, kidney, fat, heart, lungs spleen, GI tract, and brain. Two separate sets of rats were exposed to 50 and 500 ppm TRI over 2 hours and serial blood samples were obtained during the exposure and for up to 240 minutes post exposure.

TRI Extraction. Five to 100 µl of blood (depending upon analylate concentration) were transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). The vials were capped immediately with PTFE-lined butyl rubber septa and tightly crimped. The tissues were rapidly homogenized with a Tekmar Tissumizer (Tekmar Co., Cincinnati, OH). The vials were recapped, vortexed for 20 sec, and centrifuged at 2800 g for 5 min. A 5 – 10 µl aliquot of the overlying isooctane layer was transferred to a 10-ml headspace vial, that was immediately topped with a Teflon®-coated rubber cap and the metal seal crimped. This tissue extraction procedure has been detailed by Chen et al.

(1993). It was necessary to dilute the isooctane aliquot by 10- to 100-fold with isooctane when the TRI concentrations were high.

Respiratory measurements. The respiration of each animal was continuously monitored. The respiratory monitoring was conducted according to the methods previously used by Dallas et., 1989). The airflow created by the animal's inspiration was detected by a pneumotachograph located in the inhaled airstream between the influent bag and the breathing valve. The signal from the pneumotachograph and accompanying transducer was employed in recording the number of respirations per minute (f) in one channel of a physiograph. The signal was then intergrated over a 1-min interval to yield the volume of respiration per minute or minute volume (V_E). A value for the average tidal volume (V_T) during that 1-min interval was determined by dividing V_E by f for that minute. An average value for these parameters for individual animals was obtained by averaging the measurements taken at 10-min intervals during the 2-hr exposure.

TRI Analysis. Capped sample vials were placed into a 19395A Hewlett Packard headspace analyzer on a Hewlett Packard Model 5890A gas chromatograph (GC) equipped with an electron capture detector (ECD). After a 10-min equilibration time, a designated volume of vapor from each vial was automatically injected into the GC fitted with a stainless steel column (6′ X ⅛″) packed with 100/120 mesh Gas Chrom Q coated with 3% OV-17. The temperatures for the analyses were as follows: injector port 150°C; column, run isothermal at 60°C; and detector 350°C. The carrier gas was 5% argon methane at a flow rate of 60 ml/min. TRI eluted without interfering peaks with a retention time of 1.0 – 1.2 min. TRI concentrations in the samples were calculated from

the slope of a standard plot of TRI peak areas versus concentrations. Standard curves were obtained each day by injecting known amounts (1 – 15 ng) of TRI in isooctane into chilled headspace vials containing 50 µl of blank blood.

Toxicokinetic Analyses: Blood and tissue TRI concentration time profiles were analyzed by Winnonlin (Pharsight, Apex, NC). Data were analyzed by noncompartmental analysis. Area under the blood concentration time curve (AUC), apparent clearance (CL), terminal half-life ($t_{1/2}$), maximum concentration (Cmax), time to reach Cmax (Tmax) were obtained using Winnonlin.

Statistical Anaylsis: Toxicokinetic results are expressed as mean ± S.E. for n=6 rats. The data were analyzed with a Students's t-test ($p < 0.05$).

RESULTS

The target concentrations for the TRI inhalation exposures in this kinetics experiment were 50 and 500 ppm. The actual concentrations inhaled by the animals were determined by analysis of air samples taken from sampling ports immediately adjacent to the breathing valve. The inhaled TRI concentrations for the 6 rats in each group at the initiation of exposures were 51.6 ± 1.4 and 497.5 ±7.0 ppm (± S.E.) for the 50- and 500-ppm groups, respectively.

Blood TRI concentration versus time profiles revealed that inhaled TRI was rapidly absorbed into and eliminated from the body (fig. 1A and fig. 1B). TRI was rapidly absorbed from the lungs, and readily available for distribution to tissues. Substantial levels of TRI were detected in the arterial blood at the earliest sampling time (i.e. 2 min). Near steady state was achieved within the first 10 to 15 min and blood concentrations increased asymptotically for the duration of the 2 hr exposure (fig1A and fig 1B). The 10-fold increase in inhaled concentration (from 50 to 500 ppm) produced a proportional increase in both maximum concentration (Cmax) (0.8 ± 0.2 to 9.3 ± 1.1µg/ml) and area under the curve (AUC) (115 ± 19.7 to 1212 ± 278 min*µg/ml)(table 1). There was no change in half-life ($t_{½}$), clearance (Cl), or time to reach maximum concentration (tmax). TRI concentrations in the exhaled breath exceeded alveolar air concentrations during the inhalation exposure (fig. 1), due to the contribution of the unabsorbed TRI in the respiratory dead space. The converse was true post-exposure. Exhaled breath levels were relatively low due to dilution with fresh air residing in the dead space. The elimination half-lives for the 50- and 500- ppm groups were 97 and 77 min, respectively (table 1).

Systemically absorbed doses of TRI were determined from the inhalation data by subtracting the quantity of TRI exhaled from that inhaled with allowance for dead space, which yielded the amount and percentage of chemical taken up each sampling time. Percentage systemic uptake was largely independent on exposure level, but dependent on duration (data not shown). Cumulative uptake over the 2 h exposures could be determined by summation of uptake for each time period and by monitoring respiratory minute volume. Cumulative systemic uptake is plotted against duration of exposure in

figure 2. The total systemically absorbed doses at 2 h in the 50- and 500-ppm groups were 6.2 ± 1.5 and 45.8 ± 7.1 mg/kg, respectively. Thus systemic uptake was roughly proportional to inhaled concentration.

TRI tissue concentration-time profiles are given in figures 3-6 and toxicokinetic parameters are shown in Table 2. All tissues have higher TRI concentrations during the terminal phase than blood consistent with the extensive tissue distribution of TRI. The disposition of TRI in the liver, GI tract, spleen, lungs and heart mimic the disposition in the blood suggesting that these tissues are in rapid equilibrium with the blood. These tissues have similar Cmax values (0.9-2.2 ug/g) and half-lives (146-245 minutes) as compared to the blood (1.8 ug/g, 171 minutes). The brain TRI concentration-time profile is shown in Figure 4. The TRI concentrations in the blood and brain during TRI exposure are similar; however, after the initial distribution after cessation of the inhalation exposure the TRI concentrations in the brain are significantly higher during the elimination phase. TRI concentrations in the kidney were significantly higher than those in blood at all time points; however, the half-life was similar to that of blood (174 vs. 171 minutes). The increased concentrations in the kidney resulted in a 3-fold increase in AUC as compared to blood. The fat tissue serves as a depot for TRI (Figure 4) in the body. TRI peak concentration is 10-fold higher than the blood Cmax and the half-life of TRI in the fat is significantly longer than observed in the blood (Table 2) resulting in 37-fold increase in AUC.

DISCUSSION

TRI is rapidly and extensively absorbed from the lungs of rats after inhalation exposure. TRI demonstrated linear kinetics over dosage range (50-500 ppm) as

blood concentration and AUC and Cmax values were roughly proportional to inhaled TRI concentration. Warren et al. (2000) had similar findings in that male, albino Swiss-Webster (CFW) mice exposed to TRI for various amounts of time demonstrated linear kinetic over the broad exposure range as blood and brain concentrations and AUC and Cmax values were roughly proportional to the inhaled TRI concentrations. Dallas et al. (1989) also showed similar findings in that male, S-D rats exposed to TRI after 2 hr over an identical dosage range (50 and 500 pppm) exhibits linear kinetics in that exhaled breath levels and blood levels of TRI were directly proportional to the inhaled concentration. Humans exposed for 6 hr to 35 and 350 ppm TRI resulted in an almost identical observation (Nolan et al., 1984). Schumann et al. (1982) found that blood levels, tissue levels, and body burden of ^{14}C-TRI were each proportional to exposure level in both mice and rats. The pattern of uptake during inhalation where TRI is quickly absorbed, which is noted by its appearance in the blood at the first time point (i.e. 2 min) followed by a slow, but constant elimination was also seen in a previous study conducted by Schumann et al. (1982) and in Dallas et al. (1989). In the Schumann et al. (1982) study it was determined that the uptake and clearance of TRI in the blood of mice and rats exposed for 6 hr to 150 or 1500 ppm of TRI appear to reflect multiple compartmental processes. They also reported a half-life in rats after 150 and 1500ppm exposure at 139 and 258 min, respectively. In the current study, the half-life obtained after exposure to 50 and 500 ppm was 97±34.2 and 77.4±14.1, respectively. The half lives at the lower exposures are comparable, but Schumann et al. (1982) noted that the apparent longer half-lives of TRI exposed to 1500 ppm probably reflects

experimental error due to the individual animal variation encountered. In the Dallas et al. (1989) study it was determined that the initial uptake of inhaled TRI is governed by tissue loading and metabolism.

The tissue distribution of TRI was extensive with all tissues having higher concentrations during the terminal phase as compared to blood. You et al. (1994) saw similar results in male, CD-1 mice exposed to 3500 ppm TRI via inhalation for up to 100 min saw comparable AUC values in the muscle, heart, lung, gi, spleen, and blood. In this current study, however only significant differences were noted in the kidney and fat tissue, You et al. (1994) also saw the same difference in fat. The higher levels of TRI in the kidney are interesting since the kidney is not an organ of elimination for TRI. TRI is predominantly exhaled as parent compound (92.9%) with less than 3% of the dose is recovered in the urine (Reitz et al., 1988). The extensive distribution of TRI in the fat is not unexpected since TRI is a highly lipophilic compound.

The work in the study determines target organ dose by a practical method, not be using blood to predict target organ concentration, but rather measuring the area under the tissue concentration versus time curve. Dallas et al (1989) noted that it is important that target organs and mechanisms of toxicity be elucidated, so that the agents responsible for toxic effects are identified and can subsequently be quantified and correlated with the magnitude of toxicity in the target tissue(s). The current study provides a direct measurement of brain exposure, the target organ of TRI, allowing for a more rigorous validation of PBPK model predictions and also will be imperative in developing new target tissue based PBPK models.

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Table 3.1:

TRI toxicokinetic parameters

	50 PPM	500 PPM
Half-life (min)	97 ± 34.2 [a]	77.4 ± 14.1 [a]
Cl (ml/min/kg)	53.5 ± 10.1 [a]	41.3 ± 8.9 [a]
AUC (min*ug/ml)	115.2 ± 19.7 [a]	1211 ± 278 [b]
Tmax (min)	110 ± 12.5 [a]	97.5 ± 16 [a]
Cmax (ug/ml)	0.8 ± 0.2 [a]	9.2 ± 1.1 [c]

Pharmacokinetic Parameters after 2-h exposure via Inhalation 50 and 500 ppm TRI. SD (±) for rats n=6
[a-c] Means of each parameter which are significantly different are designated by different superscripts. Significant $p<0.05$

Table 3.2:

Toxicokinetic parameters for TRI in tissues after exposure to 50 PPM TRI via inhalation for 120 minutes

Tissue	Half-life (min)	AUC (ug-min/g)	Cmax (ug/g)	Tmax (min)
Liver	201	308	2.2	120
Kidney	174	582	5.5	45
Fat	295	7084	21.8	130
Heart	235	175	1	120
Lungs	182	183	1.2	130
Spleen	245	152	.9	30
GI Tract	146	228	1.4	45
Brain	207	228	1.5	120
Blood	171	190	1.8	120

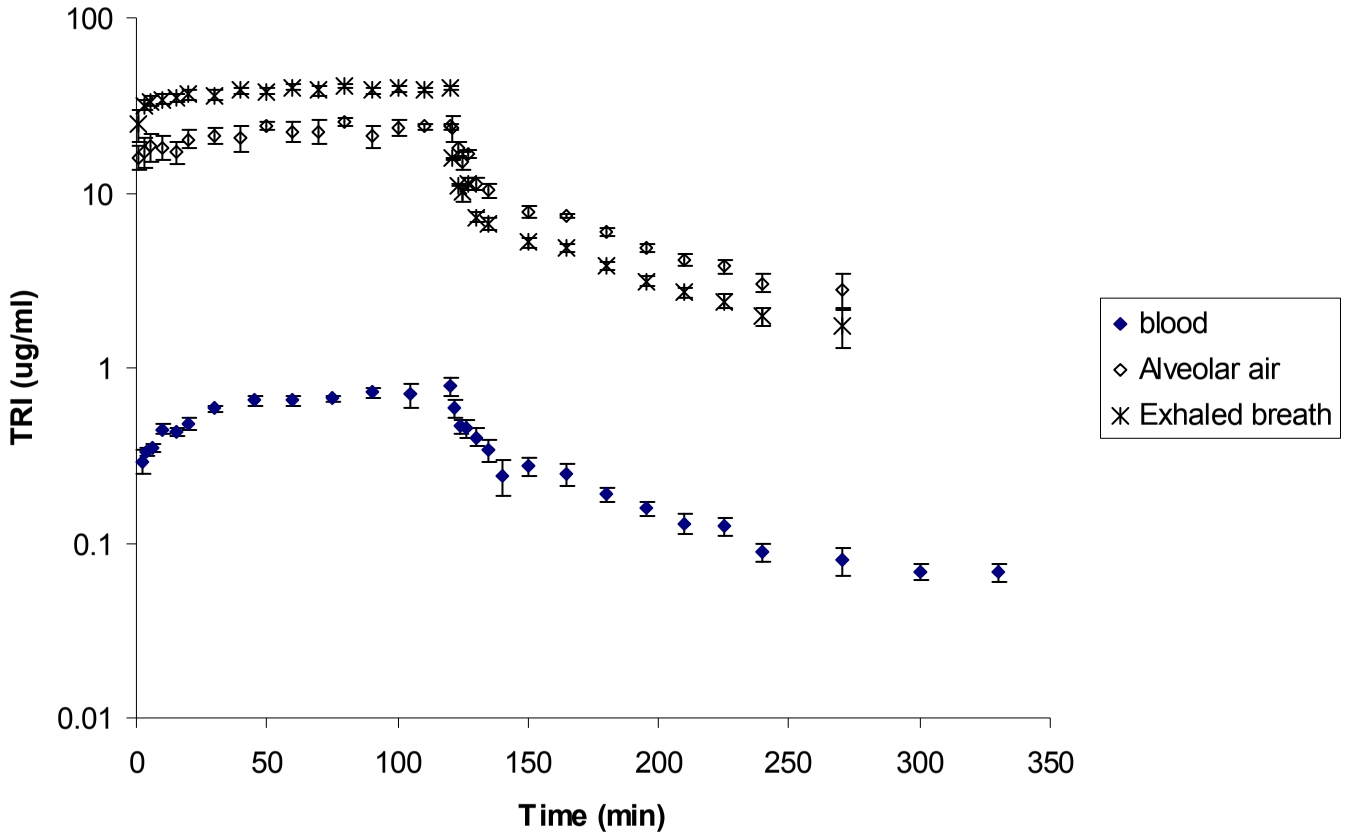

Figure 3.1A: Trichloroethane concentration after exposure to 50 ppm via inhalation. Values are means ± SE for groups of 6 rats

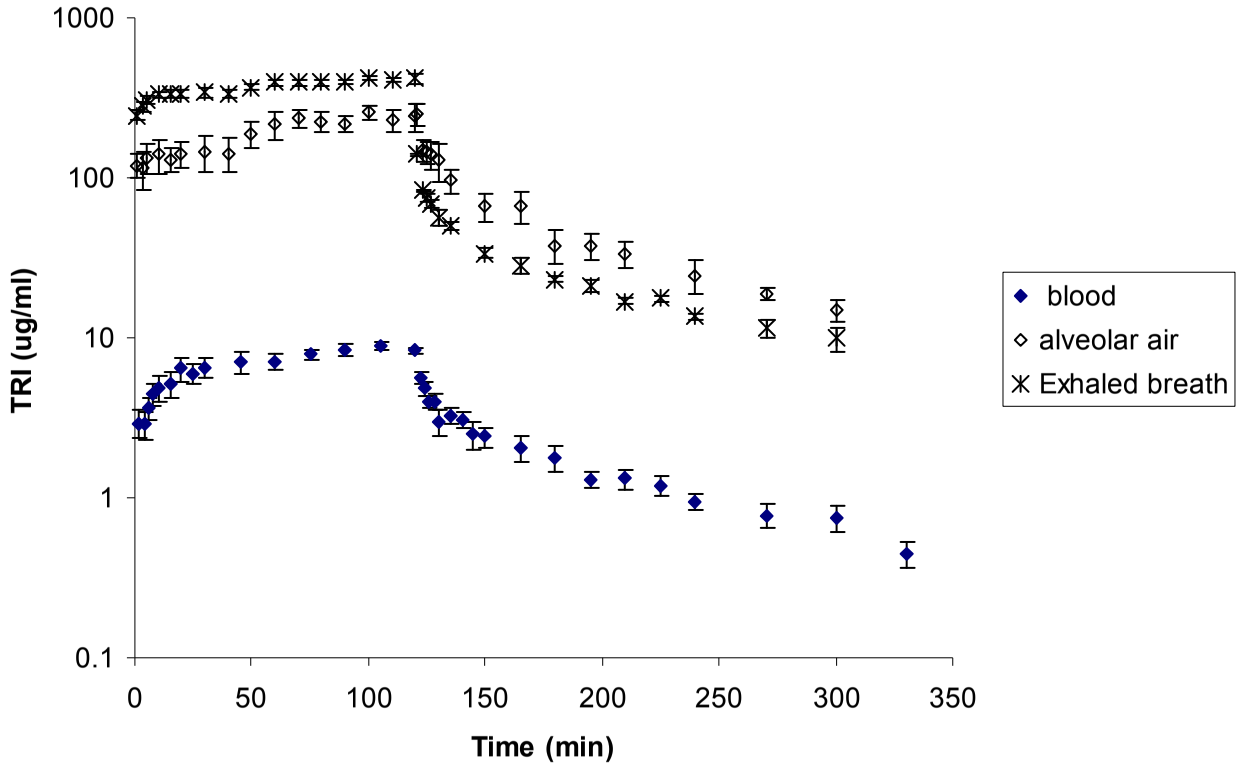

Figure 3.1B: Trichloroethane concentration after exposure to 500 ppm via inhalation. Values are means ± SE for groups of 6 rats

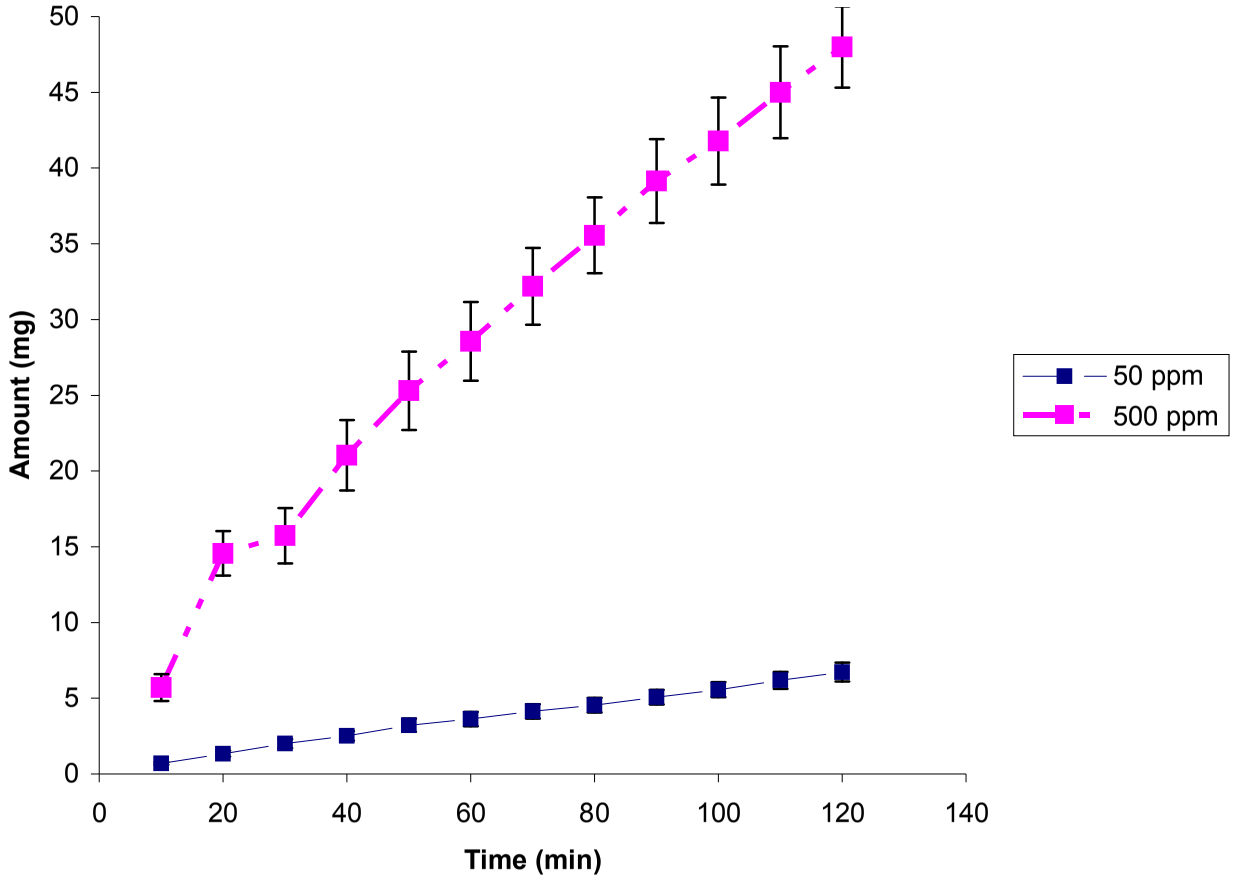

Figure 3.2: Cumulative uptake of TRI during inhalation of 50 or 500 ppm TRI for 2 h. The quantity of TRI retained during successive 2- to 20- min intervals was calculated on the basis of the measured minutes volume and the difference between inhaled and exhaled TRI concentrations. Each point represents the mean ± SE for 6 rats

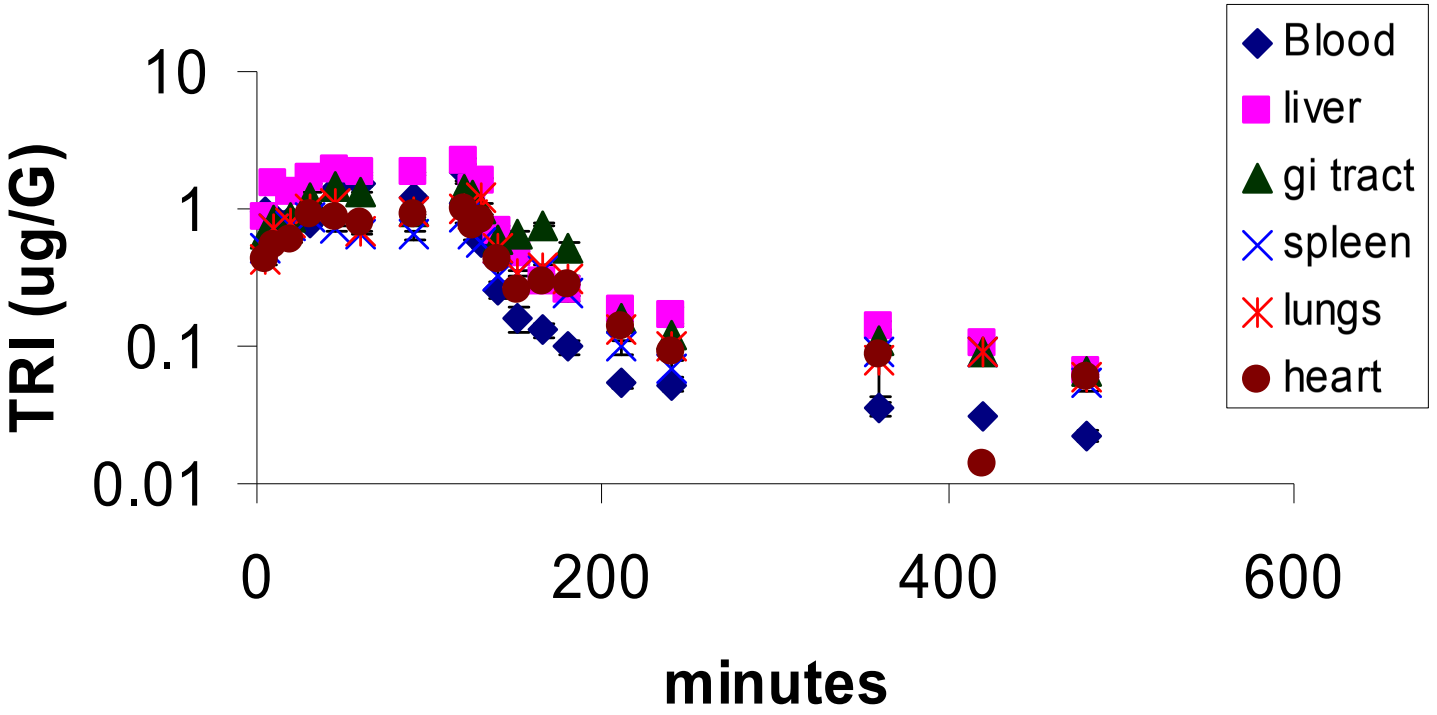

Figure 3.3: TRI concentration-time profiles for tissues after exposure to 50ppm TRI via inhalation for 120 min. Values are means ± SE for groups of 6 rats

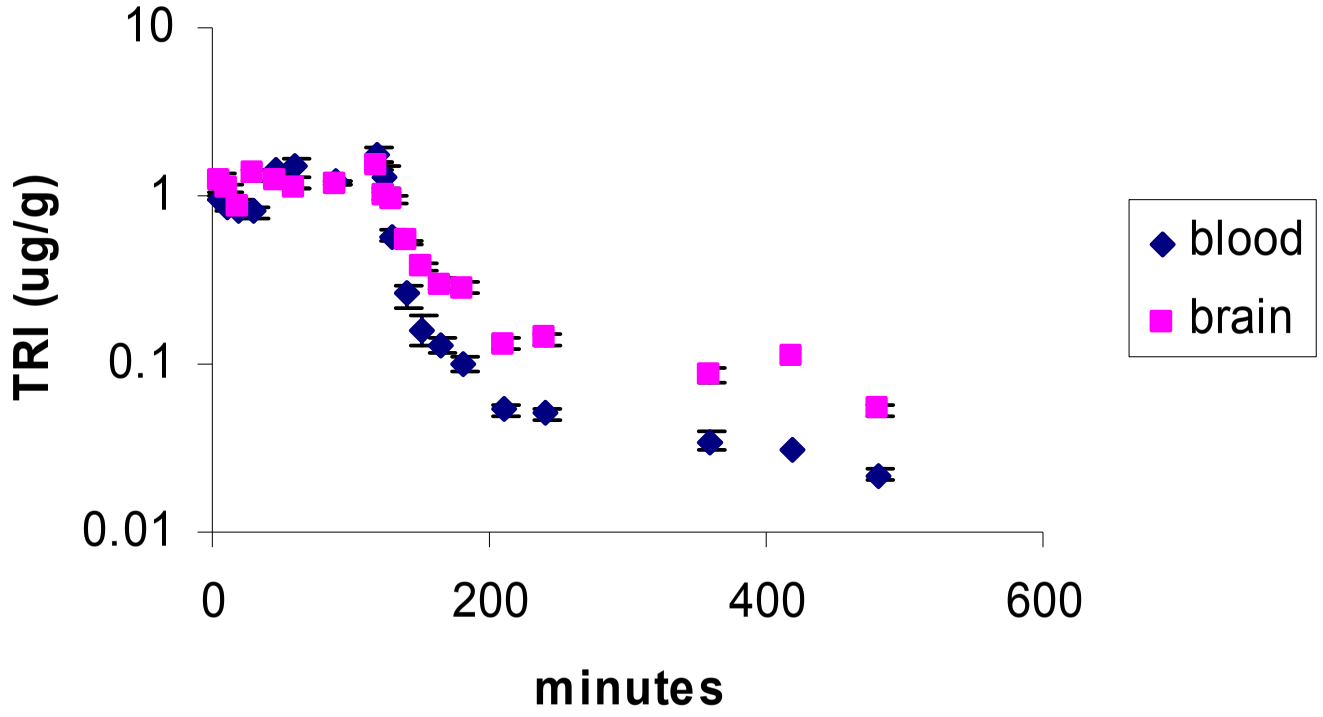

Figure 3.4: Brain:Blood concentration time profile after inhalation of 50 ppm TRI over 120 min. Values are means ± SE for groups of 6 rats

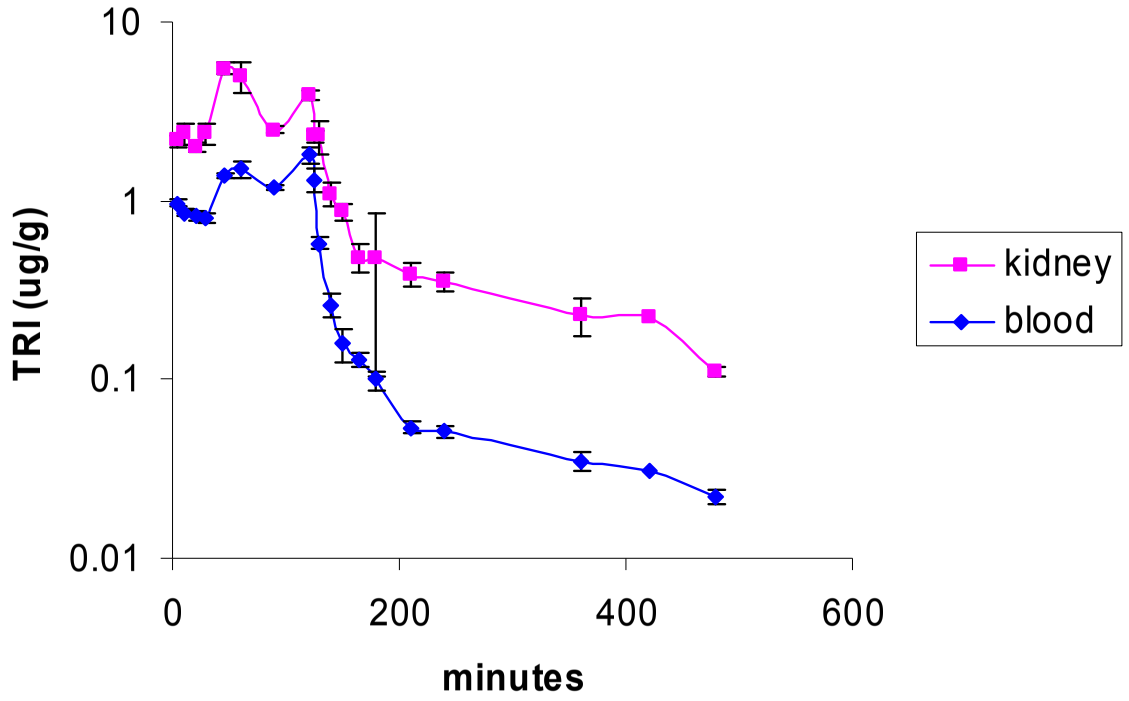

Figure 3.5: Kidney:Blood concentration time profile after inhalation of 50 ppm TRI over 120 min. Values are means ± SE for groups of 6 rats

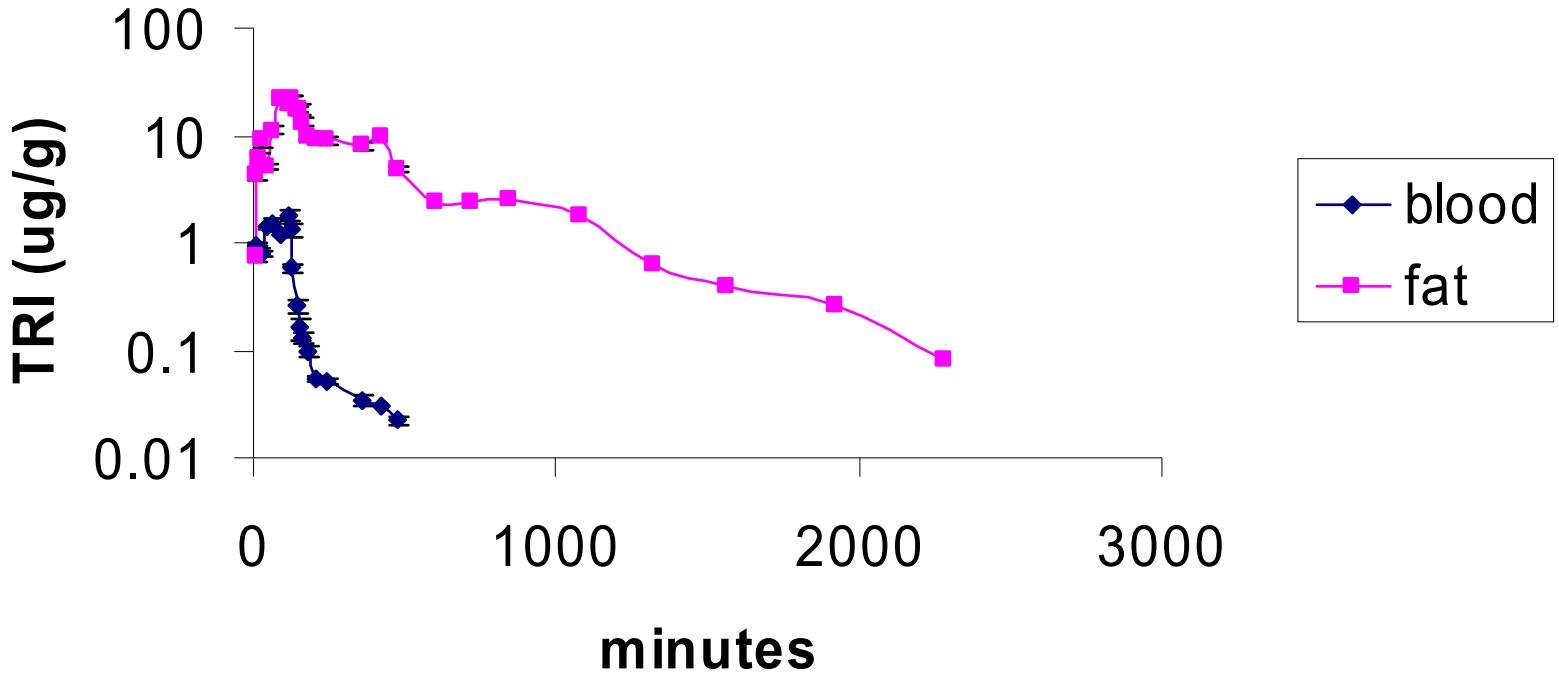

Figure 3.6: Fat:Blood concentration time profile after inhalation of 50 ppm TRI over 120 min. Values are means ± SE for groups of 6 rats

CHAPTER 4

EFFECT OF ORAL DOSAGE LEVEL AND RATE ON THE BIOAVALABILTY AND METABOLISM OF TRICHLOROTHYLENE AND 1,1,1,-TRICHLOROETHANE[56]

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ABSTRACT

Trichloroethylene (TCE) and Trichloroethane (TRI) were selected for study since they are structurally similar, have common uses, but are removed from the body differently and are believed to have different toxic effects on humans and animals. TRI is extensively exhaled through the lungs and is poorly metabolized, whereas TCE is extensively metabolized by the cytochrome P450 isozymes of the liver. Certain TCE metabolites are known to cause cancer in animals and may be carcinogenic in humans. In this study, the effect of dose and pattern of ingestion were characterized to determine if either would play a significant role in the toxicokinetics of the chemicals. Winnonlin was utilized to determine pertinent toxicokinetic parameters. TRI's blood concentrations peaked earlier when it was given as an oral bolus and at a higher concentration. One would therefore expect to see more pronounced toxic effects when TRI is given as an oral bolus verses gastric infusion. There were no significant differences in the AUCs between rates of TRI administration suggesting no changes in bioavailability. TCE AUC was both dose and rate of administration dependent. The exposure to TCA was also dependent on dose and rate of TCE administration and this effect was more pronounced at the low dose. In conclusion, this study shows that TRI's oral bioavailability and metabolism are substantially affected by presystemic elimination.

INTRODUCTION

Widespread use of volatile organic chemicals (VOCs) has resulted in their dissemination throughout the environment. Many people living in the U.S. are exposed daily to these chemicals, primarily via indoor air and drinking water. Ashley et al. (1994) found detectable levels of 1,1,1-trichloroethane (TRI), trichloroethylene (TCE), perchloroethylene (PERC), chloroform ($CHCl_3$), benzene and several other VOCs in the blood of many of a group of 600+ nonoccupationally-exposed persons. TCE and TRI were also frequently detected in a subset of 982 adults evaluated in the NHANES III survey (Churchill et al., 2001), and more recently in 951 other persons (Blount et al., 2006). High concentrations of VOCs are often present in the effluents of manufacturing facilities of chemical companies, rubber producers, petrochemical plants and paper mills. TCE is the most frequently found chemical contaminant of groundwater in the proximity of hazardous waste sites in the U.S. (Fay and Mumtaz, 1996; Fay, 2006). It is often present in combination with TRI. Levels of VOCs in surface waters usually diminish rapidly due to dilution and evaporation. In contrast, VOCs in groundwater can remain trapped for years and serve as a principal source of exposure for large populations (Wu and Schaum, 2000).

TRI is not considered to be carcinogenic (ATSDR, 2005; IRIS, 1990), but TCE is a rodent carcinogen and a potential human carcinogen (ATSDR, 1997; Bruning et al., 2003; Wartenberg et al., 2000; Wong, 2004). As both compounds are quite lipophilic, they can partition into the central nervous system (CNS) and inhibit neurological functions when present in sufficient amounts. It is commonly accepted that halogenated, short-chain, aliphatic hydrocarbons (halocarbons) must be metabolically

activated in order to produce cytotoxicity or mutagenicity (Bruckner, and Warren, 2001). TRI is poorly metabolized and primarily eliminated by exhalation by rodents (Schumann et al., 1982) and humans (Nolan et al., 1984). Humans who inhaled 35 or 350 ppm TRI for 6 h metabolized 5 – 6% of their absorbed dose to trichloroacetic acid (TCA) and trichloroethanol (TCOH), with the latter the major product (Nolan et al., 1984). Accordingly, TRI is relatively non-toxic (Bruckner et al., 2001) and is not mutagenic in most test systems (ATSDR, 2005). In contrast, TCE is extensively oxidized in the liver by cytochrome P4502E1 and certain other P450s to chloral, which is hydrated to form chloral hydrate (CH). CH is both oxidized to TCA and reduced to TCOH (Lash et al., 2000; Clewell et al., 2001). The latter metabolite contributes to TCE's CNS depressant effects. Some TCOH glucuronide that is excreted via bile is hydrolyzed by enteric bacteria and undergoes enterohepatic circulation and subsequent conversion to TCA (Stenner et al., 1997). Neither TCOH nor TCA is very cytotoxic, but TCA is hepatocarcinogenic in B6C3F1 mice. Dichloroacetic acid, a minor metabolite, is also believed to contribute to liver tumors in the mice (Bull, 2000, Bull et al., 2002).

TCE and TRI are both generally believed to be well absorbed from the gastrointestinal (GI) tract, though their bioavailability would be anticipated to differ. Ingested VOCs are subject to metabolism by the liver and exhalation by the lungs, before they reach the arterial circulation and are distributed systemically. These processes are known collectively as first-pass, or presystemic elimination. As TCE is volatile and well metabolized, oral doses that do not saturate metabolism should be efficiently removed. Indeed, Lee et al. (1996) found in rats that substantial fractions of low oral doses of TCE were removed during their initial pass through the liver and lungs

of rats. No information was found in the literature on the efficiency of first-pass hepatic or pulmonary elimination of less extensively metabolized halocarbons such as TRI. It is possible that the liver can efficiently extract TRI from the portal circulation, when exposure levels are low and ingestion is prolonged. TRI should be eliminated more efficiently than TCE by exhalation, since TRI's air:blood partition coefficient is higher (Gargas et al., 1989).

The rate of ingestion of TCE and TRI may significantly influence their bioavailability and toxicity potential. For convenience, VOCs and other chemicals are frequently administered daily to animals as a single bolus by gavage in oral toxicity and carcinogenicity studies. Actual human exposures to VOCs in drinking water are usually quite different, in that people typically consume water in divided doses over the course of the day. Chronic administration of high gavage doses of $CHCl_3$, TCE, perchloroethylene (PCE) and certain other VOCs can cause hepatocellular carcinoma in B6C3F1 mice. No increase in the incidence of this cancer over background is seen when the mice ingest the same doses of the halocarbons each day in their drinking water (Jorgenson et al., 1985; Klaunig et al., 1986). Bolus doses of these rapidly absorbed chemicals may very well result in relatively high levels of the parent compounds and reactive metabolites in the liver.

There is little information on the influence of presystemic elimination on the bioavailability of ingested VOCs, despite its potential role as an important protective mechanism against low, environmentally-encountered levels of VOCs. Knowledge of the effect of rate of oral intake on the bioavailability of VOCs and their metabolites is even more limited. Experiments were therefore designed to test the following

hypotheses: a) Hepatic first-pass elimination of VOCs is dependent upon their propensity for metabolism and their dose; b) Rate of ingestion substantially affects the disposition of low doses of both well- and poorly-metabolized VOCs; and c) Rate of ingestion of TCE, a well-metabolized VOC, substantially influences the disposition of its major hepatic metabolites, trichloroacetic acid (TCA). This information should be useful in addressing the rationale and the relevancy of commonly-used animal experimental protocols to real-life human oral VOC exposures.

METHODS

Chemicals. 1,1,2-Trichloroethylene (TCE) and 1,1,1-trichloroethane (TRI), each of 99+ % purity, were purchased from Aldrich Chemical Co. (Milwaukee, WI). Alkamuls®, formerly Emulphor®, was obtained from Rhone-Poulenc (Cranbury, WI). Sulfuric acid and methanol came from J.T. Baker (Phillipsburg, NJ). Burdick Jackson (Muskegon, WI) was the source of isooctane, while trichloroacetic acid (TCA), trichloroethanol (TCOH), and chloral hydrate (CH) were supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO).

Animals. Adult, male Sprague-Dawley (S-D) rats were obtained from Charles River Laboratories (Raleigh, NC). The animals were housed in an AAALAC-accredited animal care facility. They were acclimated to a 12-h light/dark cycle (light 0700 – 1900 h) in a temperature- (25°) and humidity– (40%) controlled room for at least 1 week prior to use. Tap water and food (Purina Rat Chow #5001) were available *ad libitum* during

this period. The experimental protocol was reviewed and approved by the University of Georgia's animal care committee.

Rats weighing 300 – 380 g were anesthetized by i.p. injection of 0.1 ml/100 g b.w. of a "cocktail" consisting of ketamine hydrochloride (100 mg/ml), acepromazine maleate (10 mg/ml) and xylazine hydrochloride (20 mg/ml) (3:2:1, V:V:V). Cannulas (PE-50 polyethylene tubing) were implanted into: the stomach for gastric infusion (g.i.) and the right carotid artery for intraarterial (i.a.) blood sampling. The cannulas were tunneled subcutaneously (s.c.) and exited at the nape of the neck, so the rats could move about freely upon recovery. Water was provided, but food was withheld during a 24-h postsurgical recovery period before dosing. Although the fasting alone resulted in increased hepatic CYP2E1 activity, fasted rats on which cannulation surgery was performed exhibited CYP2E1 activity similar to that of untreated controls (data not shown).

Dosing Protocols. Ten and 50 mg/kg of TCE, as well as 6 and 48 mg/kg of TRI were administered between 0900 and 1100 h. Alkamuls®, a polyethoxylated vegetable oil, was used to prepare stable 5% aqueous emulsions of TCE and TRI the day of dosing. Both dosages of each halocarbon were administered by: gavage (p.o.) in a volume of 1 ml/kg with a curved, ball-tipped intubation needle; and constant g.i. in a volume of 1 ml/kg over 2 h, by use of a Harvard P-22 infusion pump. Food and water were withheld for 3 h after dosing.

Blood Collection and VOC Analyses. Serial blood samples of 5 to 100 µl were collected from the arterial cannula at selected intervals until the blood concentrations fell below the analytical limit of quantitation (LOQ) of 10 ng/ml (ppb) for TRI and TCE. Each

blood specimen was immediately transferred to a 20-ml glass headspace vial (Perkin-Elmer, Norwalk, CT). The vials to be analyzed for their TRI content were empty. The vials to be analyzed for TCE and its major metabolites contained 200 µl of an esterification solution. This solution was comprised of distilled water, concentrated sulfuric acid and methanol in a ratio of 6:5:1 (V:V:V). Each vial was quickly capped with a PTFE-coated rubber septa and an aluminum cap. The seals were tightly crimped, the vials heated to 90°C for 30 min in an autosampler, and an aliquot of the vapor vented into a Perkin-Elmer 8500 gas chromatograph (GC) equipped with an electron capture detector. The GC conditions for TCE and its metabolites have been described previously by Muralidhara and Bruckner (1999). TRI analyses were carried out on a 6´ x ⅛˝ OD stainless steel column packed with a 3% OV-17 on Supelcoport® (Supelco, Bellefont, PA). The temperatures for TRI analyses were as follows: column, run isothermal at 85°C; injector 250°C; and detector 360°C. Nitrogen was used as the carrier gas at 60 ml/min. Peak areas were referenced to TRI, TCE, and TCA standard curves. Standards of TRI and TCE were prepared daily in isooctane, while distilled water was used to make TCA standards.

Pharmacokinetic Analyses. Blood TCE and TRI concentration versus time profiles were analyzed with Winnonlin (Pharsight, Apex, NC). Data were analyzed by non-compartmental analysis. Area under the blood concentration time curve (AUC), apparent clearance (CL), terminal elimination half-life ($t_{½}$), maximum concentration (C_{max}), time to reach C_{max} (T_{max}) values were obtained using Winnonlin.

Statistical Analyses. Pharmacokinetic data were expressed as mean ± S.D. in Tables 4.1 and 4.2, and shown as mean ± S.E. in Figs. 4.1 – 4.4. The data were analyzed by two-way analysis of variance, followed by Tukey's comparison test ($p < 0.05$).

RESULTS

TRI and TCE were very rapidly absorbed from the GI tract of fasted rats, when the halocarbons were given as an aqueous emulsion by gavage (p.o.). Both chemicals were readily available for distribution to body tissues, in that arterial concentrations were quite high at the initial sampling time (i.e., 2 min) (Figs. 4.1 and 4.2). Peak blood levels of TCE and TRI were observed 3 – 5 and 8 – 14 min after p.o. dosing, respectively (Tables 4.1 and 4.2). Concentrations of each parent compound then diminished quickly due to rapid tissue uptake, followed by a relatively slow decrease during the terminal elimination phase. TCE's terminal elimination half-lives for the oral dosage regimens appeared to be consistently shorter than those for TRI, though the corresponding values were not always significantly different, despite the greater extent to which TCE is metabolized except for the 50 mg/kg p.o. dose of TCE. This is consistent with the saturable metabolism of TCE in this concentration range.

The influence of pattern of ingestion and dose on blood TRI concentration versus time profiles and pharmacokinetic parameters can be seen in Figs. 4.1A and B and Table 4.1. As would be expected, the shapes of the p.o. and g.i. profiles initially differ. TRI levels peak within a few min of p.o. administration. Levels progressively rise as the chemical is infused; exceeding levels in the p.o. groups by ~ 100 min after the

exposures begin. The elimination profiles for each oral dosage regimen closely resemble one another, as do AUC and $t_{½}$ values at each dosage level. The pharmacokinetics of TRI is independent of dose between 6 and 48 mg/kg. Increases in C_{max} and AUC are directly proportional to increase in dose. Apparent elimination $t_{½}$ did not vary with dose.

The pharmacokinetics of TCE were markedly affected by its rate of oral administration and dose. Arterial TCE concentrations peaked within 5 min of p.o. dosing with 10 mg/kg, and decreased rapidly during the distribution phase (Fig. 4.2B). TCE levels in the 10 mg/kg g.i. group reached and exceeded those in the p.o. group within 60 – 70 min. TCE levels in the g.i. animals remained slightly higher for the duration of the monitoring period, but $t_{½}$ values were no different for the two patterns of oral dosing (Table 4.2). The mean g.i. AUC, however, was only ~ 6% of that for the p.o. animals. The mean g.i. AUC was a substantially higher percentage (i.e., 70%) of the p.o. AUC for the 50 mg/kg dose. The relationship of the g.i. and p.o. profiles of the higher dose (Fig. 4.2B) differed from that at the lower dose (Fig. 4.2A). Blood TCE concentrations progressively rose during g.i. of 50 mg/kg of the chemical, but did not exceed p.o. blood concentrations. The 50 mg/kg p.o. groups mean $t_{½}$ was significantly longer than that of the corresponding g.i. group (Table 4.2). The 5-fold increase in TCE dose resulted in disproportionate increases in AUC and C_{max} for each pattern of oral exposure.

Oral dosage regimen had a significant effect on the pharmacokinetics of TCA. While only low concentrations of TCE were detected in the arterial blood of the animals infused with 10 mg TCE/kg (Fig 4.2A), substantial amounts of TCA were present (Fig.

4.3A). TCA Cmax and AUC values increased 2.5- and 2-fold, respectively with the 5-fold increase in TCE dose in the g.i. groups, and 4- and 8.6-fold, respectively in the gavage groups (Table 4.3). These results seem to point to a decrease in clearance or change in formation of TCA. The TCA concentrations remained relatively constant for prolonged periods at the T_{max} and diminished very slowly thereafter. Half-lives could not be determined, because blood concentrations were not monitored long enough. AUC were truncated at the last time-point. Terminal half-lives could not be compared, but appeared to be similar (Fig. 4.4).

DISCUSSION

Both TCE and TRI are rapidly and extensively absorbed from the GI tract of fasted rats. The T_{max} of 3 – 5 min seen here for TCE is comparable to values reported by Lee et al. (2000) for fasted rats given the VOC as an aqueous emulsion. Reitz et al. (1988) also report that TRI is quickly absorbed by rats gavaged with a 14 mg/kg dose in water, but the researchers did not specify a T_{max}. Oral administration of VOCs in oil vehicles, of course, markedly delays the systemic absorption of the lipophilic chemicals (Kim et al., 1990; Withey et al., 1983).

There is little published information on the disposition of TRI following its ingestion. Unfortunately, Reitz et al's. (1988) serial blood sampling ceased 2 h post dosing, so it is not possible to ascertain bioavailability, $t_{½}$ or other kinetic parameters from their data. Blood TCA concentrations were sometimes monitored (data not shown), but aggregate TRI metabolism was not assessed. Male F-344 rats ingesting 116 mg TRI/kg in their drinking water over 8 h excreted just 3% of their dose as urinary

metabolites and CO_2, but exhaled 92.9% as the parent compound (Reitz et al., 1988). Mitoma et al. (1985) described similar findings over the course of a 4-week study, in which male Osborne-Mendel rats and B6C3F1 mice were gavaged 5 times weekly with 3,000 and 4,000 mg TRI/kg, respectively. In light of the foregoing, it can be concluded that the contribution of hepatic uptake and metabolism to presystemic elimination of TRI is quite modest, and that exhalation plays the major role.

No information on the magnitude of pulmonary first-pass elimination of oral TRI from laboratory experiments was found in the literature. Some 25 years ago, Andersen (1986) published a formula for calculation of the proportion of a circulating VOC that will be exhaled in its initial pass through the pulmonary circulation. This formula in its simplest form can be expressed as $\frac{1}{1 + PC}$, where PC is the VOC's blood:air partition coefficient. TRI's and TCE's *in vitro* blood:air PCs are 8.11 and 5.67 for rats, respectively (Gargas et al., 1989). Thus, it can be estimated that ~ 11 and 15% of TRI and TCE, respectively, will be eliminated after their initial pass through the lungs. Direct measurement studies with male S-D rats revealed that pulmonary first-pass elimination accounted for 5 – 8% of gavage doses of TCE (Lee et al., 1996). The pulmonary first pass effect for TRI was not affected by rate of drug administration and was dose independent in these studies.

Very few data are available on the kinetics of oral TRI, in contrast to a wealth of information about TCE. TRI did not exhibit dose-dependent kinetics in the oral bolus dosage range utilized here. Increases in TRI's C_{max} and AUC were directly proportional to the increase in dose. Terminal elimination $t_{½}$ did not vary significantly

with dose. The converse was true for TCE. The onset of metabolic saturation was evidenced by an increase in $t_{½}$ and disproportionate increases in C_{max} and AUC. Lee et al. (1996) found by direct measurements in male S-D rats, that hepatic first-pass elimination varied inversely with TCE dose. Prout et al. (1985) reported that male Osborne-Mendel rats metabolized most of a 10 mg/kg oral bolus dose of ^{14}C-TCE, as reflected by recovery of 80% of the radioactivity in the urine and as exhaled CO_2. Lee et al. (2000) observed the onset of non-linear kinetics in male S-D rats gavaged with 8 – 16 mg TCE/kg, with a progressive increase in the extent of metabolic saturation as the dosage rose to 432 mg/kg. Despite the limited biotransformation of TRI, Schumann et al. (1982) reported some evidence of metabolic saturation in male F-344 rats with increase in inhaled concentration from 500 to 1,500 pm. This was not evident at the relatively low oral TRI doses used in the current investigation

The rate of oral administration profoundly affected the bioavailability of TCE, but had relatively little effect on TRI. The hepatic clearance of TCE, a compound with perfusion-limited metabolism, was dependent upon dose as well as its rate of delivery to the liver. Administration of TCE as a bolus, coupled with its quick GI absorption, resulted in rapid arrival of amounts of the chemical that exceeded the liver's biotransformation capacity. In contrast, most of the 10 mg TCE/kg g.i. dose was eliminated in its first pass through the liver and lungs. Under such exposure conditions, presystemic elimination should remove virtually 100% of trace, environmentally-relevant levels of TCE before the chemical reaches the arterial blood and extrahepatic organs. Presystemic elimination, however, will have limited influence on the internal dosimetry of poorly metabolized VOCs such as TRI, irregardless of dose or the rate of ingestion.

The aforementioned findings may have important implications for hepatotoxicity and hepatocarcinogenicity risk assessments. The TCE data call into question the practice of deriving drinking water contaminant guidelines for well-metabolized VOCs from results of gavage studies. EPA's (1991) IRIS oral reference dose for carbon tetrachloride ($CC1_4$), for example, is derived by extrapolation from hepatotoxicity data from a gavage study in rats by Bruckner et al. (1986). Sanzgiri et al. (1995), however, reports that 17.5 and 179 mg $CC1_4$/kg p.o. are substantially more hepatotoxic to rats than when given over 2 h by g.i. Sanzgiri et al. (1997) see much higher liver, arterial blood and extrahepatic tissue $CC1_4$ C_{max} and AUC values in p.o. than in g.i. rats. La et al. (1996) find greater DNA binding and cellular proliferation in the liver of male B6C3F1 mice gavaged with 1,2,3-trichloropropane (TCP) than in mice ingesting the halocarbon in their water. Larson et al. (1994) report dose-dependent increases in hepatocellular necrosis and regenerative hyperplasia in female B6C3F1 mice gavaged daily with $CHCl_3$. No such changes were seen in mice consuming the same doses in water *ad libitum*. As noted in the introduction, $CHCl_3$ 1,1- and 1,2-dichloroethane produced high incidences of hepatocellular carcinoma in B6C3F1 mice gavaged with large, chronic doses. Other researchers have saw no evidence of this tumor when equivalent doses are supplied in the animals' drinking water (Jorgenson et al. ,1985; Klaunig et al.,1986). The large, oral bolus doses apparently result in concentrations of halocarbons and their active metabolites high enough to exceed the capacity of hepatocellular defense and repair systems.

Our TCE data suggest that the liver, acting in concert with the lungs, can efficiently remove well-metabolized VOCs from the systemic circulation before they

reach the arterial circulation and extrahepatic organs, when ingested amounts are low and their rate of delivery to the liver is limited. A wide variety of extrahepatic tissues contain cytochrome P4502E1 (CYP2E1) (de Waziers et al., 1990), the isoform primarily responsible for bioactivation of low concentrations of TCE and numerous other potentially carcinogenic halocarbons (Guengerich et al., 1991). CYP2E1-catalyzed metabolic activation of TCE *in situ* is important in renal (Lash et al. 2000), testicular (Forkert et al., 2002) and pulmonary (Forkert et al., 2006). DNA binding and cellular proliferation were significantly lower in the kidney and forestomach of B6C3F1 mice receiving TCP in their water than in mice gavaged with the chemical (La et al., 1996). Similarly, rats given 30 mg/kg of 1,1-dichloroethylene as an oral bolus exhibit much greater kidney and lung injury than do rats given their dose by g.i. over 2 h (Bruckner et al., 2006). If a cytotoxic and/or carcinogenic chemical reaches extrahepatic organs in reduced amounts or does not reach them at all, it follows that noncancer and/or cancer risks will be reduced or negligible. Presystemic elimination here appears to be an important protective mechanism for ingestion of trace levels of VOCs that undergo metabolic activation.

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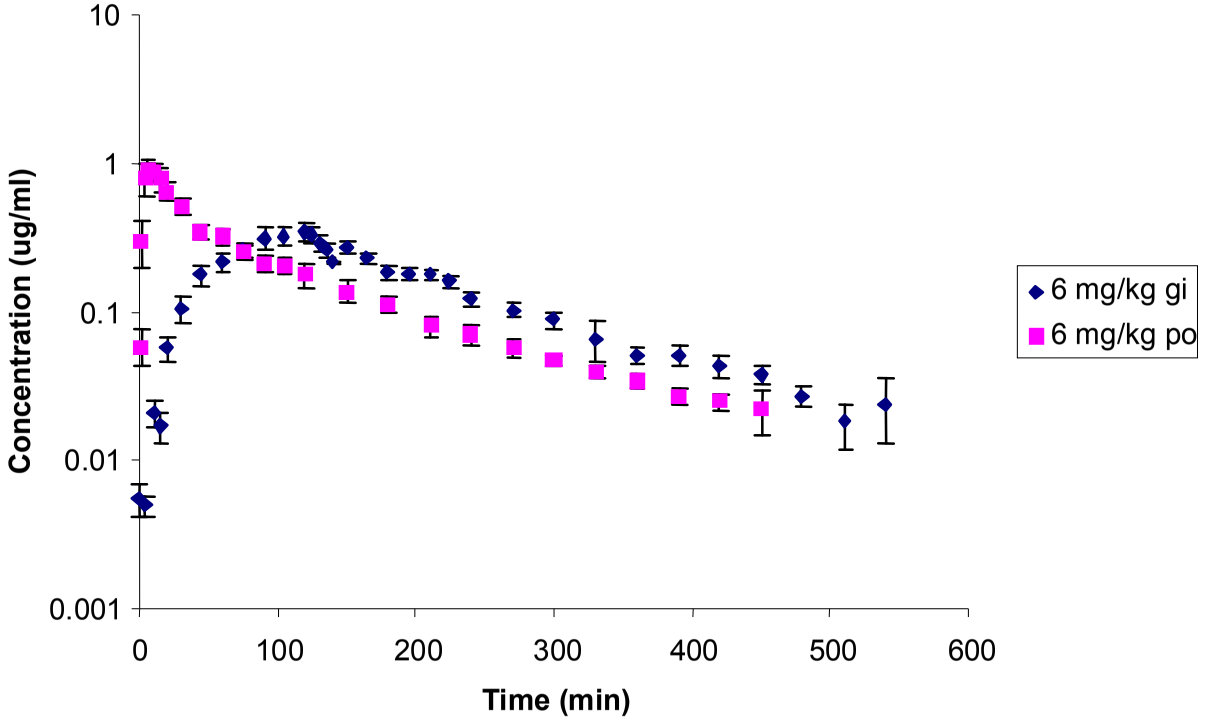

Figure 4.1A: Effect of pattern of ingestion on arterial blood TRI concentration-versus-time profiles. Rats were administered 6 mg TRI/kg in an aqueous emulsion by gavage (po) or by constant gastric infusion (gi) over 120 min. Values are means ± SE for groups of 6 rats.

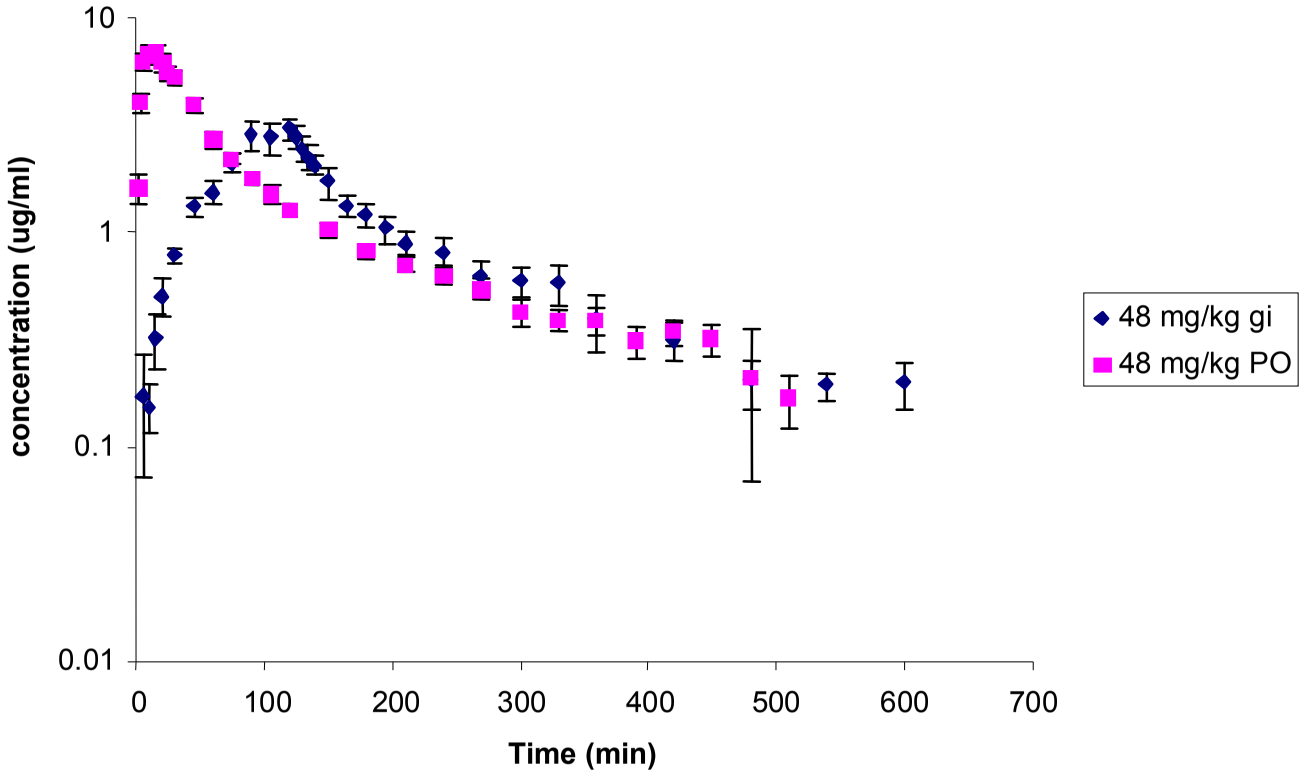

Figure 4.1B: Effect of pattern of ingestion on arterial blood TRI concentration-versus-time profiles. Rats were administered 48 mg TRI/kg in an aqueous emulsion by gavage (po) or by constant gastric infusion (gi) over 120 min. Values are means ± SE for groups of 6 rats.

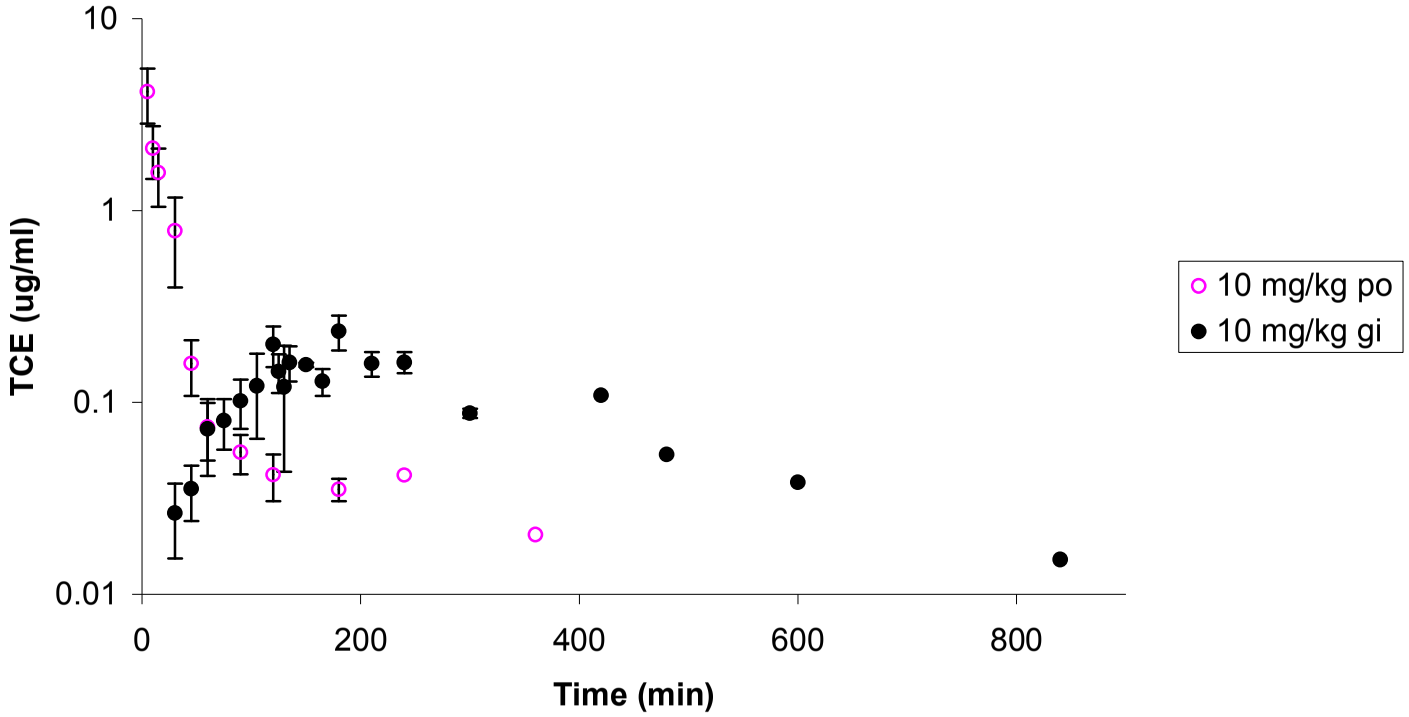

Figure 4.2A: Effect of pattern of ingestion on arterial blood TCE concentration-versus-time profiles. Rats were administered 10mg TCE/kg in an aqueous emulsion either by gavage (po) or by constant gastric infusion (gi) over 120 min. Values are means ± SE for groups of 6 rats.

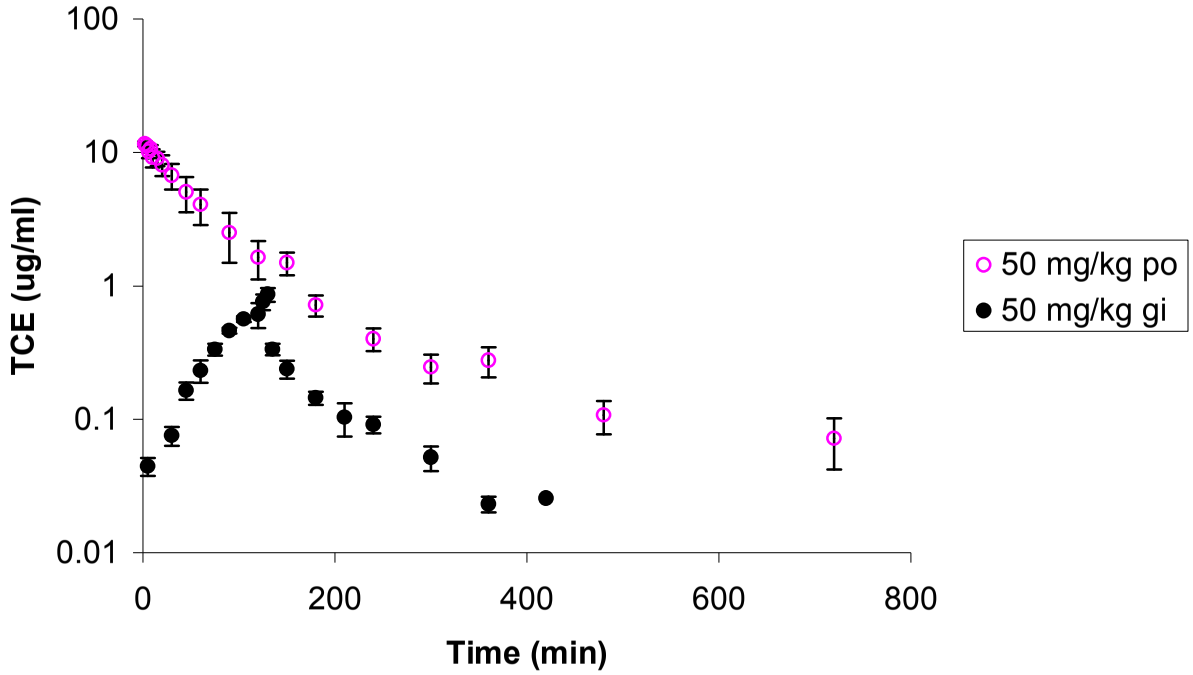

Figure 4.2B: Effect of pattern of ingestion on arterial blood TCE concentration-versus-time profiles. Rats were administered 50 mg TCE/kg in an aqueous emulsion either by gavage (po) or by constant gastric infusion (gi) over 120 min. Values are means ± SE for groups of 6 rats.

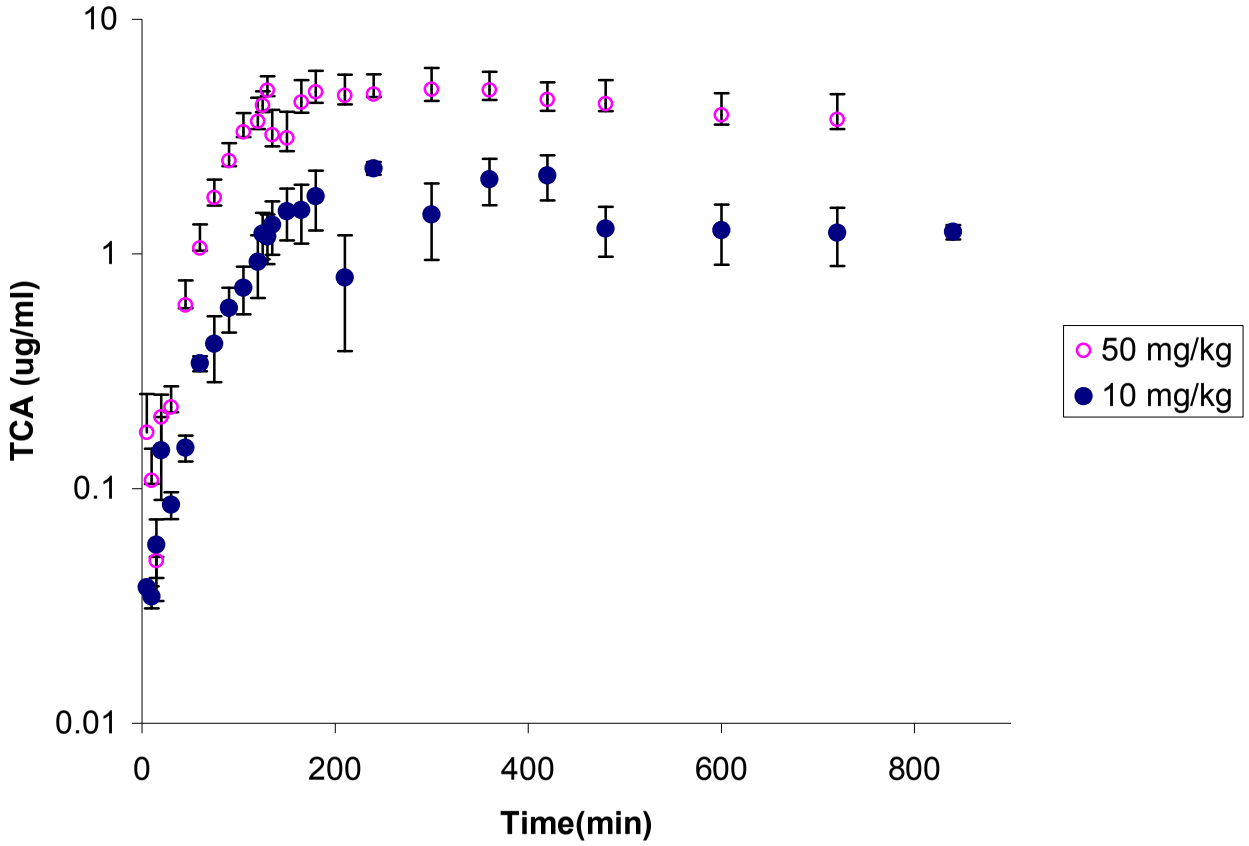

Figure 4.3A: Arterial blood TCA concentrations after constant gastric infusion of 10 or 50 mg TCE/kg over 120 min. Values are means ± SE for groups of 6 rats.

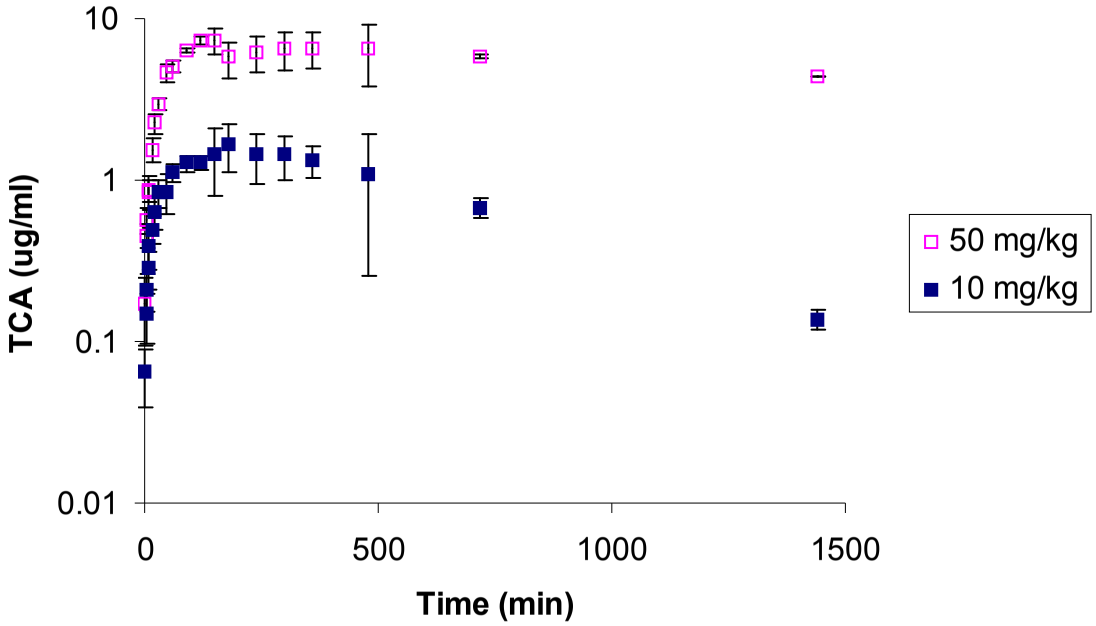

Figure 4.3B: Arterial blood TCA concentrations after gavage of rats with 10 or 50 mg TCE/kg. Values are means ± SE for groups of 6 rats.

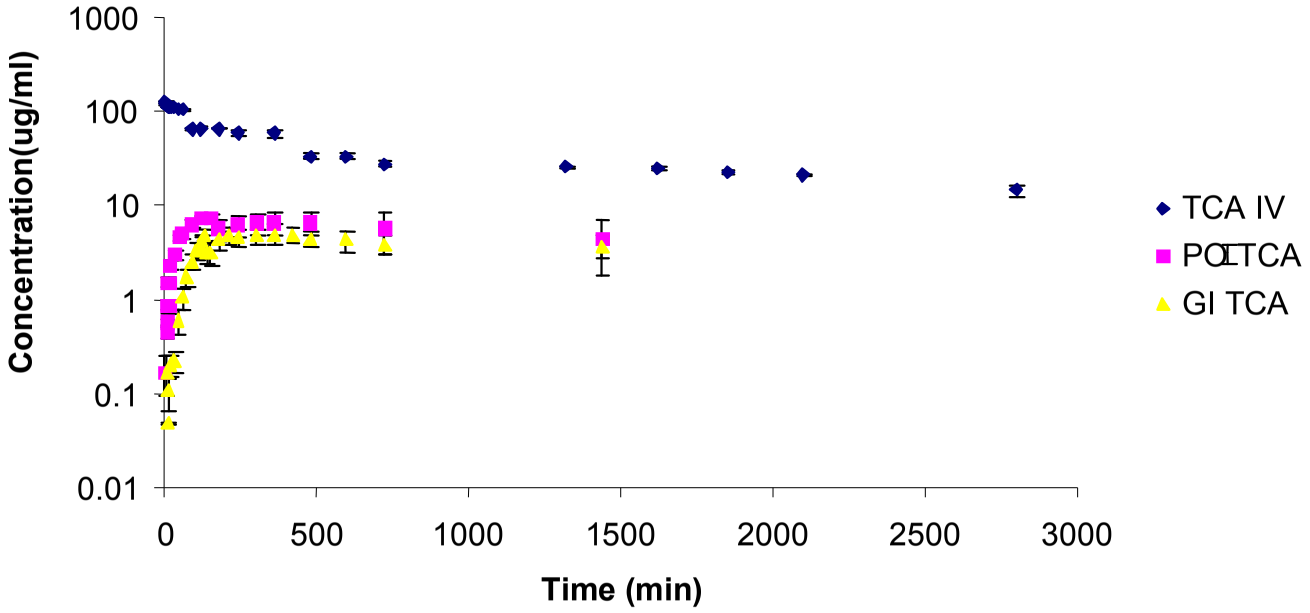

Figure 4.4: Blood concentration time profile of TCA after IV administration of 50 mg TCA/kg and 50 mg TCE/kg by oral administration (gi over 120 min and po). Values are means ± SE for groups of 6 rats.

Table 4.1: Oral Pharmacokinetics of TRI

Dose (mg/kg)	AUC (min*µg/ml)	Cl (ml/min/kg)	T ½ (min)	Tmax (min)	Cmax (µg/ml)
6 GI	**65 ± 16 [a]**	**56 ± 17 [a]**	**67 ± 30 [a]**	**118 ± 16[a]**	**0.4 ± 0.1 [a]**
6 PO	**70 ± 17 [a]**	**56 ± 11 [a]**	**99 ± 28 [a, b]**	**8 ± 2 [b]**	**1.1 ± 0.6 [a]**
48 GI	**499 ± 161 [b]**	**43 ±12 [a]**	**112 ± 24 [b]**	**108 ± 14 [a]**	**3.5 ± 1.2 [a]**
48 PO	**621 ± 80 [b]**	**69 ± 23 [a]**	**117 ± 32 [b]**	**14 ± 7 [b]**	**7.8 ± 2.3 [b]**

Pharmacokinetic parameters after 2-h gastric infusion (gi) or gavage (po) administration of 6 or 48 mg TRI/kg. to rats. Values are means ± SD for groups of 6 animals.
[a-b] Means of each parameter which are significantly different ($p<0.05$) are designated by different superscripts.

Table 4.2: Oral Pharmacokinetics of TCE

Dose (mg/kg)	AUC (min*µg/ml)	Cl (ml/min/kg)	T ½ (min)	Tmax (min)	Cmax (µg/ml)
10 gi	**5 ± 2 [a]**	**54 ± 9 [a]**	**56 ±11 [a]**	**109 ± 9 [a]**	**0.04 ± 0.02 [a]**
10 po	**80 ± 56 [b]**	**58 ± 29 [a]**	**53 ± 15 [a]**	**5 ± 0 [b]**	**5.1 ± 2.2 [b]**
50 gi	**737 ± 8 [c]**	**26 ±2.5 [b]**	**78 ± 13 [a]**	**128 ± 4[c]**	**8.7 ± 2.3 [c]**
50 po	**1057 ± 275 [c]**	**27 ± 8 [b]**	**179 ± 84 [b]**	**3 ± 1 [d]**	**12.5 ± 0.7 [c]**

Pharmacokinetic parameters after 2-h gastric infusion (gi) or gavage (po) administration of 10 or 50 mg TCE/kg to rats. Values are means ± SD for groups of 6 animals.
[a-d] Means of each parameter which are significantly different ($p<0.05$) are designated by different superscripts. Significant at $p < 0.05$

Table 4.3: Pharmacokinetics of TCA after TCE administration

Dose (mg/kg)	Cmax (ug/ml)	AUC (ug-min/ml)
10 (gi)	**2.6 ± 0.3 [a]**	**1349 ± 116 [a]**
10 (po)	**1.9 ± 0.03 [b]**	**650 ± 287 [b]**
50 (gi)	**6.3 ± 1.7 [c]**	**2442 ± 1054 [c]**
50 (po)	**7.6 ± 1.0 [c]**	**5590 ± 1936 [c]**

Pharmacokinetic parameters of TCA after 2-h gastric infusion (gi) and gavage (po) administration of 10 or 50 mg TCE/kg. to rats. Values are means ± SD for groups of 6 animals.
[a-c] Means of each parameter which are significantly different ($p<0.05$) are designated by different superscripts

CONCLUSION

Volatile organic compounds are significant contaminants of the environment and are presently an important issue in human health risk assessment. Although these compounds exhibit numerous toxicities at high doses, it is unknown what effect these compounds have on laboratory animals when exposed by various routes of administration and at different rates of oral ingestion. The goal of this research is to understand the pharmacokinetics and pharmacodynamics of DCE, TCE, TRI and some of their metabolites when exposure occurs via different routes. The objectives of these studies were to define the effect that the route of administration and rate of oral administration has on the metabolic and toxicity profile of DCE, TCE, and TRI in rats. The disposition and metabolic/toxicity profile of DCE, TCE, and TRI vary as a function route of administration.

The metabolic/toxicity profiles of DCE and TCE are significantly affected by route of administration and rate of oral ingestion due to the fact that they are both extensively metabolized by the liver. The bioavailability is significantly greater after exposure to DCE via inhalation. It is also evident that in the dosage range of 10-30 mg/kg by oral administration that the process which is responsible for removing DCE is becoming saturated. There was also a pattern of significant increase in F with an increase in dose in each oral group, indicative of saturation of first-pass metabolism. It appears the capacities of hepatocellular detoxification (GSH) and repair systems are exceeded. An enzyme that indicates liver toxicity (SDH) was slightly elevated in gi and inhalation groups and moderately elevated in the gavage group 24 h post exposure. Route of administration also had a pronounced influence on kidney injury by DCE. Kidney

toxicity indices (GGT and NAG) showed markedly elevated levels after inhaling 300 ppm DCE and slight or no difference when dosed orally with 30 mg/kg DCE. These findings demonstrate that both the route of exposure and rate of oral administration substantially influence the toxicokinetics and systemic toxicity of DCE. Bioavailability of TCE is dependent on dosage regimine. After gi bioavailability is consistently lower than gavage dosing. After oral administration of 50 mg/kg TCE metabolites (TCA and TCOH) are substantially higher in the bolus group.

Route of exposure does not have a significant effect on the toxicokinetics of TRI. While bioavailability of TRI was significantly different among the routes of exposure, it was not affected by the rate of oral ingestion. When comparing relevant pharmacokinetic parameters (AUC, CL, T1/2, and F) after a change in rate of oral ingestion (gi vs po) were similar. These findings reveal that rate of oral administration does not influence the toxicokinetics and bioavailability of TRI, but route of administration significantly alters the bioavailability.

Many of the previous toxicokinetic studies with volatile organic compounds have been done by inhalation exposure. This is due to the fact that this is the primary route of exposure, but also due to the expense and time associated with oral gavage and gastric infusion. Compounds entering the body through the lung are only affected by one elimination organ which is the lung before reaching systemic circulation, where as, when a compound is ingested it must pass through three elimination organs: Gut (efflux transporters), liver, and the lungs before reaching systemic circulation.

The most important contribution of the findings in these studies are that they illustrate the importance of designing experimental protocols that are relevant to actual human exposures.