# APPENDIX C
# INFORMATION ON CHEMICAL MIXTURES AND THEIR COMPONENT CHEMICALS

## Introduction

This appendix summarizes data on chemicals considered for examples in the present analysis. These data can be used in a component-based approach to cumulative risk, particularly in developing an understanding of toxicokinetics relevant to determining the likelihood of an interaction. For the data contained in this appendix, several points bear emphasis.

1. No data on either of the two example chemical mixtures was located. Thus, this report contains information only on each of 10 components chemicals (*i.e.*, 6 component chemicals for Mixture 1 and 4 component chemicals for Mixture 2).
2. This summary of data is by no means an exhaustive review for each of these 10 component chemicals. Only brief information is provided for each of the sections for each chemical. We placed a great deal of emphasis on not repeating the effort of existing reviews; rather, we provide an update on each chemical with current information.
3. We focused more on pharmacokinetics/pharmacodynamics with special emphasis on physiologically-based pharmacokinetic (PBPK) modeling.

In this report, the toxicological and pharmacokinetic characteristics of ten chemicals are discussed. The ten chemicals consist of two groups that can potentially form mixtures in drinking water. The first mixture consists of the organophosphorus pesticides parathion, methyl parathion, chlorpyrifos, diazinon, fenthion, and fenitrothion. The second mixture consists of the chlorinated hydrocarbons chloroform, trichloroethylene, tetrachloroethylene, and 1,1,1 trichloroethane. Each chemical is discussed separately in one section of the report. In each section, a brief description of the toxicology of the chemical is provided as a background to the selection of appropriate dose metrics for risk assessments that can be quantified using PBPK modeling. Subsequently, available data describing the pharmacokinetics (PKs) of each chemical in laboratory animals and humans is provided. Finally, available studies regarding potential PK interactions between the chemicals are provided.

The studies incorporated in this review are necessarily limited. The review is based on a detailed search of the open literature. However, inevitably there are additional studies to be considered, especially those that are not published.

The principal purpose of this review is to compile data that may be useful in performing a PBPK model-based cumulative risk assessment (CRA) for the two groups of chemicals in drinking water.

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Yang, R. S. H. (2004). Final Report and Final Approach. A Report to USEPA under USEPA Contract No. 3C-R102-NTEX. May 24, 2004.

# Parathion

## 1.0 Introduction

Parathion (*O*, *O*-diethyl 4-nitrophenyl phosphothioate) is a phosphorothionate insecticide that has no registered uses in the U.S. but is widely used elsewhere in agriculture and is present in food and environments (Brack *et al.* 1999; Fenske *et al.* 2002; Leblanc *et al.* 2000; Lifshitz *et al.* 1999; Ripley *et al.* 2000; Simcox *et al.* 1995).

## 1.1 Toxic effects

Parathion exerts its toxicological effects via inhibition of acetylcholinesterase (Nigg and Knaak 2000; Thiermann *et al.* 1997). Metabolism of parathion exploits CYP450 as a metabolizing enzyme (Atterberry *et al.* 1997; Attia 2000; Attia *et al.* 1995; Besser *et al.* 1993; Halpert *et al.* 1980; Halpert and Neal 1981a, b; Howard and Pope 2002; Jett *et al.* 1994; Katz *et al.* 1997). Other toxicities such as reproductive toxicities, immunotoxicity, cytotoxicity, carcinogenicity, and other effects have also been shown (Bustos-Obregon and Diaz 1999; Bustos-Obregon *et al.* 2001; Cabello *et al.* 2001; Cao *et al.* 1999; Carlson and Ehrich 2001; Carlson *et al.* 2000; Galloway and Handy 2003; Grellner and Glenewinkel 1997; Ivens *et al.* 1998; Levario-Carrillo *et al.* 2001; Li and Zhang 2001; Liu *et al.* 1999; Melendez Camargo and Lopez Hernandez 1998; Olivier *et al.* 2001a; Olivier *et al.* 2001b; Padungtod *et al.* 1999; Padungtod *et al.* 1998; Padungtod *et al.* 2000; Rojas *et al.* 1998; Saleh *et al.* 2003; Segura *et al.* 1999; Selgrade *et al.* 1984; Senel *et al.* 2001; Tong *et al.* 1988; Undeger *et al.* 2000; Van Den Beukel *et al.* 1997; van den Beukel *et al.* 1998; Wagner *et al.* 2003; Zaidi *et al.* 2000).

## 1.2 Pharmacokinetics

There are a number of pharmacokinetic studies of parathion and its toxic metabolite, paraoxon, conducted both in non-mammalian and mammalian species such as mice, rat, pig and dog via many routes of exposure including intravenous, oral and dermal exposure (Braeckman *et al.* 1983; Brimer *et al.* 1994; Chang *et al.* 1997; Chang *et al.* 1994a; Chang and Riviere 1991, 1993; Chang *et al.* 1994b; Denga *et al.* 1995; Eigenberg *et al.* 1983; Hurh *et al.* 2000a; Hurh *et al.* 2000b, c; Lessire *et al.* 1996; Oneto *et al.* 1995; Pena-Egido *et al.* 1988a; Pena-Egido *et al.* 1988b).

Parathion at the dose of 3 mg/kg was intravenously administered to a rat. From the pharmacokinetic analysis, the terminal half-life and clearance of parathion were 3.4 hr and 93 ml/min/kg respectively (Eigenberg *et al.* 1983). Similar results were obtained from another study in rats, where the terminal half-life, AUC and clearance of parathion were 321 min., 52.5 μg-min/mL and 57.1 ml/min/kg respectively (Hurh *et al.* 2000a; Hurh *et al.* 2000b, c). In these studies, paraoxon levels were lower than their detection limits.

Parathion pharmacokinetics in dogs were somewhat different from that in rats. After 30 mg intravenous dosing, plasma clearance and terminal half-life were 21 ml/min and 8.5-11.2 hr respectively. The plasma clearance in dogs appeared to be less than one-third of the plasma clearance for the rats.

### 1.2.1 Absorption

In a pharmacokinetic study in dogs (Braeckman *et al.* 1983), parathion was administered at 5 mg/kg intravenously and 10 mg/kg orally to determine its absolute bioavialability (F). The fraction absorbed was high (57-98%). However, the bioavailability of parathion appeared to have a comparatively large variation because of its first pass metabolism and intersubject variation in parathion hepatic extraction

ratio (range = 63-97%) (Braeckman *et al.* 1983). Oral absorption of parathion was also studied in rats (Beubler *et al.* 1985).

Numerous dermal exposure studies have been performed (Antunes-Madeira and Madeira 1984; Bucks *et al.* 1990; Campbell *et al.* 2000; Carver and Riviere 1989; Carver *et al.* 1989; Fisher *et al.* 1985; Gyrd-Hansen *et al.* 1993; Hawkins and Reifenrath 1986; Knaak *et al.* 1984; Murphy 1980; Qiao *et al.* 1996; Qiao *et al.* 1994; Reifenrath *et al.* 1984; Reifenrath *et al.* 1991; Riley and Kemppainen 1985; Shah and Guthrie 1983; Skinner and Kilgore 1982; Wester *et al.* 2000; Williams *et al.* 1990; Williams *et al.* 1996). After dermal application of 50 mg/kg parathion was performed along the midline of the entire back of pigs, the dermal bioavailability (F) was 0.0993. Tissue distribution of parathion in back skin, back fat, liver, kidney, muscle, adipose tissue was also determined. It appeared that 25.0-60.8% of the administered dose remained at the application site (Brimer *et al.* 1994).

### 1.2.2 Distribution

Protein binding in dog serum and in human serum were 99% and 98% respectively (Braeckman *et al.* 1983). Tissue distributions were also reported in some species (Brimer *et al.* 1994).

### 1.2.3 Metabolism

Parathion is metabolized into paraoxon and 4-nitrophenol by desulfuration and dearylation (Fig. 1), respectively. 4-Nitrophenol formation is considered as the inactivation pathway, whereas paraoxon formation is considered as an activation pathway (Benke GM 1975; Bulusu and Chakravarty 1986, 1988; Butler and Murray 1993, 1997; Chambers and Forsyth 1989; Chambers *et al.* 1994; Chaturvedi *et al.* 1991; Contreras *et al.* 1999; Halpert *et al.* 1980; Halpert and Neal 1981a, b; Hou *et al.* 1996; Kulkarni and Hodgson 1982; Kuo and Perera 2000; Lapadula *et al.* 1984; Levi and Hodgson 1985; Martinez-Zedillo *et al.* 1979; Monnet-Tschudi *et al.* 2000; Morgan *et al.* 1994; Murray and Butler 1994, 1995; Mutch *et al.* 1999; Mutch *et al.* 2003; Nadin and Murray 1999; Pond *et al.* 1995; Pond *et al.* 1998; Purshottam and Srivastava 1987; Ramos and Sultatos 1998; Rowland *et al.* 1991; Soranno and Sultatos 1992; Sultatos 1986; Sultatos *et al.* 1984; Sultatos and Gagliardi 1990; Sultatos and Minor 1986; Sultatos *et al.* 1985; Sultatos and Murphy 1983; Tang and Chambers 1999; Vargas Loza *et al.* 1997; Venera *et al.* 1978; Vitarius *et al.* 1995; Wallace and Dargan 1987; Watson *et al.* 1994; Zhang and Sultatos 1991; Zhu and Liu 1994). The primary metabolizing organ is the liver by the enzyme cytochrome P450 3A4.

In mouse liver microsomes, the apparent Km's for the formation of paraoxon and p-nitrophenol were 29.6 and 26.5 μM respectively, and the apparent Vmaxs were 5.8 and 6.7 nmols/100 mg liver/min respectively (Sultatos 1986; Sultatos *et al.* 1984; Sultatos and Gagliardi 1990; Sultatos and Minor 1986; Sultatos *et al.* 1985; Sultatos and Murphy 1983).

In rat liver microsomes, the kinetic curve for desulfuration of parathion is baphasic with apparent Km's of 0.23 and 71.3 μM and Vmaxs of 3.62 and 4.56 nM/min/mg protein. For the dearylation reaction, parathion has an apparent Km and Vmax of 56 μM and 1.49 nM/min/mg protein, respectively (Ma and Chambers 1994, 1995).

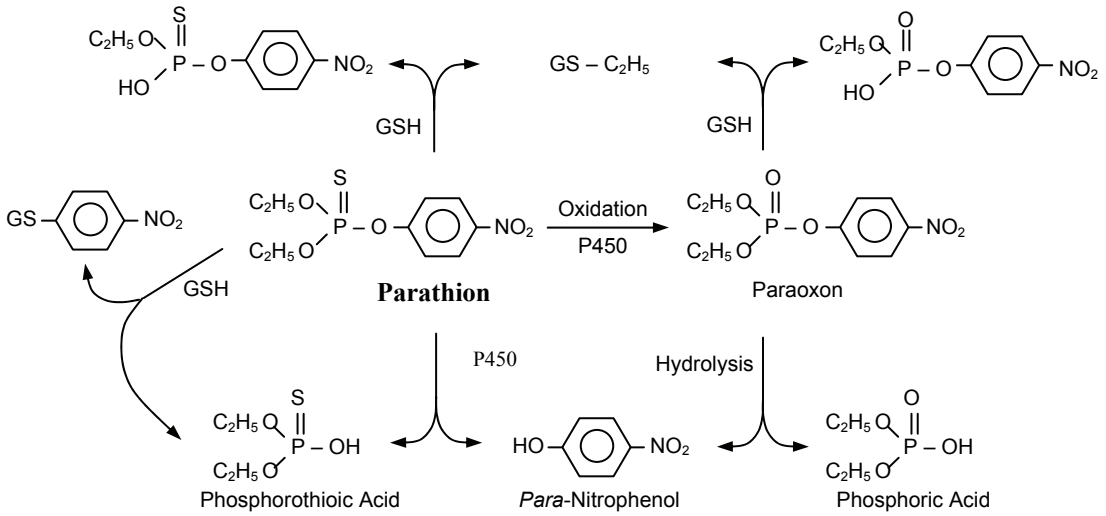


Figure 1. Metabolic pathway of parathion (adapted from Benke 1975).

In a human microsomal study, parathion demonstrated biphasic behaviors in both individual microsomal and pooled samples. Its apparent $Km_1$ and $Km_2$ in individual microsomes were 0.30 and 165.5 μM and $Vmax_1$ and $Vmax_2$ were 290 and 821 pmol oxon/mg protein/min respectively. In pooled liver microsomes, parathion's apparent $Km_1$ and $Km_2$ were 9.0 and 69.6 μM and $Vmax_1$ and $Vmax_2$ were 106.6 and 2,478 pmol oxon/mg protein/min, respectively (Buratti *et al.* 2003; Ma and Chambers 1994, 1995). Another study in human liver microsomes indicated that CYP3A4 is the major enzyme responsible for catalyzing parathion oxidation to paraoxon (Butler and Murray 1993, 1997).

In addition to the liver, the brain is also capable of metabolizing parathion in various regions such as cortex, olfactory bulb/hypothalamus, striatum, cerebellum, midbrain, medulla and pons, and hippocampus. However, the total activity appears to be highest in the cortex (Soranno and Sultatos 1992).

### 1.2.4 Pharmacokinetic studies in special population

There have been a number of pharmacokinetic studies in specific populations (Benjaminov *et al.* 1992; Jaramillo and Reyes 1990). Neilsen *et al.* conducted a pharmacokinetic study in neonatal and young pigs. Intravenous parathion (0.5 mg/kg) was administered to newborn, 1 week and 8 weeks old piglets. The total body clearance was 7, 35 and 121 ml/min/kg, respectively. Tissue distribution in all groups was also presented. Interestingly, the newborn piglets seemed to retain parathion in significant amounts in many organs such as the liver, lung, brain, heart and muscle indicating that reduced total body clearance in the newborn markedly influenced tissue distribution (Nielsen *et al.* 1991).

Pregnancy also affects parathion disposition and its toxicity. Concentrations of parathion were significantly higher in blood and brain of pregnant mice at most times after administration (5 mg of parathion/kg) of parathion when compared to non-pregnant mice (Weitman *et al.* 1983, 1986a, b).

## 1.3 Pharmacokinetic interaction between parathion and other compounds

Due to its metabolic pathway via CYP450, there are many possibilities that parathion pharmacokinetics may be affected by certain compounds particularly drugs, CYP450 inducers, inhibitors, other environmental pollutants and foods (Agyeman and Sultatos 1998; Carr *et al.* 2002; Chakravarty and Sreedhar 1982; Costa and Murphy 1984; Delaunois *et al.* 1999; Gelal *et al.* 2001; Graziano *et al.* 1985; Guilhermino *et al.* 1998a, b; Hurh *et al.* 2000a; Hurh *et al.* 2000b, c; Joshi and Thornburg 1986; Karanth *et al.* 2001; Miranda *et al.* 1998; Mourelle *et al.* 1986; Murphy 1980; O'Shaughnessy and Sultatos 1995; Purshottam and Kaveeshwar 1982; Purshottam and Srivastava 1984; Ramos and Sultatos 1998; Sawahata and Neal 1982; Siller *et al.* 1997; Wester *et al.* 2000).

Cimetidine, a non-specific inhibitor of CYP450, was capable of antagonizing methyl parathion toxicity but failed to decrease parathion-induced toxicity in mice and rats (Weitman *et al.* 1983). Rats pretreated with dexamethasone, a specific inducer of CYP3A23, showed faster clearance of parathion than control rats (Hurh *et al.* 2000a).

## 1.4 PBPK modeling of parathion and Monte Carlo simulation

A PBPK model of parathion was developed by Gearhart *et al*. In brief, the model describes the metabolism of parathion to paraoxon by the liver, the inhibition of acetylcholinesterase, butyrylcholinesterase, and carboxylesterase by paraoxon in the brain, liver, kidneys, rapidly perfused tissues and the arterial and venous blood (Gearhart 1994). Physiologic parameters are available in the literature. (Jepson *et al.* 1994; Kousba and Sultatos 2002). Due to the existence of paraoxonase polymorphisms (Costa *et al.* 2003; Diepgen and Geldmacher-von Mallinckrodt 1986; Eaton 2000; Furlong *et al.* 2000; Haber *et al.* 2002; Laplaud *et al.* 1998; Lee *et al.* 2003; Shih *et al.* 1998), a subsequent study was conducted by using this existing PBPK model with Monte Carlo simulation to elaborate the effect of polymorphic paraoxonase (PON1) on its toxicity (Gentry *et al.* 2002).

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# Methyl Parathion

## 2.0 Introduction

Methyl parathion (*O*,*O*-dimethyl *O*-4-nitrophenylphosphorothioate) is a highly toxic organophosphorus insecticide approved for specific agricultural crops. Its use is restricted by appropriately trained certified pesticide applicators (Garcia *et al.* 2003). However, it has been used illegally indoors in certain areas of the Southern and Midwestern parts of the United States due to its effectiveness and low cost (ATSDR 2001; Rubin *et al.* 2002), leading to an increased health risk in non-workers, children and pregnant women.

## 2.1 Toxic effects

Neurotoxicity is the major toxic effect of methyl parathion (MP) or its metabolite, methyl paraoxon, in various species caused by inhibition of acetylcholinesterase (AChE) enzymes, resulting in acetylcholine accumulation at postsynaptic receptors and overstimulation of cholinergic systems (Chambers and Carr 1993; Gupta *et al.* 2000; Hahn *et al.* 1991; Ma *et al.* 2003). The median lethal dose ($LD_{50}$) of MP in mice applied orally and dermally was 14.5 and 1200 mg/kg body weight, respectively, while the dermal median effective dose ($ED_{50}$) that caused 50% reduction in AChE was 550 mg/kg at 24 hours after dosing (Haley *et al.* 1975; Skinner and Kilgore 1982). The developing animals are more sensitive to acute toxicity of MP than adults, indicating the age-related differences in sensitivity to MP exposure (Liu *et al.* 1999; Pope and Chakraborti 1992; Pope *et al.* 1991).

In humans, manifestations of exposure to MP such as shortness of breath, nose bleeding, vomiting, diarrhea, abdominal cramps, headache, eye pain, blurred vision, sweating, confusion, muscle contraction, contact burns and erythema multiforme eruption (following dermal exposure) were reported. The severe neurotoxic effects include loss of coordination, slurred speech, fatigue and death caused by respiratory or cardiac arrest (Azaroff and Neas 1999; Fisher 1986; Karki *et al.* 2001; Rehner *et al.* 2000). Cranial nerve palsies and intermediate syndromes have also been reported in certain patients (Karki *et al.* 2001; Narendra *et al.* 1989).

Other effects reported include genotoxic and mutagenic effects (Bartoli *et al.* 1991; Breau *et al.* 1985; Chen *et al.* 1981; de Cassia Stocco *et al.* 1982; Degraeve and Moutschen 1984; Dolara *et al.* 1993; Griffin and Hill 1978; Grover and Malhi 1985; Lodovici *et al.* 1994; Lodovici *et al.* 1997; Mathew *et al.* 1990; Mathew *et al.* 1992; Nehez *et al.* 1994; Rashid and Mumma 1984; Rupa *et al.* 1990; Rupa *et al.* 1991; Singh *et al.* 1984; Tripathy *et al.* 1987; Undeger *et al.* 2000; Velazquez *et al.* 1990; Vijayaraghavan and Nagarajan 1994; Wiaderkiewicz *et al.* 1986), effects on calmodulin (Pala *et al.* 1991), effects on liver and muscle enzymes (Della Morte *et al.* 1994; Gupta *et al.* 1994; Jabbar *et al.* 1990), hematoxicity (Parent-Massin and Thouvenot 1993), immunotoxic effects (Crittenden *et al.* 1998; Institoris *et al.* 1995; Institoris *et al.* 1992; Lee *et al.* 1979; Sunil Kumar *et al.* 1993; Undeger *et al.* 2000), hormonal effects (Asmathbanu and Kaliwal 1997; Fatranska *et al.* 1978; Lukaszewicz-Hussain *et al.* 1985; Sortur and Kaliwal 1999), reproductive and developmental effects (Basha and Nayeemunnisa 1993; Desi *et al.* 1998; Dhondup and Kaliwal 1997; Garcia *et al.* 2003; Gupta *et al.* 1985; Gupta *et al.* 1984; Kumar and Desiraju 1992; Mahaboob Basha *et al.* 2001; Mahaboob Basha and Nayeemunnisa 1993; Nagymajtenyi *et al.* 1995; Nayeemunnisa and Begum 1992; Sortur and Kaliwal 1999), embryotoxicity (Tanimura *et al.* 1967; Uzokwu 1974), cardiac toxicity (Howard and Pope 2002), and behavioral effects (George *et al.* 1992; Liu *et al.* 1994; Schulz *et al.* 1990; Zhu *et al.* 2001).

## 2.2 Pharmacokinetics

### 2.2.1 Absorption

Because of its lipid solubility, MP can be absorbed through skin; therefore, the most likely route of human exposure is dermal, particularly from agricultural field reentry (Abu-Qare *et al.* 2000). Oral exposure can also occur via contaminated food or water consumption and suicidal attempt (Garcia *et al.* 2003) while exposure to MP via inhalation during spraying is questionable (Kummer and van Sittert 1986).

*Oral absorption*

MP is well and rapidly absorbed through the gastrointestinal tract following oral gavage in mice (Hollingworth 1967), rats (Garcia-Repetto *et al.* 1997; Kramer and Ho 2002; Kramer *et al.* 2002; Miyamoto 1963), guinea pigs (Miyamoto 1963), dogs (Braeckman *et al.* 1983), and humans (Morgan *et al.* 1977). However, the oral bioavailability is very low (5-20%), which can be explained by a significant hepatic first-pass effect. The oral absorption rate constant after 1.5-2.5 mg/kg administration of MP in rats was 1.2 $h^{-1}$ (Kramer and Ho 2002; Kramer *et al.* 2002).

*Dermal absorption*

An *in vitro* model using human skin in a static diffusion cell system demonstrated that 5.2% of the applied dose of MP from a commercial formulation was present after 24 h (Sartorelli *et al.* 1997). In adult female rats and pregnant rats, 20-50% of administered dose was absorbed following a single dermal dose of 10-50 mg/kg MP with the absorption rate constant of 0.41 $h^{-1}$ (Abu-Qare *et al.* 2000; Kramer and Ho 2002; Kramer *et al.* 2002).

### 2.2.2 Distribution

Following oral and dermal administration, MP is extensively bound to plasma protein and rapidly distributed to tissues including placenta and fetus. Then it is slowly redistributed to the central compartment (Abu-Qare *et al.* 2000; Garcia-Repetto *et al.* 1997). The highest level of MP is found in adipose tissue. Distribution coefficients of adipose tissue, liver and brain in rats and mice have been published (Garcia-Repetto *et al.* 1995; Sultatos *et al.* 1990). The terminal half-life varies from 7.2 h to 15 days, depending on species and gender (Abu-Qare *et al.* 2000; Braeckman *et al.* 1980; Garcia-Repetto *et al.* 1997; Kramer and Ho 2002; Kramer *et al.* 2002). The volume of distribution is relatively high (9.6 l/kg in dogs and 10.1 l/kg in female rats) (Braeckman *et al.* 1980; Kramer and Ho 2002).

### 2.2.3 Metabolism

MP is metabolized by hepatic and extrahepatic phase I and phase II enzymes (Figure 2.1) (Abu-Qare *et al.* 2000; Garcia *et al.* 2003). Phase I metabolism include dearylation of MP, leading to the formation of *p*-nitrophenol and dimethyl thiophosphoric acid, which promotes detoxification. On the other hand, desulfuration by cytochrome P450 can activate MP to methyl paraoxon, the neurotoxic metabolite (Yamamoto *et al.* 1983; Zhang and Sultatos 1991). This oxidation process is the major metabolic pathway of MP in the liver (Anderson *et al.* 1992; Sultatos 1987). Methyl paraoxon is also formed in the brain. CYP2B has been demonstrated to be responsible for MP activation in rat brain extracts (Albores *et al.* 2001). The activities of dearylation and desulfuration of MP were reduced when a low dose of MP was given repeatedly in rats (Yamamoto *et al.* 1982).

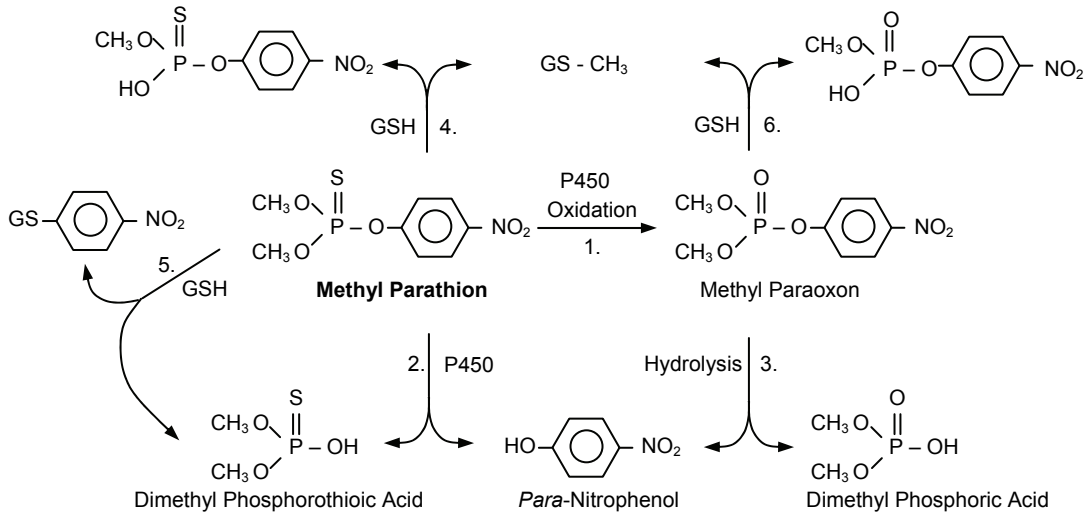


Figure 2.1. Metabolic pathways of MP (Benke and Murphy 1975; Garcia *et al.* 2003).

Methyl paraoxon is then hydrolyzed by liver and plasma paraoxonase to form *p*-nitrophenol and dimethyl phosphoric acid (Garcia *et al.* 2003). The correlation between $LD_{50}$ of MP in rats of several ages and reaction rates of metabolism of methyl paraoxon, both hydrolysis and GSH-dependent pathways, was reported, indicating that these pathways contributed to age-related differences in MP toxicity (Benke and Murphy 1975). *p*-Nitrophenol further undergoes glucuronidation and sulfuric conjugation.

MP is also conjugated by glutathione *S*-aryl transferase to form *p*-nitrophenyl mercapturic acid (Di Ilio *et al.* 1995; Huang and Sultatos 1993; Sultatos and Woods 1988) and by glutathione *S*-alkyl transferase to yield *S*-methyl glutathione (Radulovic *et al.* 1987; Radulovic *et al.* 1986). Furthermore, a study has reported another non mixed-function oxidative pathway of MP in brain tissue subfractions that transformed MP to its isomer (de Lima *et al.* 1996).

### 2.2.4 Excretion

MP is rapidly eliminated after oral and dermal administration. Renal excretion is the major route of MP elimination. In rats, 75-90% of administered dose was recovered in urine and less than 10% was found in feces (Abu-Qare *et al.* 2000; Abu-Qare and Abou-Donia 2000; Hollingworth 1967; Miyamoto 1963). In humans, the urinary metabolites of MP are *p*-nitrophenol, dimethylphosphate, and unidentified metabolites (Morgan *et al.* 1977). Therefore, *p*-nitrophenol has been used as a biomarker of MP exposure in humans (Barr *et al.* 2002; Chang *et al.* 1997; Esteban *et al.* 1996; Hryhorczuk *et al.* 2002; Rubin *et al.* 2002).

## 2.3 Interactions

MP has been reported to produce behavioral alterations when given in combination with endosulfan (Castillo *et al.* 2002) or toxaphene (Crowder *et al.* 1980) and more likely to induce intermediate syndrome when combined with parathion (De Bleecker *et al.* 1992). Conversely, the inhibition of cholinesterase enzyme activity was significantly lowered when MP was administered with either chlorpyrifos or diazinon, which could be due to competition for cytochrome P-450 enzymes, resulting in

inhibition of oxon formation (Abu-Qare *et al.* 2001; Abu-Qare and Abou-Donia 2001). Moreover, cimetidine, chlordecone, mirex and linuron, gentamicin and rifamycin, polychlorinated biphenyls (PCBs), and permethrin have also been demonstrated to change the toxicity of MP (Carr *et al.* 2002; Joshi and Thornburg 1986; Ortiz *et al.* 1995; Tvede *et al.* 1989; Youssef *et al.* 1987). No interactions with acetaminophen and hexachlorocyclohexane (HCH) have been reported (Costa and Murphy 1984; Dikshith *et al.* 1991).

## 2.4 PBPK models

One- to three-compartment classical models has been used to fit the blood concentration data following intravenous, oral, and dermal administration (Abu-Qare *et al.* 2000; Braeckman *et al.* 1983; Braeckman *et al.* 1980; Kramer and Ho 2002; Kramer *et al.* 2002). No PBPK models for MP have been published.

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# Chlorpyrifos

## 3.0 Introduction

Chlorpyrifos (*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothiolate) is an OP pesticide with restricted uses in the U.S. It is produced in the U.S. and marketed under various trade names, including Dursban®, Lorsban®, and other names (ATSDR 1997). Chlorpyrifos (CP) has been used against pests in turfgrass, commercial agriculture, and in residential settings, although indoor uses have been restricted. CP has been found in drinking water supplies, although often at low levels (ATSDR 1997).

## 3.1 Toxic Effects

Numerous systemic effects of CP have been reported, but the critical effect for risk assessment is inhibition of acetylcholinesterase by CP or CP-oxon (ATSDR 1997). This can result in headache, diaphoresis, nausea, vomiting, diarrhea, epigastric cramping, bradycardia, blurred vision, miosis, bronchoconstriction and excess mucous secretions, pulmonary edema, dyspnea, muscle fasciculations, salivation, lacrimation, urination, tremors, anxiety, drowsiness, confusion, ataxia, abnormal gait, hypotension, and memory impairment (Ballantyne and Marrs 1992).

Neurodevelopmental effects have also been described (Auman *et al.* 2000; Campbell *et al.* 1997; Carr *et al.* 2001; Chakraborti *et al.* 1993; Chanda and Pope 1996; Crumpton *et al.* 2000; Dam *et al.* 1999; Dam *et al.* 1998, 2000, 2003; Das and Barone 1999; Garcia *et al.* 2002; Garcia *et al.* 2003; Gore 2001, 2002; Howard and Pope 2002; Jett *et al.* 2001; Lassiter *et al.* 1998; Levin *et al.* 2002; Levin *et al.* 2001; Olivier *et al.* 2001; Qiao *et al.* 2002; Qiao *et al.* 2001; Qiao *et al.* 2003; Raines *et al.* 2001; Richardson and Chambers 2003; Roy *et al.* 1998; Sachana *et al.* 2001; Slotkin *et al.* 2001; Slotkin *et al.* 2002; Song *et al.* 1998; Tang *et al.* 1999; Whitney *et al.* 1995; Won *et al.* 2001). Some of these studies suggested that developmental neurotoxicity occurred at lower exposure levels than did acetylcholinesterase inhibition in adult animals, and developmental neurotoxicity may be worthy of consideration in future risk assessments (Abdel-Rahman *et al.* 2002).

## 3.2 Pharmacokinetics

### 3.2.1 Absorption

CP is well absorbed through the gut after oral exposure to CP in drinking water or food. In humans or rodents, 70-90% of the oral dose was absorbed (Ahdaya *et al.* 1981; Bakke *et al.* 1976; Nolan *et al.* 1984; Smith *et al.* 1967). Only 3% of the dermal dose was absorbed; (Nolan *et al.* 1984) however, this is dependant on the vehicle. When acetone was used as vehicle in another study, 46-99% of the dose was absorbed (Shah *et al.* 1987). Absorption rates through the gut (Cook and Shenoy 2003) and skin (Griffin *et al.* 2000; Sartorelli *et al.* 1998) have been quantified.

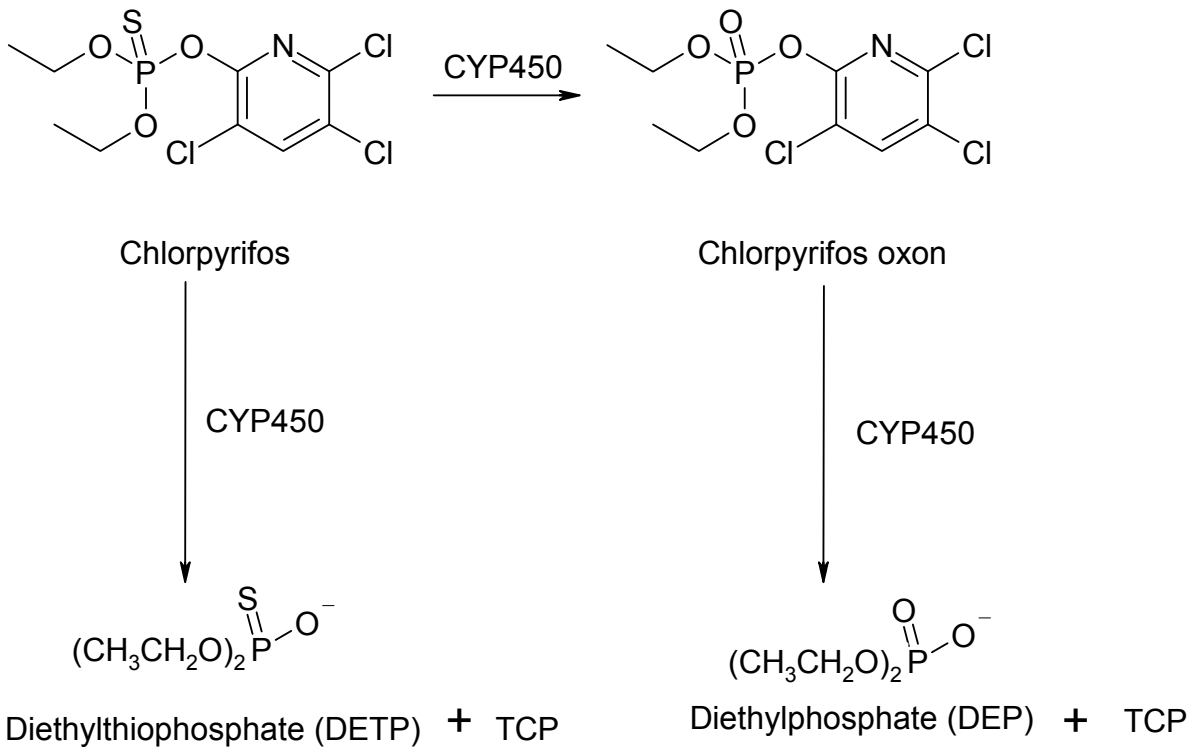
### 3.2.2 Distribution

CP rapidly distributes to tissues after absorption (Shah *et al.* 1987; Shah *et al.* 1981; Smith *et al.* 1967).

### 3.2.3 Metabolism

CP is primarily metabolized in the liver due to the high concentration of cytochrome (CYP) P450s in that organ (Ma and Chambers 1994; Sultatos and Murphy 1983b). CP is rapidly bioactivated to CP-oxon by multiple isoforms of CYP P450. Both CP and CP-oxon are hydrolyzed by acetylcholinesterase to 3,5,6-trichloro-2-pyridinol (TCP), diethyl thiophosphate, and diethyl phosphate (Bakke *et al.* 1976; Nolan *et al.* 1984; Smith *et al.* 1967; Sultatos *et al.* 1985; Sultatos and Murphy 1983a, b). The principal metabolic pathways are shown in Figure 3.1 below.

**Figure 3.1**
**Principal Metabolic Pathways for Chlorpyrifos**




Modified from ATSDR (1997)

For risk assessment or PBPK model development, studies of metabolism are often used, but further incorporation of pharmacodynamic responses such as acetylcholinesterase inhibition would improve the risk assessment or PBPK model development. Therefore, either type of study is included in this review. Several human pharmacokinetic (PK) or biomonitoring studies have been conducted (Cocker *et al.* 2002; Drevenkar *et al.* 1993; Fenske *et al.* 2002; Sams and Mason 1999).

A number of animal studies have been conducted addressing PKs, binding, cholinestase inhibition, or other endpoints with CP (Abdel-Rahman *et al.* 2002; Atterberry *et al.* 1997; Bushnell *et al.* 1994; Bushnell *et al.* 1993; Carr and Chambers 1996; Carr *et al.* 2002; Carr *et al.* 1995; Chanda *et al.* 1997; Chiappa *et al.* 1995; Cowan *et al.* 2001; Hunter *et al.* 1999; Karanth and Pope 2000; Lassiter *et al.* 1999; Li *et al.* 2000; Li *et al.* 1995; Liu *et al.* 1999; Mattsson *et al.* 2000; Mortensen *et al.* 1996; Mortensen *et al.* 1998; Moser *et al.* 1998; Moser and Padilla 1998; Padilla *et al.* 2000; Padilla *et al.* 1994; Pond *et al.* 1995; Pond *et al.* 1998; Stanton *et al.* 1994).

Fetal transfer of CP or metabolites was assessed in several studies, including two recent ones (Abdel-Rahman *et al.* 2002; Ashry *et al.* 2002).

*In vitro* PK studies with CP have been conducted. These include studies using cell lines (Barber and Ehrich 2001; Ehrich *et al.* 1997; Monnet-Tschudi *et al.* 2000), microsomes (Buratti *et al.* 2003; Katz *et al.* 1997; Ma and Chambers 1994; Poet *et al.* 2003; Sams *et al.* 2000; Tang *et al.* 2001; Usmani *et al.* 2003), antibodies (Buratti *et al.* 2003), tissue slices (Liu *et al.* 2002), or other systems (Amitai *et al.* 1998).

Genetic polymorphisms relevant to CP metabolism have been described (Brophy *et al.* 2000; Costa *et al.* 1999; Costa *et al.* 2003; Dai *et al.* 2001; Furlong *et al.* 2000a; Furlong *et al.* 1998; Furlong *et al.* 2000b).

### 3.2.4 Excretion

Most metabolites are found as conjugated metabolites of TCP in the urine. The half-life of elimination in rats was 10-16 hours in most tissues and 62 hours for fat (Smith *et al.* 1967). The half life for elimination in humans was estimated at 27 hours (Nolan *et al.* 1984).

## 3.3 Interactions with other OP pesticides

Interactions of CP with other OP pesticides have been studied (Axelrad *et al.* 2002; Karanth *et al.* 2001; Richardson *et al.* 2001; Tang *et al.* 2002; Usmani *et al.* 2002).

## 3.4 PBPK models for chlorpyrifos

One group has developed PBPK models for CP (Timchalk *et al.* 2002a; Timchalk *et al.* 2002b). These models were based on adult rat and human exposures in gavage, dietary, or dermal studies in a seven-compartment model structure. They included saturable metabolism of CP by CYP and "a-esterases," and binding of CP-oxon to "b-esterases" as a second order process. Regeneration of b-esterases was also included.

A classical PK model for CP was also described (Rigas *et al.* 2001).

The Timchalk *et al.* CP PBPK model could be adapted for use in a PBPK-model based risk assessment. Adaptation should include alteration for drinking water scenarios. Also, during the process of determining the common mechanism of toxicity for a mixture of OP pesticides, if developmental neurotoxicity is an important part of the risk assessment, PBPK models for relevant endpoints in the fetus or neonates should be considered.

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# Diazinon

## 4.0 Introduction

Diazinon (*O*,*O*-diethyl *O*-2 isopropyl-6-methylpyrimidinyl phosphothiolate) is an organophosphate insecticide that is used in agriculture and as a topically applied pesticide in animal use (ATSDR 1996; Garfitt *et al.* 2002; USEPA 2003). It is a colorless liquid and was available as granules, emulsifiable concentrate, dust, and wettable powder. It is soluble in most organic solvents and is stable in neutral media but is slowly hydrolyzed in alkaline media and more rapidly in acidic media (HSDB 2003).

## 4.1 Toxic effects

Many effects have been determined to occur in chronic bioassays in laboratory animals. Some of the target organs affected include the respiratory, kidney, cardiovascular, gastrointestinal, hematological, hepatic, endocrine, lymphatic, reproductive (ATSDR 1996), immune (Galloway and Handy 2003), and nervous systems (Gordon and Mack 2003).

Acute oral LD50s in rats between 76 and 408 mg/kg have been reported (ATSDR 1996). Dermal LD50's in rats ranged between 455 and 1100 mg/kg (ATSDR 1996).

Diazinon has been reported to cause genotoxicity in a number of assays, including in the *S. typhimurium*, mouse lymphoma cell forward mutation assay, and Chinese hamster cell chromosomal aberration assay, but was negative in several other assays (ATSDR 1996; Hatjian *et al.* 2000).

Risk assessment for exposure to diazinon, however, has generally been based on inhibition of brain acetylcholinesterase (AChE) as the critical endpoint of toxicity (ATSDR 1996). As with other OP pesticides, the mode of action of diazinon is inhibition of AChE in the central and peripheral nervous system. Diazinon is a weak inhibitor of AChE while the oxon analog is much more potent. Therefore, activation by mixed function oxygenases, primarily in the liver, is an important bioactivating step. Other metabolic pathways (see Figure 4.1 below) are generally detoxifying. Symptoms of acute toxic exposure include vomiting, unconsciousness, giddiness, sweating, diarrhea, tachycardia, muscle fasciculations, abdominal pain, and bronchospasm (ATSDR 1996).

ATSDR has published an oral Minimal Risk Level for intermediate term exposure to diazinon of 0.0002 mg/kg/day (ATSDR 1996). USEPA established a chronic reference dose (RfD) of 0.0002 mg/kg/day in the diet (USEPA 2000).

## 4.2 Pharmacokinetics

Diazinon pharmacokinetics are qualitatively similar to other organophosphate pesticides described in this report.

### 4.2.1 Absorption

Several oral absorption studies have been performed. 85% of the single oral dose of 4.0 mg/kg diazinon was absorbed by Beagle dogs in one study (Iverson *et al.* 1975). Other oral absorption studies were

conducted in rats, goats, sheep and cows (Abdelsalam and Ford 1986; Janes *et al.* 1973; Machin *et al.* 1974; Machin *et al.* 1971; Mount 1984; Wu *et al.* 1996a). In a dermal study, human volunteers absorbed 34% of the dose applied to the abdomen or forearm for 24 hours (Wester *et al.* 1993).

### 4.2.2 Distribution

Diazinon is found widely distributed in all tissues examined after oral absorption (Abdelsalam and Ford 1986; de Blaquiere *et al.* 2000; Janes *et al.* 1973; Machin *et al.* 1974; Machin *et al.* 1971; Mucke *et al.* 1970; Tomokuni and Hasegawa 1985; Tomokuni *et al.* 1985). No studies of distribution after inhalation or dermal exposures are available. After an *i.v*. dose of 0.2 mg/kg in ethanol, the terminal halflife was 1.5 hours (Iverson *et al.* 1975). Distribution coefficients for diazinon were reported (Garcia-Repetto *et al.* 1995).

### 4.2.3 Metabolism

The principal metabolic pathways of diazinon are shown in Figure 4.1.

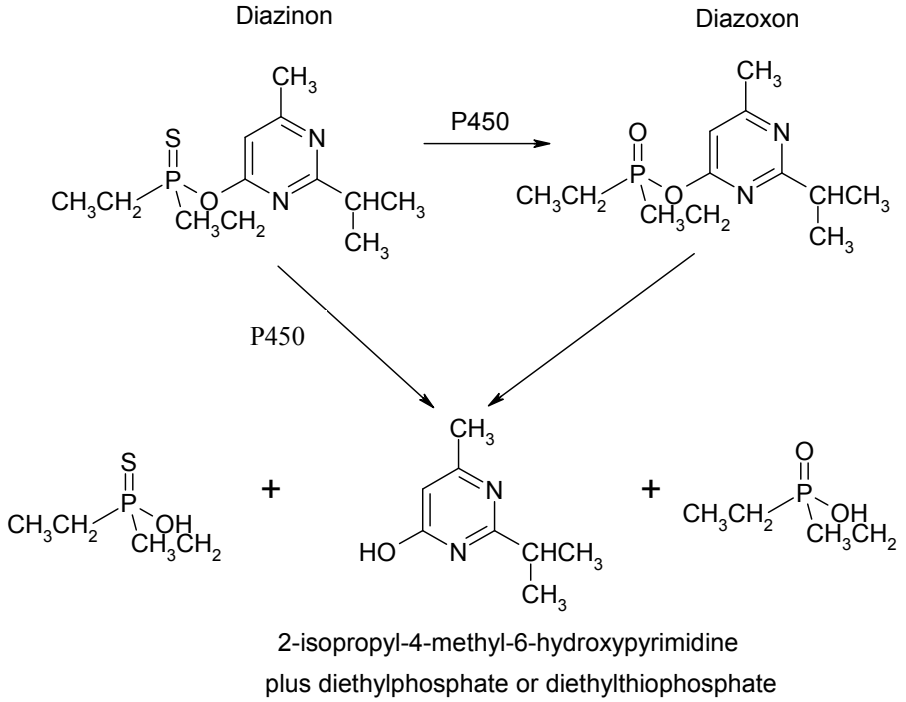


Figure 4.1 Principal metabolic pathways for diazinon. Adapted from Poet, 2003.

Diazinon is subject to oxidative desulfurization and hydrolysis of the ester. Hydrolysis of the ester can occur before or after desulfurization, *i.e*., either diazinon or diazoxon can be hydrolyzed to yield 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) and either diethylphosphate or diethylthiophosphate (Iverson *et al.* 1975; Machin *et al.* 1975; Mucke *et al.* 1970); however, the P450 catalyzed oxidative cleavage of phosphorothioate (*i.e.,* diazinon) triester bond is much more efficient (Yang *et al.* 1971). Desulfurization is mediated by cytochrome P450 isoenzymes while oxidative cleavage or hydrolysis is mediated by cytochrome P450 or various esterases, respectively (Poet *et al.* 2003b; Walker and Mackness 1987). Diazinon and its metabolites may also be oxidized at alkyl carbons (Aizawa 1989; Yang *et al.* 1971).

In a human self-poisoning case, diazinon was found in serum and monoethyl phosphate, diethyl phosphate, and diethyl phosphorothioate were detected in the urine (Klemmer *et al.* 1978). In rat liver microsomes, diazoxon hydrolysis occurred without NADPH (Yang *et al.* 1971).

Rates of metabolism in rat liver and intestinal microsomes of diazinon and diazoxon were recently reported by Poet and coworkers. The authors measured CYP450 mediated oxidative desulfurization (to form diazoxon) or hydrolysis (to form IMHP and diethylthiophosphate) as well as hydrolysis by esterase (PON1) in microsomes from both tissues. Based on the measured rates, the authors conclude that intestinal metabolism may be important, especially for low level oral doses (Poet *et al.* 2003b). Also in microsomes, Vittozzi *et al*. measured the activity of expressed cytochrome P450s for desulfurization and hydrolysis (Vittozzi *et al.* 2001). Significant activity was obtained for all nine cytochrome P450s tested that varied over about one order of magnitude (CYP2C19, 3A4, 2B6, 1A2, 1A1, 2C8, 2C9, 2D6, and 2A6). A separate study by the same group indicated that CYP2C11, 3A2, and 2B1/2 were involved but that 2E1 and 1A1 were not (Fabrizi *et al.* 1999). Kappers *et al*. indicated that CYP2C19 was the major isoform involved in diazinon metabolism, but that others such as CYP1A2 and CYP3A4 may also be showing some activity (Kappers *et al.* 2001). However, using immunoinhibition and other techniques, Buratti and coworkers found that the principal isoforms involved in diazinon metabolism were CYP3A4, 1A2, and 2B6 (Buratti *et al.* 2003), while Sams *et al*. felt that CYP 2D6 and 3A4 were the most important isozymes (Sams *et al.* 2000).

Toxicity and acetylcholinesterase inhibition was studied in PON1 knockout mice (Li *et al.* 2000).

Rates of inhibition of acetylcholinesterase were measured in some studies (Kamal and Al-Jafari 2000).

#### 4.2.4 Excretion

Most diazinon is excreted as metabolites in urine, while smaller amounts are excreted in feces or, after extensive metabolism, as CO2 in expired air. Approximately 75% of a 4.0 mg/kg oral dose to rats was excreted as urinary metabolites, 20% in the feces, and about 6% as CO2 (Mucke *et al.* 1970). Approximately 85% of total label was recovered in a 24 hour urine sample from dogs receiving a single oral dose of diazinon. After an *i.v*. dose, the dogs excreted approximately 58% of the label in urine (Iverson *et al.* 1975). In human volunteers, urinary excretion of diethyl phosphate and diethyl thiophosphate was reported after oral and dermal dosing (Cocker *et al.* 2002; Garfitt *et al.* 2002). Diazinon has been found in hair (of rabbits) as a potential biomarker of exposure (Tutudaki *et al.* 2003). Blood cholinesterase inhibition has also been used as a biomarker for diazinon exposure (Nigg and Knaak 2000).

#### 4.2.5 Special populations and variability

Several studies have suggested high variability in human metabolism of diazinon (Buratti *et al.* 2003; Kappers *et al.* 2001). Polymorphisms in PON1 have been described (Brophy *et al.* 2000; Cherry *et al.* 2002; Costa *et al.* 2003; Davies *et al.* 1996; Mackness *et al.* 2003).

### 4.3 Interactions with other chemicals

The toxicity was increased and pharmacokinetics of diazinon were affected by cimetidine (Wu *et al.* 1996b). Interactions between diazinon and methyl parathion were reported in the blood and brain of pregnant rats and the fetus after a single dermal dose (Abu-Qare and Abou-Donia 2001). Neurite outgrowth was assessed for mixtures of diazinon and chlorpyrifos (Axelrad *et al.* 2002).

## 4.4 Diazinon PBPK models

One PBPK model for diazinon has been published in abstract form (Poet *et al.* 2003a). The model incorporated an oral exposure route, desulfurization and "hydrolysis" (an oxidation reaction actually) by a CYP450 enzyme, hydrolysis of the oxon by PON1 in liver and blood, and second order binding and inhibition and regeneration of B-esterases in the liver, blood, diaphragm, and brain.

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# Fenthion

## 5.0 Introduction

Fenthion [*O,O*-dimethyl *O*-(4-(methylthio)-m-tolyl) phosphorothioate, (DMTP), Figure 1] is an organophosphorus insecticide used against mosquitoes, pests and bugs (EXTOXNET 1996). It is available in dust, emulsifiable or liquid concentrate, and granular and wettable powder formulations. Fenthion is soluble in organic solvents such as DMSO, acetone, methanol and ether, but not in water (NTP 2003).

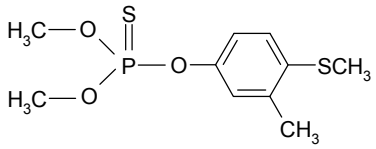


Figure 5.1: Chemical structure of fenthion

## 5.1 Toxic effects

Fenthion is moderately toxic to laboratory animals (mice, rats, guinea pigs and rabbits). The acute oral and intraperitoneal $LD_{50}$'s varied from approximately 125 to >1000 mg/kg body weight (DuBois and Kinoshita 1964; IPCS 1971; Ma 1995). Mortality of rats orally treated with fenthion in subchronic studies (30 mg/kg for 13 weeks, or 5.0 mg/kg for 3 months) was reported (NIH 1979).

Fenthion predominately causes cholinergic toxicity in animals and humans. Its oxon inhibits plasma, erythrocyte and brain cholinesterase activity (Bai *et al.* 1990; De Bleecker *et al.* 1994; Dellinger and Mostrom 1988; Ma 1995; Misra *et al.* 1985; Misra *et al.* 1994; Tsatsakis *et al.* 2002; Tsatsakis *et al.* 1998). An acute no-observed-adverse –effect-level (NOAEL) of 0.07 mg/kg/day was determined in a 2-year oral monkey study (USEPA 1999a, 2001). Other effects unrelated to cholinergic mechanisms, however, were also reported (Bagchi *et al.* 1995; Bagchi *et al.* 1996; Cova *et al.* 1995; Kojima *et al.* 1992).

Fenthion did not show mutagenic effect in the bacterial reverse mutation test or the *in vitro* chromosome aberration test in Chinese hamster ovary cells, but did in unscheduled DNA synthesis study and mouse micronucleus assays (USEPA 1999a). In a 103-week chronic feeding study, no elevated incidence of tumor was observed in both sexes of F344 rats and female B6C3F1 mice; a slightly increased incidence of sarcomas, fibrosarcomas, and rhabdomyosarcomas of the integumentary system in male B6C3F1 mice was observed (NIH 1979). Fenthion is not considered a carcinogen (Ma 1995; USEPA 1999a).

## 5.2 Pharmacokinetics

### 5.2.1 Absorption

Depending on application, fenthion may be absorbed from the gastrointestinal tract, skin and respiratory tract. The former two pathways, however, have been more intensively studied. Generally, fenthion is readily absorbed from GI tract. Blood levels peak a few hours after oral dosing in rats (Ma 1995), rabbits (Emteres *et al.* 1985) and lactating goat (Ma 1995). Absorption was almost complete (96-100% at 72

hours) and not dose-dependent (at 10 or 100 mg/kg) in Wistar rats fasted for 16-24 hour before gavage (Ma 1995).

Dermal absorption of fenthion is slow and incomplete. Eighteen hours after a single dermal dose, prepared as an application formulation, in pigs or lactating cows, the tissue residue levels were generally low, whereas at the application site the levels were much higher (Ma 1995). USEPA set a dermal absorption factor as 20% in 1996 and re-set it as 3% in 1999 based on the LOAEL's (lowest observed adverse effect level) of cholinesterase inhibition from an oral development toxicity study and a 21-day dermal toxicity study in rabbits (USEPA 1999b).

### 5.2.2 Distribution

From the limited information on its distribution in body, fenthion and its metabolites had relatively high concentration in fat, liver and kidney (EXTOXNET 1996). In milk from fenthion-treated dairy cows, the fenthion level was 50 times higher in the "fat" fraction than that in the "non-fat" fraction (O'Keeffe *et al.* 1983).

### 5.2.3 Metabolism

Principal metabolic pathways are shown in Figure 5.2. Fenthion has several possible oxidative metabolites in body such as sulfoxide (SO), sulfone ($SO_2$), oxygen analogue (P=O), oxygen analogue sulfoxide (P=O, SO) and sulfone (P=O, $SO_2$) (Wright and Riner 1979). Of them, the oxygen analogues are bioactivated forms with higher anti-cholinesterase activity (IPCS 1971, 1976).

Incubated with rat liver microsomes, fenthion was oxidized to oxygen analogue and fenthion sulfoxide. Fenthion sulfone, however, was not detected. The main enzymes involved were cytochrome P450s (especially CYP1A1) and flavin-containing monooxygenase (Kitamura *et al.* 2003; Venkatesh *et al.* 1991). In liver cytosol of rats, fenthion sulfoxide was reduced to fenthion catalyzed by aldehyde oxidase (Kitamura *et al.* 2003).

$^{14}$C-Fenthion was extensively metabolized in rats (Ma 1995). No unchanged parent compound was detected in the urine and very little (< 2%) in the feces. The major group of metabolites (about 60% of the total label) was composed of the three phenols (phenol fenthion, phenol sulfoxide and phenol sulfone) and their glucuronide and sulfate conjugates. Four demethyl metabolites accounted for about 30% of the label, whereas the oxygen analogue sulfoxide constituted only 1-4%. The metabolite profiles were not affected by dosing route, dose, sex or pre-treatment with fenthion.

In pigs, fenthion was oxidized to fenthion sulfoxide, fenthion sulfone, oxygen analogue and oxygen analogue sulfoxide and sulfone. These metabolites were further hydrolyzed and excreted via urine in conjugated forms (Ma 1995).

No data from human studies is available.

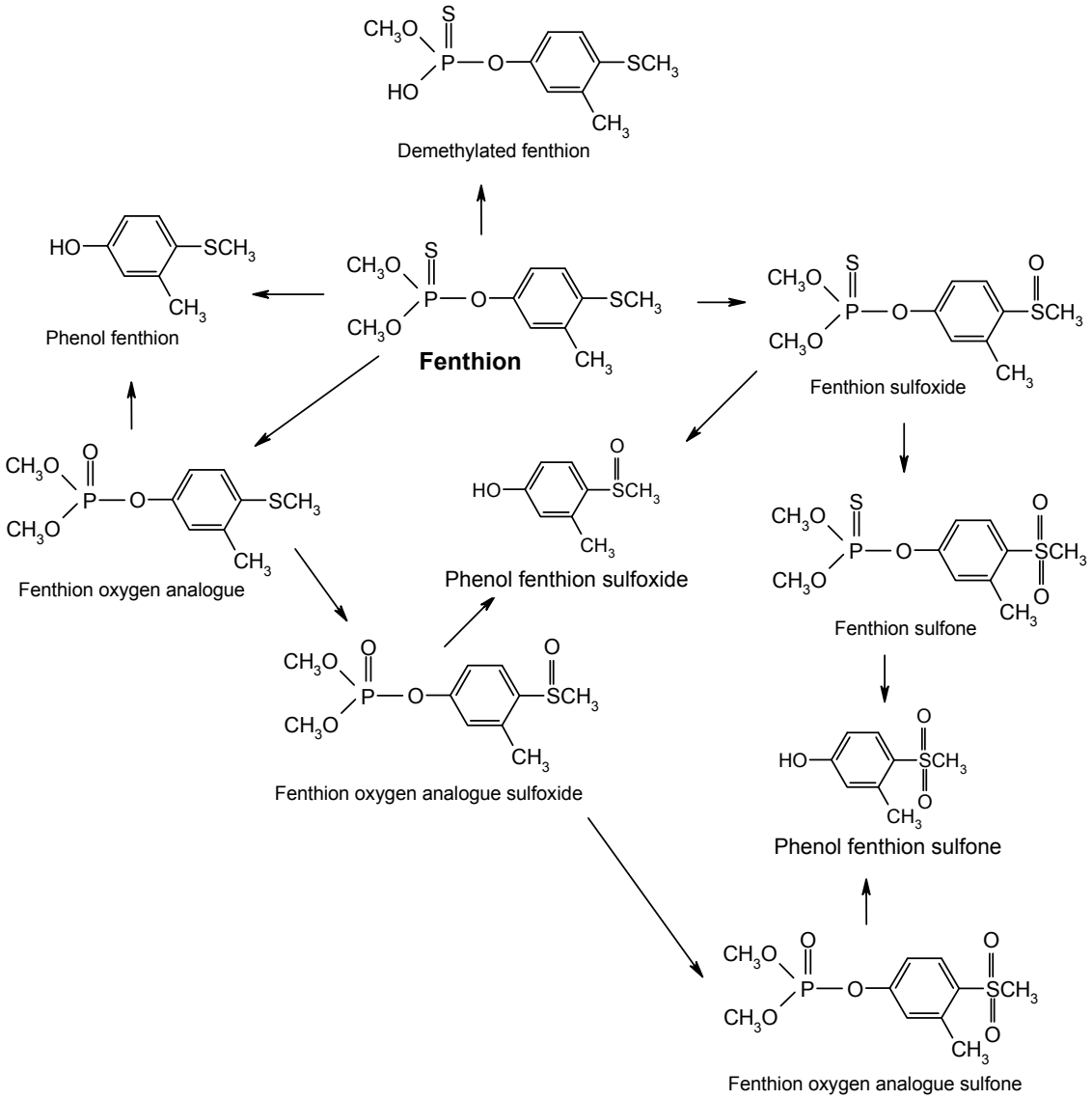


Figure 5.2: Proposed metabolism pathways of fenthion in the rats.
Adapted from Ma, 1995.

## 5.2.4 Excretion

Fenthion was rapidly eliminated after a single dose in Wistar rats, over 90% of the administered radiolabel being excreted within 48 hours, and less than 1% retained 72 hours after treatment (Ma 1995). In pigs and dairy cows rapid elimination and low bioaccumulation were also observed (Ma 1995). In New Zealand white rabbits, the halflife of a single dose (20mg/kg) was about 11-12 hours regardless the route of administration (Emteres *et al.* 1985).

The polar metabolites of fenthion are mainly excreted via urine in rats, pigs and dairy cows (Ma 1995). Milk is a significant pathway for the elimination of the parent compound from lactating dairy cows (IPCS 1971; O'Keeffe *et al.* 1983; Wright and Riner 1979).

## 5.3 Interactions of fenthion with other OP pesticides

Fenthion potentiated the acute intraperitoneal toxicity of malathion, dioxathion, and coumaphos in rats, but intraperitoneal administration of 13 other organophosphate or carbamate insecticides to rats in combination with fenthion did not result in greater than additive toxic effects (Ma 1995). Dietary combination of equitoxic doses (2 mg/kg) of fenthion with coumaphos, neither of which alone affected cholinesterase activity when fed to dogs for six weeks, was found to potentiate the anticholinesterase activity in serum and erythrocytes by 75 and 30%, respectively. The potentiation was less evident with malathion, and no potentiation was noted with dioxathion (Ma 1995). Pretreatment with fenthion significantly potentiated the acute toxicity of 2-*sec*-butylphenyl *N*-methycarbamate (BPMC) in mice and dogs, which may be a result of the inhibited detoxification of the carbamate (Ma 1995; Miyaoka *et al.* 1984; Miyaoka *et al.* 1987).

## 5.4 PBPK models

No PBPK models on fenthion have been reported yet.

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# Fenitrothion

## 6.0 Introduction

Fenitrothion (*O*,*O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorodithioate), also commonly called Sumithion™, is an organophosphorus insecticide and was registered for use in ant and roach bait. There are no approved domestic food or feed uses for fenitrothion, and exposure to fenitrothion in the U.S. is minimal. However, fenitrothion is used in countries to control pests on crops, stored grains, and cotton. Fenitrothion is also used elsewhere in forest spraying and public health campaigns. As a result, the human health effects associated with exposure to fenitrothion remain a concern, especially among pesticide workers and applicators whose acute exposure to organophosphorus pesticides can sometimes occur at levels high enough to inhibit blood acetylcholinesterase activity (Nigg and Knaak 2000; Ohayo-Mitoko *et al.* 2000; Satoh and Hosokawa 2000).

Degradation rates reported for fenitrothion are as follows:

- Soil and groundwater $\rightarrow T_{1/2}$ < one week (Meister 1994; U.S.EPA 1987)
- Surface water $\rightarrow T_{1/2}$ = 1.5-2 days (Novathion 1987)
- Surface water in dark $\rightarrow T_{1/2}$ = 21.6 - 49.5 days (Novathion 1987)
- Plants $\rightarrow T_{1/2}$ = 1-2 days (Möllhoff 1968)
- Plants (fenitrooxon) $\rightarrow T_{1/2}$ = few hours (Möllhoff 1968)

## 6.1 Toxic effects

Like other organophosphorus compounds, fenitrothion acts in the organism as a cholinesterase inhibitor, after conversion to fenitrooxon. Some evidence indicates that acetylcholinesterase inhibition in brain depends more on the rate of penetration than on the rate of oxidation and decomposition of fenitrothion (JMPR 1988; Miyamoto 1969). Fenitrothion appears to affect cytochrome P450 enzyme activity in the liver and testes of rats (Clos *et al.* 1994; Gradowska-Olszewska *et al.* 1984).

Fenitrothion has anticholinesterase activity and moderate acute toxicity with oral $LD_{50}$ values in rats and mice ranging from 330 to 1,416 mg/kg body weight (Miyamoto *et al.* 1963b). Acute dermal toxicity in rodents is reported to range from 890 to more than 2,500 mg/kg body weight (WHO 1992). The $LC_{50}$ value in rats exposed for 8 h is estimated to be more than 186 mg/m$^3$ (WHO 1992). In short-term studies on rats and dogs, no-observed-adverse-effect levels (NOAELs) based on brain cholinesterase activity were 10 mg/kg diet and 50 mg/kg diet, respectively. Long-term studies on rats and mice indicated a NOAEL of 10 mg/kg diet (WHO 1992). An acceptable daily intake (ADI) of 0.003 mg/kg body weight was established in 1984 (WHO 1986), but no occupational exposure limits (OEL) have been published. No carcinogenic effects were found in any of the long-term fenitrothion studies (WHO 1992). Fenitrothion was not found to be mutagenic in *in vitro* and *in vivo* studies or teratogenic at doses of up to 30 mg/kg body weight in rabbits and up to 25 mg/kg body weight in rats (Benes *et al.* 1975; WHO 1992). Other toxicity studies have been conducted (Chevalier *et al.* 1982; Groszek *et al.* 1995; Khan *et al.* 1990; Misu *et al.* 1966; Myatt *et al.* 1975; Trottier *et al.* 1980; Yoshida *et al.* 1987). Fenitrothion has also been shown to be neurotoxic, immunosuppressive, a pulmonary toxicant, and cause disturbances of prenatal development (Berlinska and Sitarek 1997; Kunimatsu *et al.* 1996; Khan *et al.* 1990; Lehotzky and Ungvary 1976).

In a field spraying operation in Nigeria and Kenya, humans exposed to fenitrothion exhibited depressed plasma cholinesterase activity (Ohayo-Mitoko *et al.* 2000; Vandekar 1965; Wilford *et al.* 1965). A study of fenitrothion on 24 human subjects was also conducted and showed that both plasma and cholinesterase activity was not depressed in all but one case (Nosal and Hladka 1968). Fenitrothion has been reported as causing "intermediate syndrome" due to acute poisoning (Groszek *et al.* 1995).

The presence of chemicals in the environment that have antiandrogenic activity and thus the ability to disrupt the endocrine system is a source of concern. In several studies fenitrothion has been shown to be a competitive androgen receptor antagonist both *in vivo* and *in vitro* (Curtis 2001; Sohoni *et al.* 2001; Tamura *et al.* 2001; Turner *et al.* 2002). One study, however, exhibited fenitrothion not having significant androgenic or antiandrogenic activity *in vivo* (Sunami *et al.* 2000). Fenitrothion might also alter estradiol metabolism by inhibition of certain P450 enzymes and produce changes in adrenal function (Berger and Sultatos 1997; Yamamoto *et al.* 1982b).

## 6.2 Pharmacokinetics

Various studies in mouse, rat, guinea pig, and humans have dealt with the pharmacokinetic and biochemical aspects of fenitrothion and its metabolites (Aprea *et al.* 1999; Douch *et al.* 1968; Hladka and Nosal 1967; Hollingworth *et al.* 1967; Meaklim *et al.* 2003; Miyamoto 1964a; Miyamoto 1964b; Miyamoto and Sato 1969; Miyamoto 1969; Miyamoto *et al.* 1963a; Nishizawa *et al.* 1961; Vandanis and Crawford 1964).

### 6.2.1 Absorption

Fenitrothion is presumably rapidly absorbed from the mammalian intestinal tract when given orally. Additionally, it can also be absorbed by the intact skin and by inhalation. (Kohli *et al.* 1974; Moody and Franklin 1987; Moody *et al.* 1987).

### 6.2.2 Distribution

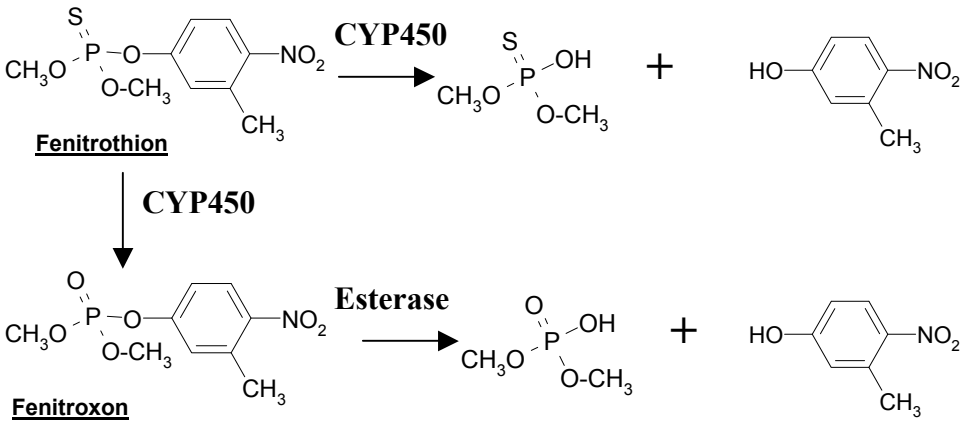
The presence of the oxygen analogue was demonstrated in all tissues examined (brain, heart, lung, liver, kidney, spleen, and muscle), and it was detectable in blood one min after intravenous injection of fenitrothion (Muller 2000).

### 6.2.3 Metabolism

The oxygen analogue is the most important metabolite with respect to toxicity. It is formed in the microsomal fraction of the cell, the main organs responsible for the transformation being the liver and kidney. The major excretion product found is 3-methyl-4-nitrophenol which can be oxidized further to 3-carboxyl-4-nitrophenol. Other metabolites are the dimethyl derivatives, which, with increasing dose, are excreted in increasing amounts. Nine metabolites have been isolated, most of which have also been identified. *In vitro*, formation of the oxygen analogue depended on the availability of reduced nicotine adenine dinucleotide phosphate ($NADPH_2$) and oxygen (Miyamoto *et al.* 1963a; Miyamoto 1969). Liver slices incubated with fenitrothion did not produce measurable amounts of fenitrooxon, while liver homogenates and the supernatant fraction of such homogenates appreciably activated added fenitrothion (Miyamoto *et al.* 1963a; Miyamoto 1969). No correlation between the toxicity and rate of formation of fenitrooxon could, however, be demonstrated (JMPR 1988; Miyamoto *et al.* 1963a; Miyamoto 1969). No observations were made in these studies on the distribution into fatty tissues, but studies of residues in milk, meat, and fat from cattle indicated the presence of approximately 0.001 mg/kg in these samples

(JMPR 1988; Miyamoto and Sato 1969). Other studies involving the metabolism of fenitrothion have been described (Anjum and Qadri 1986; Kasagami *et al.* 2002; Sultatos 1991; Yamamoto *et al.* 1983; Yamamoto *et al.* 1982; Yoshida *et al.* 1975).

Figure 6.1: Metabolic pathway of fenitrothion *in vivo* (Kumar *et al.* 1993).




6.2.4 Excretion

Fenitrothion and its metabolites are excreted mainly in the urine (90-95%) (Aprea *et al.* 1999; Hollingworth *et al.* 1967). Up to 10% was recovered in feces (Hollingworth *et al.* 1967). Within three days nearly complete recovery of an orally administered dose (15 mg/kg) could be obtained (Hollingworth *et al.* 1967). The ratios between the amounts of metabolites was dependent upon the dose given (Hollingworth *et al.* 1967). Other urinary excretion studies have been described (Aprea *et al.* 1999; Hladka and Nosal 1967; Kojima *et al.* 1989; Nosal and Hladka 1968).

## 6.3 Interactions of fenitrothion with other chemicals

Interactions of fenitrothion with other compounds such as malathion (Hladka *et al.* 1974), diethyl maleate (Sultatos *et al.* 1991), 2-sec-butylphenol methylcarbamate (BPMC) (Takahashi *et al.* 1984), and N,N-diethyl-m-toluamide (DEET) (Moody *et al.* 1987) have also been studied.

## 6.4 PBPK models

To date there are no published PBPK models for fenitrothion; however, there are numerous pharmacokinetic data that could be used in model development (Aprea *et al.* 1999; Douch *et al.* 1968; Hladka and Nosal 1967; Hollingworth *et al.* 1967; Kojima *et al.* 1989; Meaklim *et al.* 2003; Meaklim and McNeil 1999; Miyamoto 1964a; Miyamoto 1964b; Miyamoto and Sato 1969; Miyamoto 1969; Miyamoto *et al.* 1963a; Muller 2000; Nishizawa *et al.* 1961; Nosal and Hladka 1968; Vandanis and Crawford 1964).

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# Chloroform

## 7.0 Introduction

Chloroform (trichloromethane, $CHCl_3$) is a dense liquid that is insoluble in water and volatile under environmental conditions (McCulloch 2003). The major domestic use for chloroform is in the manufacture of refrigerant HCFC-22 (Chemical Marketing Reporter 1995). Chloroform is also used as a laboratory reagent and extraction solvent for pharmaceuticals. A significant amount of chloroform has been released to the environment as a by-product of the treatment of drinking and waste waters and through reactions of chlorine with organic chemicals (Meek *et al.* 2002).

## 7.1 Mechanisms of toxicity

Oral and inhalation exposures to chloroform cause toxicity to the liver, kidney, and nasal epithelium (USEPA 2001). Chloroform can also cause reproductive or developmental toxicity, although most of the effects are secondary to maternal toxicity (USEPA 2001). Increased incidences of liver and kidney tumors have been observed in several animal species after exposures to chloroform via several routes, although there is no adequate human data for carcinogenicity (USEPA 2001). The mode of toxicity of chloroform is probably through oxidative metabolism to form phosgene (Pohl *et al.* 1977), which can react to form covalent bonds with microsomal proteins (Corley *et al.* 1990; Rosenthal 1987).

## 7.2 Pharmacokinetics

### 7.2.1 Absorption

Chloroform is generally absorbed rapidly in humans and animals. It is easily absorbed into the blood from the lungs after inhalation exposures. Human inhalation studies include exposures via surgical anesthesia (Smith *et al.* 1973), indoor swimming pools (Aggazzotti *et al.* 1993; Cammann and Hubner 1995; Levesque *et al.* 1994; Levesque *et al.* 2000), and shower air (Jo *et al.* 1990; Levesque *et al.* 2002). Chloroform can also be absorbed through the skin easily. Dermal exposures were considered in conjunction with inhalation exposures in some of the indoor swimming pool studies (Cammann and Hubner 1995; Levesque *et al.* 1994; Levesque *et al.* 2000) and shower air studies (Jo *et al.* 1990; Levesque *et al.* 2002). Other human studies of dermal-only exposures include showering with facemask (Corley *et al.* 2000; Gordon *et al.* 1998) and topical administration of chloroform to volunteers (Dick *et al.* 1995). Dermal absorption in animals was studied in guinea pigs (Jakobson *et al.* 1982) and hairless rats (Islam *et al.* 1995). Gastrointestinal absorption of chloroform is also fast and extensive (USEPA 2001). Oral exposure studies of humans were done in volunteers using $^{13}C$-labeled chloroform (Fry *et al.* 1972) and additional information is available from an accidental chloroform poisoning case (Rao *et al.* 1993). Animal studies via oral exposure were reported in mice and rats by Withey et al. (Withey *et al.* 1983) and Pereira (Pereira 1994), respectively.

### 7.2.2 Distribution

Chloroform is widely distributed throughout the body after being absorbed. Radiolabeled chloroform in mice was reported to distribute to the liver, kidney, lungs, spleen, body fat, muscle, and nervous tissue (Bergman 1979; Cohen and Hood 1969). The highest levels of chloroform detected in human postmortem samples are in the body fat (5–68 μg/kg) and lower levels (1–10 μg/kg) were detected in the kidney, liver, and brain (McConnell *et al.* 1975). In a study with $^{14}C$-chloroform injected in male mice

intraperitoneally, the maximum radioactivity levels were observed in the liver, kidney, and blood within 10 minutes of dosing (Gemma *et al.* 1996). It was also found that the presence of testosterone affected chloroform accumulations in mouse kidney (Ilett *et al.* 1973; Pohl *et al.* 1984; Smith *et al.* 1973) and resulted in higher nephrotoxicity in male mice.

### 7.2.3 Metabolism

The major metabolic pathway of chloroform in humans and animals (Figure 7.1) is oxidative metabolism that produces reactive phosgene and the minor pathway is reductive metabolism that forms dichloromethyl free radical (USEPA 2001). In the presence of oxygen, chloroform is converted to trichloromethanol, which spontaneously dehydrochlorinates to produce phosgene (Pohl *et al.* 1981; Stevens and Anders 1981). These reactions are catalyzed by cytochrome P450 in liver and kidneys (Ade *et al.* 1994; Branchflower *et al.* 1984; Smith and Hook 1984). Phosgene can in turn react with nucleophilic groups in cellular macromolecules and form covalent adducts (Noort *et al.* 2000; Pereira and Chang 1981; Pereira *et al.* 1984; Pohl *et al.* 1977; Pohl *et al.* 1981; Pohl *et al.* 1980). Phosgene can also undergo hydrolysis to form carbon dioxide and hydrochloric acid, or react with glutathione to form diglutathionyl dithiocarbonate, gluathione disulfide, and carbon monoxide (Pohl *et al.* 1981; USEPA 2001). In the absence of oxygen, chloroform is converted to dichloromethyl free radical, which can form covalent adducts with microsomal enzymes and can also cause lipid peroxidation (USEPA 2001). Metabolic pathways of chloroform overlapping with the other three volatile organics in Mixture 2 are shown in Figure 8.1 under trichloroethylene.

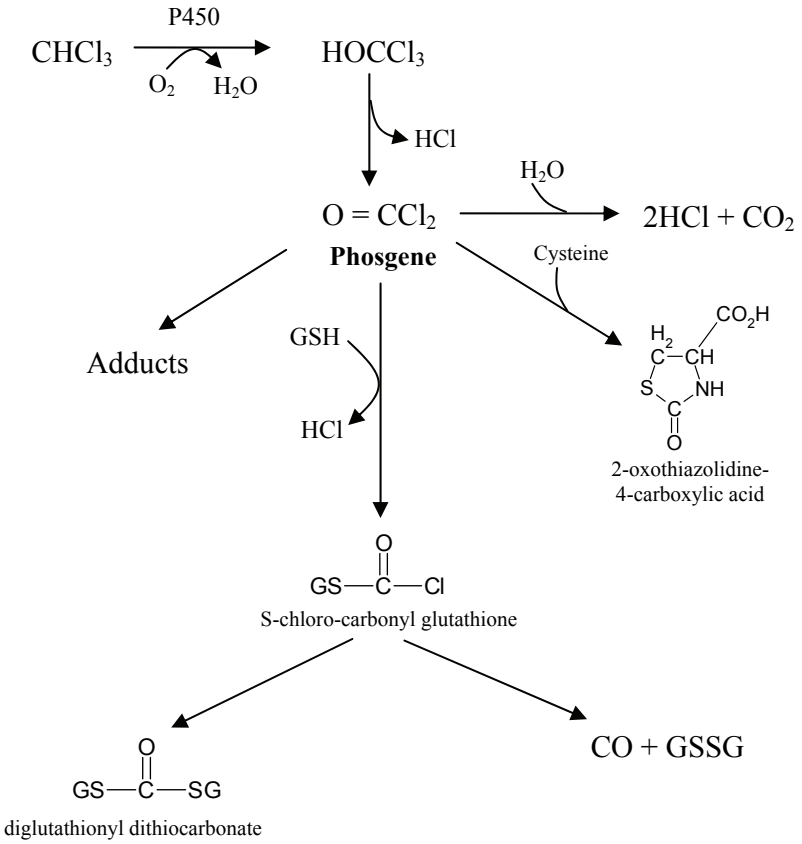


*Figure 7.1* Major metabolic pathways of chloroform (adapted from USEPA (2001))

### 7.2.4 Excretion

Chloroform is excreted through the lungs either unchanged or as carbon dioxide, with small amounts detected in urine after inhalation (Corley *et al.* 1990; Fry *et al.* 1972; Gordon *et al.* 1988), oral (Fry *et al.* 1972), and dermal (Dick *et al.* 1995) exposures.

## 7.3 Physiologically based pharmacokinetic (PBPK) models

The first PBPK model for chloroform was developed by Corley and colleagues (Corley *et al.* 1990) to describe the fate of chloroform in several species via numerous exposure routes. Several subsequent PBPK models (Chinery and Gleason 1993; Corley *et al.* 2000; Gearhart *et al.* 1993; Levesque *et al.* 2000; McKone 1993; Roy *et al.* 1996) were developed based on the Corley model to include a variety of exposure scenarios. A schematic representation of a general PBPK model for chloroform is shown in Figure 7.2.

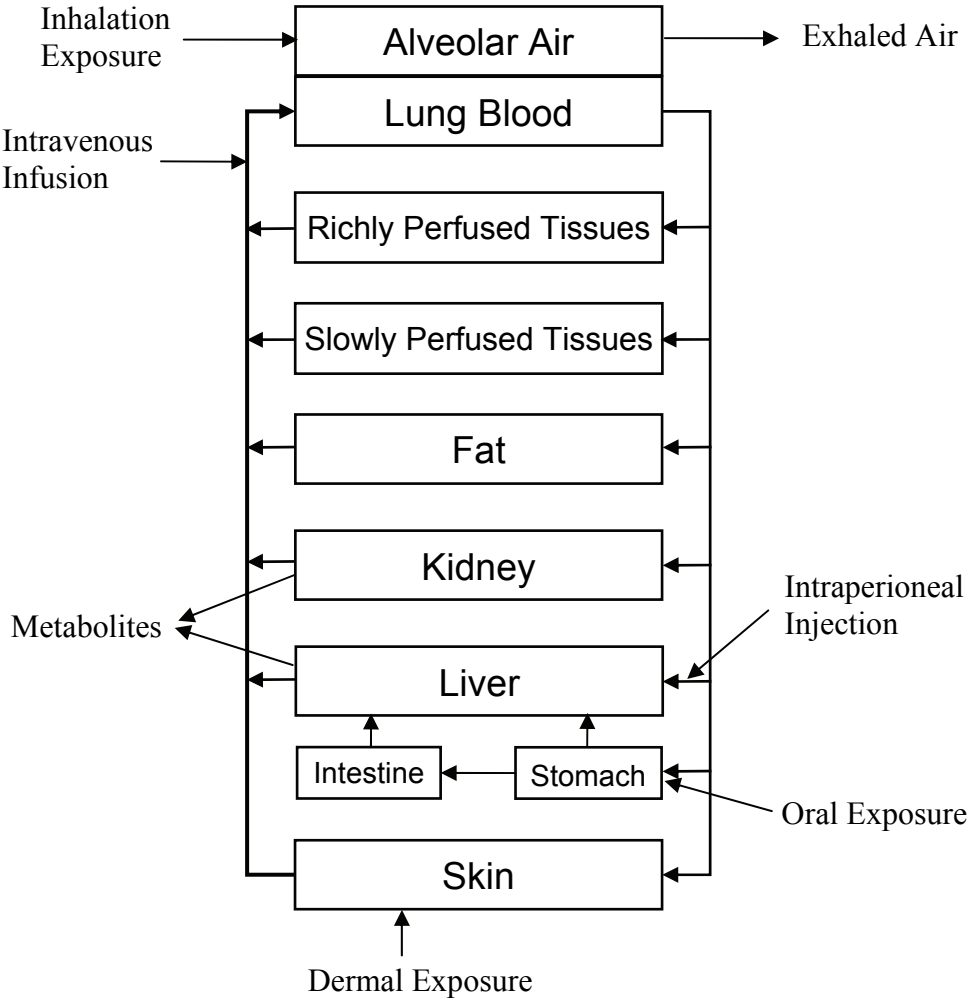


*Figure 7.2* PBPK model for chloroform in rats, mice, and human (adapted from (Corley *et al.* 2000))

In the Corley model (Corley *et al.* 1990), the exposure routes include oral, inhalation, and intraperitoneal. Liver and kidney were both sites of metabolism for chloroform. The amount of

metabolite binding to cellular macromolecules was used as the indicator for chloroform toxicity. Due to the lower rates of metabolism, ventilation, and cardiac output in larger species than in smaller species, the relative potency of chloroform was predicted as mice > rats > humans in the Corley model (Corley *et al.* 1990).

Reitz and colleagues (Reitz *et al.* 1990) extended the Corley model to include pharmacodynamic endpoints for cancer risk assessment. Two dose metrics were used for the liver compartment, while the kidney compartment was not considered. The first type of dose metric used was the average daily macromolecular binding. The other type of dose surrogate was cytotoxicity due to the formation of reactive chloroform metabolite, phosgene. It was concluded that cytotoxicity is the dose metric best reflecting carcinogenicity (Reitz *et al.* 1990). These two dose metrics were later analyzed for interindividual variability and parameter uncertainty by Allen and colleagues (Allen *et al.* 1996). The cytotoxicity dose metric was much more sensitive to interindividual variability than the average daily macromolecular binding was.

Gearhart and colleagues (Gearhart *et al.* 1993) adjusted partition coefficients, rate of metabolism, cardiac output, and minute ventilation according to body temperature. These adjustments strengthened the Corley model (Corley *et al.* 1990) according to the fitting of gas uptake data of mice by loosening the assumption of enzyme loss and resysthesis.

Chinery and Gleason (Chinery and Gleason 1993) further included the skin compartment to describe the fate of chloroform after adsorption through dermal exposure. The skin compartment was further divided into three subcompartments: the aqueous solution, stratum corneum, and viable skin. The model was able to predict the concentration of chloroform in the exhaled air from humans exposed while showering through inhalation only and the combination of dermal and inhalation routes.

In a PBPK model similar to Chinery and Gleason's, McKone (McKone 1993) assumed skin to be only one compartment. It was demonstrated that chloroform metabolism by the liver was not linear with respect to higher exposure concentrations (60-100 mg/L).

Based on the Corley model (Corley *et al.* 1990), Levesque and colleagues (Levesque *et al.* 2000) used a PBPK model to predict the fate of chloroform for individuals exposed while swimming through dermal and inhalation routes. Dermal exposure was described using an overall skin permeability constant. The levels of macromolecular binding in swimmers calculated from the model are much lower than the smallest no-observed-effect level for liver tumors in animals.

Corley and colleagues (Corley *et al.* 2000) added a single skin compartment to the original Corley model (Corley *et al.* 1990) and described the kinetics of human dermal exposure to chloroform while bathing. With the adjustment of model parameters according to temperature, the model can predict the relationship between water temperature (30-40°C) and exhaled chloroform observed from experiments (Gordon *et al.* 1998).

Constan and colleagues (Constan *et al.* 2002) used cytolethality and regerative cell proliferation as pharmacodynamic endpoints to perform a chloroform inhalation cancer risk assessment. The NOAEL for chloroform-induced hepatotoxicity in humans was estimated to be 110 ppm using experimental data from B6C3F1 mouse and PBPK-PD model calculations.

Meek and colleagues (Meek *et al.* 2002) recently performed an assessment of exposure-response analyses and risk characterization using PBPK models. Inhalation, oral, and dermal exposures were

considered from ten-minute shower, discrete periods of water and food consumption, as well as inhalation of chloroform at a variety of concentrations. Dose metrics used for carcinogenicity were the maximum rate of metabolism per unit kidney cortex volume and mean rate of metabolism per unit kidney cortex volume during each dose interval. For non-neoplastic effects, the dose metrics used were the mean rate of metabolism per unit centrilobular region of the liver and the average concentration of chloroform in the non-metabolizing centrilobular region of the liver.

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# Trichloroethylene

## 8.0 Introduction

Trichloroethylene (TCE) is one of the most important industrial chemicals of our time. It is an organic solvent that has been used widely in dry cleaning, metal degreasing, and as a solvent for oils and resins. Because of the large production volume and its wide applications, TCE is one of the top, if not the top, environmental pollutants in ground water. Recently, an entire supplemental volume (Volume 108, Supplement 2, May 2000) of *Environmental Health Perspective* was devoted to *Trichloroethylene Health Risks*. This volume contains many excellent review articles which cover the areas of toxicology and risk assessment extensively. The USEPA, in its re-assessment of TCE health risks, devoted a great deal of effort to publishing a document on *Trichloroethylene Health Risk Assessment: Synthesis and Characterization* (EPA/600/P-01/002A; quoted in this write-up as USEPA, 2001). This document and its related Science Advisory Board review (www.epa.gov/science1/pdf/ehc03002.pdf) also provided an excellent source of information on TCE. Thus, the summary below represents a brief update of the current information.

## 8.1 Toxic effects

### 8.1.1 Noncancer effects

Neuro- or neuro-behavioral (Boyes et al., 2000; Ohta et al., 2001; USEPA, 2001; Waseem et al., 2001; Kilburn, 2002; Moser et al., 2003), male reproductive (Kumar et al., 2000; 2001; Forkert et al., 2002; 2003), developmental (Boyer et al., 2000; Rodenbeck et al., 2000; USEPA, 2001; Johnson et al., 2003), liver (USEPA, 2001), renal (USEPA, 2001; Mensing et al., 2002), immuno- (Griffin et al., 2000a,b,c; Kaneko et al., 2000; USEPA, 2001) toxicities in experimental animals and/or humans are reported or implicated. *In vitro* studies using isolated cell cultures have demonstrated and reconfirmed many of the species-, sex-, and tissue-dependent differences in hepato- and renal- toxicities observed *in vivo* (Cummings et al., 2000a,b; Lash et al., 2001).

Halogenated hydrocarbons such as TCE are among the most common water supply contaminants in the U.S. and elsewhere. Epidemiological studies have found an association, but not a cause-and-effect relation, between halogenated hydrocarbon contamination and increased incidence of congenital cardiac malformations or other defective birth outcomes. However, some animal studies in birds and rats as well as in tissue cultures had demonstrated statistically significant increased incidence of congenital cardiac malformations or other defective birth outcomes (Boyer et al., 2000; Johnson et al., 2003) while others turned out negative (Fisher et al., 2001). The most recent study (Johnson et al., 2003) reported that maternal rats exposed to more than 250 ppb TCE, a very low dose study, showed an associated increased incidence of cardiac malformations in their developing fetuses.

### 8.1.2 Cancer effects

TCE causes liver, lung tumors and lymphomas in mice and kidney and testicular tumors in rats (Bull, 2000; Green, 2000; Lash et al., 2000a; USEPA, 2001). In humans, TCE was implicated to be a carcinogen (Wartenberg et al., 2000; USEPA, 2001). It is well established that two metabolites of TCE, dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are important contributors to carcinogenicity of TCE (Bull, 2000; Tao et al., 2000; Bull et al., 2002).

Regarding renal cancer in humans, German epidemiological studies of prevalence of renal cancer following high exposure of TCE in workers have been the subject of considerable scientific debate, re-evaluation, and repeated studies (Brauch et al., 1999; Bruning et al., 1999; Green and Lash, 1999; Schraml et al., 1999; Brauch et al., 2000; Bruning and Bolt, 2000; Green, 2000; Lash et al., 2000a; Wartenberg et al., 2000; USEPA, 2001; Bruning et al., 2003).

## 8.2 Pharmacokinetics

The pharmacokinetics of TCE has been reviewed thoroughly (ATSDR, 1997; Fisher, 2000). More recent updates are provided below.

### 8.2.1 Absorption

Dose-dependent gastrointestinal absorption of TCE and its kinetics in male Sprague-Dawley rats over a wide range of oral bolus doses were characterized by Lee et al. (2000b). Dietary incorporation of guar gum, a thickener and stabilizer in foods and pharmaceuticals, was found to decrease TCE accumulation in the body by reducing absorption and fat tissue mass (Nakashima and Ikegami, 2001).

### 8.2.2 Distribution

PBPK models for the systemic transport of TCE to various tissues and organs with a special emphasis to fat tissues were established by Albanese et al. (2002).

### 8.2.3 Metabolism

*Human and animal studies*

The principal metabolic pathways for TCE and metabolic steps where interactions with chloroform (CHL), tetrachloroethylene (PERC), and/or 1,1,1 trichloroethane (MC) may occur are denoted in Figure 8.1. The metabolism of TCE has been reviewed thoroughly (Lash et al., 2000b; USEPA, 2001); more recent updates are provided below.

Lash et al. (1999a) reported direct, *in vivo*, evidence of GSH conjugation of TCE in human volunteers exposed to 100 ppm TCE and demonstrated markedly higher amounts of S-(1,2-dichlorovinyl) glutathione (DCVG) in males than females. However, Bloemen et al. (2001) studied urinary concentrations of metabolites from GST-dependent pathway in human volunteers exposed to 50 and 100 ppm TCE for 15 min or occupationally exposed (0.4 to 21 ppm TWA) workers. They found little or no such metabolites and suggested the glutathione-mediated metabolism is of minor importance in humans.

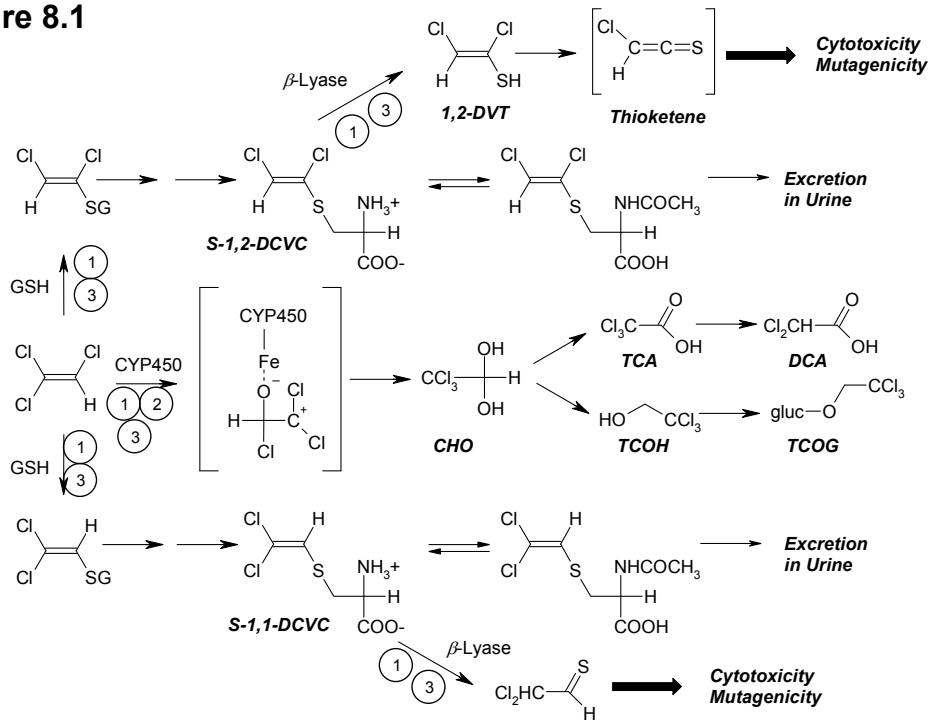
There were evidences suggesting that TCE is metabolized in the reproductive tract of the mouse and monkey; the fact that TCE and its metabolites accumulated in seminal fluid in human diagnosed with clinical infertility also suggested associations between production of TCE metabolites, reproductive toxicity, and impaired fertility (Forkert et al., 2003).

*In vitro studies*

Extensive *in vitro* biotransformation studies have been published on a variety of enzyme preparations and cell culture systems ranging from cell free tissue preparations (Lipscomb *et al.*, 1997, 1998; Lipscomb and Garrett, 1998; Lash et al., 1999b; Cai and Guengerich, 2000; Snawder and Lipscomb,

2000; Cummings et al., 2001; Lipscomb *et al.*, 2003a) to highly purified human enzymes (Cai and Guengerich, 2001), to primary and other cell cultures (Lash et al., 1999b; Cummings and Lash, 2000; Cummings et al., 2000a,b; Walgren et al., 2000; Cummings et al., 2001) including collagen gel sandwich cultures of rat hepatocytes (De Smet et al., 2000).

**Figure 8.1**




(1) Potential interaction between PCE and TCE
(2) Potential interaction between MC and TCE
(3) Potential interaction between CHL and TCE

Figure 8.1 Superimposed on a metabolic schematic for TCE (Dobrev, et al., 2001), metabolic steps where pharmacokinetic interactions could occur are denoted by symbols. Identification of additional potential interactions should occur during CRA Steps 3 and 6.

Cai and Guengerich (2001) demonstrated that the direction reaction of TCE oxide with either human P450 2E1, P450 2B1, or NADPH-P450 reductase was shown to lead to enzyme inactivation, and no recovery of either enzyme occurred.

### 8.2.4 Elimination and Excretion

Presystemic elimination of TCE has been shown by Lee et al. (1996) to be inversely related to dose. When relatively high doses were administered to rats via the portal vein, first-pass hepatic extraction became negligible. This phenomenon could result not only from metabolic saturation, but from suicidal destruction of cytochrome P450 and hepatocellular injury as well (Lee et al., 2000a). Subsequent pharmacokinetic analysis by Lee et al. (2000b) indicated that TCE was eliminated by capacity-limited hepatic metabolism, no evidence for P450 2E1 destruction, with incursion into nonlinear kinetics with bolus doses greater or equal to 8 to 16 mg/kg.

## 8.3 Interactions of TCE with other chemicals

Interaction studies reported in the recent literature covered diverse subject areas. Each of the relevant papers is discussed briefly below.

Dobrev et al. (2001; 2002) studied "Interaction Thresholds" in rats and humans using interactive PBPK modeling of a ternary mixture of TCE, tetrachloroethylene, and 1,1,1-trichloroethane. Because of competitive inhibition of the primary metabolic system, P450 2E1, an alternative pathway, the GST conjugation system, becomes important. It was demonstrated that at or below the current threshold limit values (TLVs) for these three chemicals, the coexposure to these chemicals would result in significant interactions.

Very high doses (2000 to 5000 mg/kg, ip) of TCE induced anticonvulsive effect of a number of drugs (Shih et al., 2001); it was suggested that this effect might be predominantly mediated by GABA receptors.

A full-factorial design for neurobehavioral evaluations of mixtures of TCE, heptachlor, and di (2-ethylhexyl) phthalate in F344 rats was carried out by Moser et al. (2003). In general, significant overall interactions that deviated from response additivity were detected for most endpoints (11 of 14). Most of the interactions are antagonistic in nature.

Pretreatment of TCE in Sprague-Dawley rats altered drug kinetics of theophylline, quinidine, and pentobarbital (Kukongviriyapan et al., 2001).

Dietary incorporation of guar gum, a thickener and stabilizer in foods and pharmaceuticals, was found to decrease TCE accumulation in the body by reducing absorption and fat tissue mass (Nakashima and Ikegami, 2001).

## 8.4 PBPK models

TCE is undoubtedly one of the chemicals, which were most extensively studied using PBPK modeling technique. The initial development of PBPK models was reported by Andersen et al. (1987). This initial PBPK model for TCE was followed by a number of variations by others for different goals (Fisher et al., 1989; 1990; Koizumi, 1989; Dallas et al., 1991). As the science advances, more and more sophistication were incorporated into the later PBPK models. Thus, PBPK models with incorporation of TCE metabolites, as well as reproductive physiology and toxicology (Fisher et al., 1989; 1990; 1991; Abbas et al., 1996; Abbas and Fisher, 1997; Fisher et al., 1998; Greenberg et al., 1999), and pharmacokinetic and pharmacodynamic interactions (Elmasri et al., 1996; Byczkowski et al., 1999) were seen in the literature. Furthermore, the application of PBPK modeling in risk assessment received progressively more emphasis (Allen and Fisher, 1993; Fisher and Allen, 1993; Gearhart et al., 1993; Clewell et al., 1995; Cronin et al., 1995; Bogen and Gold, 1997; Simon, 1997). The 2000 Monograph in EHP and more recent PBPK modeling efforts included its application in risk assessment (Clewell et al., 2000; Fisher, 2000), statistical analyses for variability and uncertainty (Bois, 2000a,b), further toxicological interaction studies to define "Interaction Thresholds" (Dobrev et al., 2001; 2002). PBPK modeling studies for other specific purposes or toxic endpoints are also seen. Thus, Poet et al. (2000) utilized PBPK modeling for assessing percutaneous absorption of TCE in rats and humans. PBPK models for the transport of TCE in adipose tissues were reported by Albanese et al. (2002) and PBPK modeling for male Long-Evans rats to aid in evaluation of neurotoxicity data was published by Simmons et al. (2002).

## 8.5 Risk assessment related

Because TCE is a very important industrial chemical and a prevalent environmental pollutant, the risk assessment, particularly cancer risk assessment became an area of much scientific debate. Consequently, quite a number of publications, review articles, and documents are available specifically dealing with mechanisms of toxicity of TCE and PBPK modeling in relation to risk assessment, as well as the process of risk assessment of TCE (Allen and Fisher, 1993; Fisher and Allen, 1993; Gearhart et al., 1993; Clewell et al., 1995; Cronin et al., 1995; Bogen and Gold, 1997; Simon, 1997; Brauch et al., 1999; Bruning et al., 1999; Green and Lash, 1999; Motohashi et al., 1999a,b; Schraml et al., 1999; Barton and Clewell, 2000; Bois, 2000a,b; Bruning and Bolt, 2000; Bull, 2000; Chen, 2000; Clewell et al., 2000; Fisher, 2000; Green, 2000; Lash et al., 2000a,b; Moore and Harington-Brock, 2000; Pastino et al., 2000; Rhomberg, 2000; Wartenberg et al., 2000; Ruden, 2001a,b; Stewart, 2001; USEPA, 2001; Ruden 2001a,b; 2002a,b; Bruning et al., 2003; Lipscomb *et al.*, 2002; 2003b; Ruden, 2003).

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# Tetrachloroethylene

## 9.0 Introduction

Tetrachloroethylene is made by direct chlorination or oxychlorination of certain hydrocarbons. Tetrachloroethylene is used as a chemical intermediate, as solvent for metal cleaning and vapor degreasing, and for dry-cleaning and textile processing. (Aggazzotti *et al.* 1994) It is found in many household products, including paint removers, water repellents, silicone lubricants, spot removers, adhesives, and wood cleaners (ATSDR 1997).

## 9.1 Toxic effects

Liver, kidney, blood, and the central nervous system are the target organs for systemic effects (Calabrese 1983; Chen *et al.* 2002; Echeverria *et al.* 1995; Ferroni *et al.* 1992; Umezu *et al.* 1997; Utzinger and Schlatter 1977; Zavon 1967). Exposure to high concentrations of tetrachloroethylene induces dizziness, headache, sleepiness, confusion, nausea, unconsciousness, and death. Irritation could occur when skin is exposed to tetrachloroethylene. Breathing the vapor may irritate the lungs, causing coughing and/or shortness of breath (Stewart *et al.* 1961). Animal studies showed that tetrachloroethylene can cause liver and kidney damage (Schimmelpfennig *et al.* 1987; Kylin *et al.* 1963; Kylin *et al.* 1965; Lash *et al.* 2002). The developing fetus and children may be particularly susceptible to the toxic effects of tetrachloroethylene (Ahlborg 1990; Fredriksson *et al.* 1993; Motohashi *et al.* 1993; Spector *et al.* 1999). Exposure to pregnant rodents induces behavioral deficits in pups (Mattsson *et al.* 1998; Seeber 1989).

The neurotoxicities of tetrachloroethylene may result from the alterations of fatty acid patterns in the brain (ATSDR 1997; Burger *et al.* 1991). In contrast to the nervous system, the effects on the liver including cancer are thought to be a result of the metabolite, trichloroacetic acid (ATSDR 1997). It is believed that trichloroacetic acid may play a role in inducing hepatocellular peroxisomes, resulting in the production of hydrogen peroxide as a by-product (Bentley *et al.* 1993). The increased hydrogen peroxide may increase DNA damage. Kidney cancer may in part be a result of the formation of the genotoxic metabolites from S-(1,2,2-trichlorovinyl) glutathione by β-lyase (Birner *et al.* 1997; Cooper *et al.* 2002; Green *et al.* 1990). Tetrachloroethylene is classified as a group 2A carcinogen (probably carcinogenic to human) (Aschengrau *et al.* 1993; Aschengrau *et al.* 1998; Aschengrau *et al.* 2003; Wartenberg *et al.* 2000).

## 9.2 Pharmacokinetics

Tetrachloroethylene is readily absorbed through oral, skin, and inhalation exposure (Ward *et al.* 1988). Once it is absorbed, tetrachloroethylene is distributed to fatty tissues because of high lipophilicity (fat/blood partition coefficient is about 140) (Dallas *et al.* 1994c). The half-life of tetrachloroethylene in fat tissues is 55 hours (ATSDR 1997). One to three percent of absorbed tetrachloroethylene is metabolized to trichloroacetic acid in the liver (ACGIH 1991). Unmetabolized tetrachloroethylene is exhaled (ATSDR 1997). This is the primary route of excretion. Trichloroacetic acid is excreted in the urine (ATSDR 1997).

### 9.2.1 Absorption

Tetrachloroethylene is readily absorbed in the G.I. tract and lungs. Pulmonary uptake is proportional to ventilation rate, duration of exposure, and the concentration in the inspired air (ATSDR 1997). In rats, the proportion absorbed was approximately 55-70% after 1 minute, gradually declining to 40-50% after 2 hours (Dallas *et al.* 1994b). Dermal absorption has been studied in guinea pigs (Bogen *et al.* 1992).

### 9.2.2 Distribution

Tetrachloroethylene is preferentially stored in fat tissues. In rats, distribution to brain, liver, and kidneys has also been demonstrated (Frantz and Watanabe 1983; Dallas *et al.* 1994a; Dallas *et al.* 1994b). In animal studies, transplacental and lactational transport of unchanged tetrachloroethylene has been reported (Byczkowski *et al.* 1994; Hamada and Tanaka 1995).

### 9.2.3 Metabolism

The metabolic pathways of tetrachloroethylene are summarized in Figure 9.1. The overlapping pathways with the other three volatile organics in Mixture 2 can be seen in Figure 8.1 under trichloroethylene.

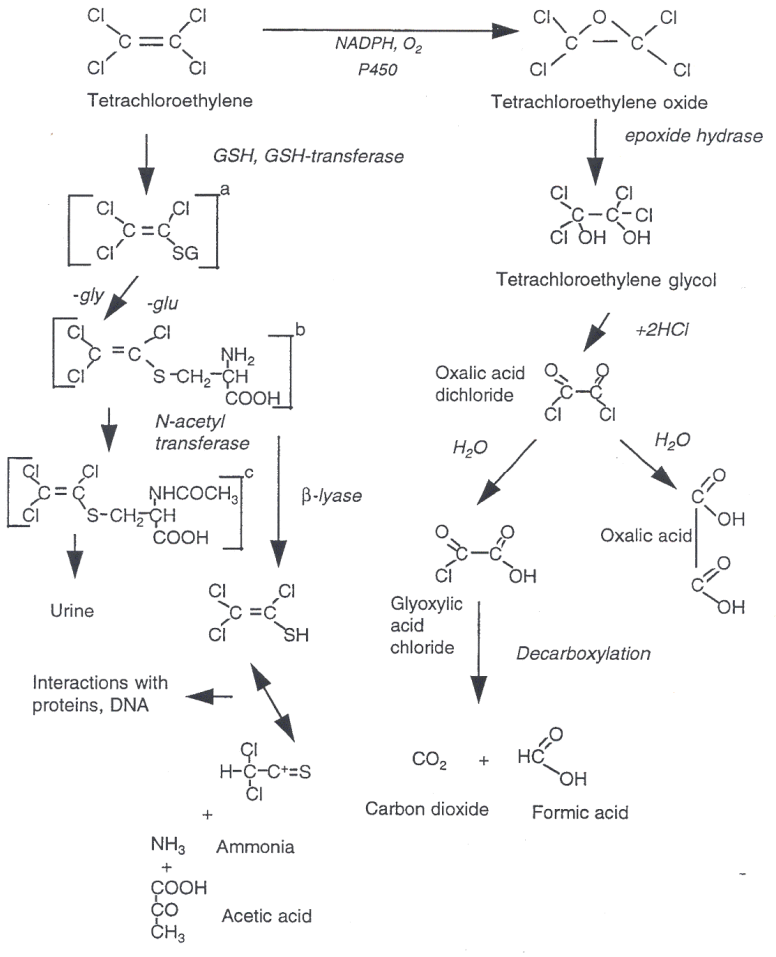


Fig 9.1 Metabolic pathways of tetrachloroethylene (ATSDR, 1997)

Human pharmacokinetic studies have been performed in volunteers and workers. The pharmacokinetics of tetrachloroethylene by inhalation exposure has been described (Ikeda 1977; Monster *et al.* 1979;

Ohtsuki *et al.* 1983; Imbriani *et al.* 1988). One study described the pharmacokinetics of tetrachloroethylene in a boy who ingested the chemical (Koppel *et al.* 1985).

The pharmacokinetics of tetrachloroethylene following inhalation exposure have been described for rodents (Pegg *et al.* 1978; Schumann and Watanabe 1979). The dermal pharmacokinetics of tetrachloroethylene in hairless guinea pigs was also studied (Bogen *et al.* 1992). Pharmacokinetics of tetrachloroethylene following oral exposure were reported in several studies including rats, mice, and dogs (Frantz and Watanabe 1983; Dallas *et al.* 1994c).

*In vitro* metabolic studies of tetrachloroethylene have been conducted using rat hepatic microsome and other subcellular systems (Huang *et al.* 2001; Costa and Ivanetich 1980; Reitz *et al.* 1996; Dekant *et al.* 1998). Some studies focused on the interaction of tetrachloroethylene with rat hepatic microsomal P450 enzymes (Hanioka *et al.* 1995a; Hanioka *et al.* 1995b; Hanioka *et al.* 1997).

### 9.2.4 Excretion

In humans and animals, the major part of the absorbed amount is exhaled unchanged. In humans, 80-100% of the amount was exhaled as parent compound. In rats, about 70% was exhaled in same conditions (ATSDR 1997). Excretion of metabolites in urine is 2% of exposed dose with a half-life of 75-80 hours (Ikeda *et al.* 1972; Imbriani *et al.* 1988; ATSDR 1997). In rats, elimination via maternal milk was high (Byczkowski *et al.* 1994; Byczkowski and Fisher 1995).

## 9.3 Interactions with other chemicals

The hepatic monooxygenase system is mainly responsible for oxidation of tetrachloroethylene. Thus, chemicals that affect the monooxygenase system could affect the metabolism and toxicity of tetrachloroethylene. Two papers were published dealing with pharmacokinetic interactions between tetrachloroethylene and other chlorinated contaminants (Dobrev *et al.* 2001, 2002). Toxicological interactions between tetrachloroethylene and ethanol or other chemicals were also reported (Koizumi *et al.* 1982; Dobrov and Poluekto 1971; Kobayashi *et al.* 1982; Seiji *et al.* 1989; Giovannini *et al.* 1992).

## 9.4 PBPK models

Several PBPK models for the disposition of tetrachloroethylene were presented in animals and humans (Gelman *et al.* 1996; Haddad *et al.* 2000; Ward *et al.* 1988; Koizumi 1989; Bois *et al.* 1990; Gearhart *et al.* 1993; Dallas *et al.* 1994b; Dallas *et al.* 1994c; Wilson and Knaak 1994; Dallas *et al.* 1995; Reitz *et al.* 1996; Poet *et al.* 2000; Loizou 2001; Poet *et al.* 2002). The majority of the available PBPK models are concerned with the carcinogenesis of tetrachloroethylene. One model has been developed to predict brain concentrations following exposure to tetrachloroethylene during showering (Rao and Brown 1993). PBPK models for the lactational transfer of tetrachloroethylene through breast milk were developed to estimate the risk of tetrachloroethylene exposure to infants (Byczkowski *et al.* 1994; Byczkowski and Fisher 1995).

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# 1, 1, 1-Trichloroethane

## 10.0 Introduction

1, 1, 1-Trichloroethane (TCA; Figure 10.1) is a common organic solvent, often used commercially for industrial degreasing as well as dry-cleaning. The 2001 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Priority List of Hazardous Substances includes TCA in the top 100 hazardous substances based upon its environmental distribution, especially at hazardous waste sites (ATSDR, 2001a). Furthermore, TCA ranks 13$^{th}$ in the CERCLA Completed Exposure Pathway; therefore, humans are frequently exposed to TCA (ATSDR, 2001b). Because of its ability to induce central nervous system depression, TCA has been abused, and thus purposeful human exposure also occurs. TCA is considered to be a group III carcinogen due to lack of adequate evidence of carcinogenicity in rodents and humans (IARC, 1999).

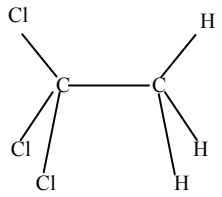


Figure 10.1. Structure of 1, 1, 1-trichloroethane.

## 10.1 Toxic effects

TCA has various systemic effects, most notably central nervous system (CNS) depression, hepatotoxicity, and cardiovascular complications. Central nervous system depression is the principal CNS effect observed in individuals and animals following exposure to TCA (Hall and Hine, 1966; Stahl *et al.*, 1969; Jones and Winter, 1983; Bowen and Balster, 1998; Bowen *et al.*, 1998; Bruckner *et al.*, 2001). Descriptions of plausible mode(s) of action for CNS depression are given in numerous reports (Rosengren *et al.*, 1985; Nilsson, 1986b; Nilsson, 1986a; Nilsson, 1987; Fernicola *et al.*, 1991; Beckstead *et al.*, 2000; Warren *et al.*, 2000; You and Dallas, 2000; Beckstead *et al.*, 2001; Okuda *et al.*, 2001; Beckstead *et al.*, 2002; Wiley *et al.*, 2002; Lopreato *et al.*, 2003).

Various reports cite changes in serum enzyme chemistry, which serve as indicators of hepatotoxicity for both humans and animals (Halevy *et al.*, 1980; Hodgson *et al.*, 1989). Another marker for hepatotoxicity observed following exposure to TCA is accumulation of fat in the liver (Hall and Hine, 1966; Caplan *et al.*, 1976; Hodgson *et al.*, 1989). However, many contradictory studies on both humans and animals report failure of serum enzymes to change or extremely mild changes, indicating no apparent hepatotoxic effects (Domette and Jones, 1960; Carlson, 1973; Kramer *et al.*, 1978; Kelafant *et al.*, 1994; Wang *et al.*, 1996). Although observed hepatic alterations are reversible, they tend to indicate mild hepatotoxicity induced by TCA and/or a metabolite (Halevy *et al.*, 1980; Bruckner *et al.*, 2001). Cardiac sensitization to epinephrine, resulting in arrhythmia, has been linked with exposure to TCA in both humans and animals (Clark and Tinston, 1973; Guberan *et al.*, 1976; Macdougall *et al.*, 1987). Additionally, cardiac depression, resulting in decreased blood pressure, is caused by exposure to TCA. Toraason and coworkers demonstrated decreases in contractility of cultured cardiac cells occurred in a dose-dependent manner following treatment with TCA (Toraason *et al.*, 1990). Some reproductive effects have also been reported, ranging from increased mammary adenocarcinomas to decreases in

sperm motility, however effects were usually slight and in some cases confounded by exposure to chemical mixtures (Rudolph and Swan, 1986; Swan *et al.*, 1989; Yang, 1993; Coleman *et al.*, 1999; Lemasters *et al.*, 1999; NTP, 2000; Wang *et al.*, 2002). Other studies found no association between TCA exposure and reproductive effects (George *et al.*, 1989; Wrensch *et al.*, 1990a; Wrensch *et al.*, 1990b)

## 10.2 Pharmacokinetics

### 10.2.1 Absorption

Exposure to TCA primarily occurs through inhalation, and has been described in both humans and animals (Morgan *et al.*, 1972a; Morgan *et al.*, 1972b; Monster *et al.*, 1979; Hobara *et al.*, 1982; Jakobson *et al.*, 1982; Hobara *et al.*, 1983; Koizumi *et al.*, 1983; Nolan *et al.*, 1984; Dallas *et al.*, 1989; Boman *et al.*, 1995). Dermal and gastrointestinal exposures are plausible as TCA is a groundwater contaminant, though due to the volatility of TCA, the most common exposure route is inhalation (ATSDR, 1995). Alternative routes of TCA exposure have been explored by many researchers, as TCA is efficiently and rapidly absorbed via the lung, skin, and gastrointestinal tract of humans and animals (Stewart and Dodd, 1964; Riihimaki and Pfaffli, 1978; Mitoma *et al.*, 1985; RTI, 1987; Reitz *et al.*, 1988; Morgan *et al.*, 1991; Yoshida *et al.*, 1998; Giardino *et al.*, 1999; Kezic *et al.*, 2000; Poet *et al.*, 2000; Kezic *et al.*, 2001). Steady-state blood levels in rats exposed to 50 or 500 ppm TCA were approached at 2 hours following initiation of continuous exposure (Dallas *et al.*, 1989). Reitz and colleagues noted achievement of maximal blood levels of TCA at 10-15 minutes following administration of a 14.2 mg/kg dose of TCA in water via gavage (Reitz *et al.*, 1988). Following the initial phases, absorption rates plateau as steady-state levels are approached in blood and tissues; generally, blood levels approach steady-state within a few hours following onset of exposure (Monster *et al.*, 1979; Nolan *et al.*, 1984).

### 10.2.2 Distribution

TCA is widely distributed, with preferential distribution to fatty tissues due to its lipophilic nature, regardless of exposure scenario (Takahara, 1986; RTI, 1987; Shimada, 1988; Katagiri *et al.*, 1997; You and Dallas, 1998). Detectable levels of TCA are found in the fat, liver, kidney, spleen, blood, lung, heart, brain, placenta, and fetus following inhalation exposure (Danielsson *et al.*, 1986; Takahara, 1986; Shimada, 1988). In mice exposed for 1 hour to 1,000 ppm TCA, tissue concentrations of TCA immediately following exposure resulted in preferential accumulation of TCA (in descending order) in the fat, liver, kidney, spleen and blood, followed by lung, heart and brain (Takahara, 1986). Schumann and coworkers supported these findings, as they reported significantly higher TCA concentrations in fatty tissues than in the liver and kidneys following exposure of mice and rats to either 150 or 1,500 ppm TCA for 6 hours (Schumann *et al.*, 1982b). Distribution of TCA is regulated by various factors, including tissue blood flow rate, tissue volume and tissue:blood partition coefficients, the latter likely being most influential (ATSDR, 1995).

### 10.2.3 Metabolism

Metabolism of TCA has been studied extensively (Carlson, 1973; Ivanetich and Van den Honert, 1981; Casciola and Ivanetich, 1984; Takano *et al.*, 1985; Takano *et al.*, 1988; Kawai *et al.*, 1991; Durk *et al.*, 1992; Baker and Ronnenberg, 1993). Regardless of exposure route, TCA is metabolized at low rates (<10%), mainly to four metabolites: trichloroethanol, trichloroethanol glucuronide, trichloroacetic acid, and carbon dioxide (Monster, 1979; Schumann *et al.*, 1982a; Nolan *et al.*, 1984; Mitoma *et al.*, 1985; Reitz *et al.*, 1988; Dallas *et al.*, 1989; Kawai *et al.*, 1991); Figure 10.2). Oxidative metabolism of TCA

by the cytochrome P-450 mixed-function oxidase system combined with other metabolic dehydrogenase enzymes forms trichloroethanol and trichloroacetic acid; trichloroethanol may be further metabolized via conjugation to form a glucuronide derivative. Cytochrome P450 2E1, specifically, is believed to play a role in TCA metabolism (Nakajima and Sato, 1979; Guengerich *et al.*, 1991; Kaneko *et al.*, 1994). Monster and coworkers found in humans exposed to 70 or 145 ppm of TCA for 4 hours, trichloroethanol and trichloroacetic acid excreted in the urine only accounted for 2 and 0.5%, respectively, of absorbed TCA (Monster *et al.*, 1979). Another byproduct, acetylene, may also be formed from TCA in mammals via reductive dechlorination, though only under hypoxic conditions (Durk *et al.*, 1992). The metabolic pathway of TCA is shown in Figure 10.2, as well as in Figure 8.1 where interactive reaction network with the other three volatile organics is evident.

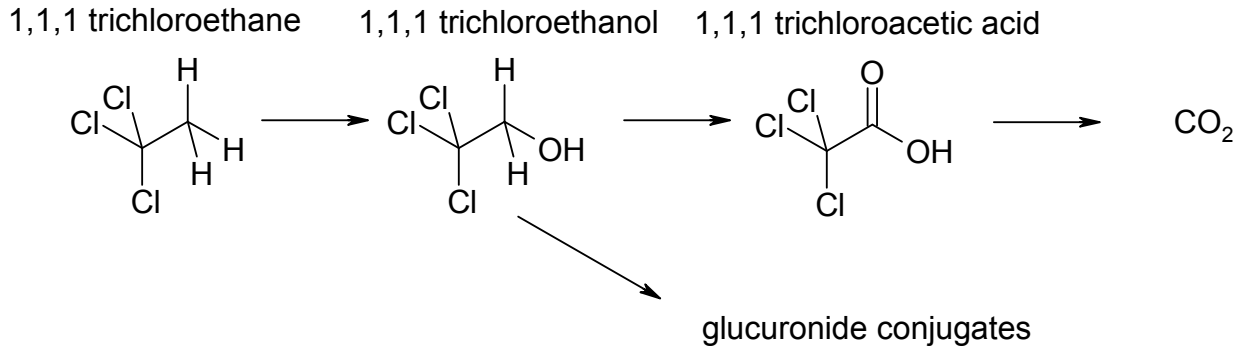


Figure 10.2. Metabolism of 1, 1, 1-Trichloroethane, reproduced from Agency for Toxic Substances and Disease Registry's toxicological profile (ATSDR, 1995).

### 10.2.4 Elimination

The primary route of TCA elimination is exhalation of the parent compound, which occurs fairly rapidly following exposure due to TCA's highly volatile nature (Monster, 1979; Monster *et al.*, 1979; Hobara *et al.*, 1982; Schumann *et al.*, 1982c; Schumann *et al.*, 1982a; Schumann *et al.*, 1982b; Nolan *et al.*, 1984). In humans exposed to 35 or 350 ppm for 6 hours, more than 91% of TCA absorbed was eliminated, unchanged, in exhaled air (Nolan *et al.*, 1984). Similarly, in animals given 20 daily doses of TCA by gavage in vegetable oil followed by a single $^{14}$C-labeled bolus, 85.1 and 92.3% of TCA was excreted as parent compound via exhalation, from rats and mice, respectively, (Mitoma *et al.*, 1985). Both acetylene and carbon dioxide are excreted in expired air (Durk *et al.*, 1992; ATSDR, 1995). The other major metabolites, trichloroethanol, trichloroethanol glucuronide, and trichloroacetic acid are mainly eliminated via urinary excretion, though fecal excretion has also been observed (Caperos *et al.*, 1982; Mitoma *et al.*, 1985; Ghittori *et al.*, 1987; Imbriani *et al.*, 1988; Kawai *et al.*, 1991).

### 10.2.5 Species variations

Because physiologically based pharmacokinetic (PBPK) modeling attempts to extrapolate between various species for risk assessment purposes, variations among species can affect model precision and accuracy. Most aspects of TCA pharmacokinetics are similar among species, including absorption and elimination route. However, quantitative differences in blood:air partition coefficients as well as metabolism rates have been noted (Schumann *et al.*, 1982b). Specifically, mice tend to have higher rates of TCA metabolism compared to rats and humans. Furthermore, blood:air partition coefficients, which

dramatically effect inhalation absorption differ: 2.53, 5.76, and 10.8 for humans, rats, and mice respectively (Reitz *et al.*, 1988).

## 10.3 PBPK modeling

Attempts to construct PBPK models appropriate for TCA's disposition have commonly used approaches similar to those developed in 1984 by Ramsey and Andersen (Ramsey and Andersen, 1984; Reitz *et al.*, 1988). Based upon the Ramsey and Andersen model (RAM), a modified model was used to estimate metabolic kinetic constants using a closed, recirculated atmosphere representative of those used for gas uptake studies (Gargas *et al.*, 1986). This study found that to adequately describe TCA disposition, its metabolism required only a first-order pathway, which was abolished when oxidative microsomal metabolism is inhibited. Reitz and colleagues utilized a model similar to the RAM to simulate exposure to TCA via inhalation, intravenous administration, bolus gavage, and in drinking water, and demonstrated the plausibility of using PBPK models in TCA risk assessment, based upon successful interspecies extrapolation (Reitz *et al.*, 1988).

Bogen and Hall used a derivation of the RAM with an additional compartment for skin to assess risk associated with TCA in drinking water, and found PBPK modeling predicted nontoxic TCA concentrations lower than the existing NOAELs (Bogen and Hall, 1989). Attempts to determine metabolic constants via PBPK modeling concluded that, due to low metabolism of TCA, gas uptake study techniques were too insensitive to sufficiently form a TCA PBPK model (Gargas and Andersen, 1989). Absorption and elimination of TCA across time following an inhalation exposure was measured, and a PBPK model was built to predict TCA levels in blood and expired air (Dallas *et al.*, 1989). As TCA contaminates both water and soil, percutaneous absorption has been modeled in rats and humans, including simulations specific for exposure to children (Poet *et al.*, 2000). Notably, combination of quantitative structure-property relationships with traditional PBPK modeling has successfully predicted inhalation pharmacokinetics for TCA, as well as other volatile organic chemicals (Beliveau *et al.*, 2003).

Within the context of utilizing biological monitoring to assess exposure, especially in a work environment, PBPK models of TCA have been applied. Droz and coworkers first developed a population physiological model to investigate variability in biological monitoring, then applied the model to assess how alterations in components such as workload, organ function, and body build affected the model's ability to accurately determine TCA exposure (Droz *et al.*, 1989a,b). Comparison of various exposure scenarios on alterations in biological monitoring using PBPK modeling has been used to determine which biological indices, *i.e.,* parent compound versus metabolite in various biological media, are best suited to assess exposure to TCA (Lapare *et al.*, 1995). A linear four-compartment mass-balance model was used to not only assess uptake and elimination of TCA in human subjects at environmentally feasible levels, but also predict exhaled TCA concentrations in another human study (Wallace *et al.*, 1997). Analysis of various PBPK models for a series of chemicals, including TCA, has allowed analysis of pharmacokinetic model output sensitivity to variability in both biochemical and metabolic input parameters (Hetrick *et al.*, 1991).

Because exposure to a single chemical compound in industrial or environmental exposure settings is unlikely, examination of chemical in mixtures is necessary. Koizumi and coworkers performed inhalation studies to investigate co-exposure of TCA with perchloroethylene, and found significant decreases in formation of TCA metabolites due to co-exposure with perchloroethylene (Koizumi *et al.*, 1983). Further, Tardif and Charest-Tardif noted decreases in excretion of TCA metabolites following co-exposure with *m*-xylene (Tardif and Charest-Tardif, 1999). Dobrev and associates successfully modeled competitive inhibition of trichloroethylene by TCA and tetrachloroethylene, likely due to a

shared metabolic pathway with limited enzymatic capacity, specifically the cytochrome P450s (Dobrev *et al.*, 2001). Further work by Dobrev and associates also used a combination of tetrachloroethylene, perchloroethylene, and TCA to assess possible interactions which might change observed toxicity (Dobrev *et al.*, 2002). The findings indicated that co-exposure to the three chlorinated hydrocarbons lead to a nonlinear increase in toxic conjugative metabolites of tetrachloroethylene (which are associated with renal toxicity and/or carcinogenicity), possibly indicating a greater than additive risk associated with exposure to the chemical mixture. Although metabolism of TCA is relatively low (<10%), its ability to interact with essential metabolic enzymes may confer TCA the ability to inhibit or decrease metabolism/detoxification of other chemicals, especially other organic solvents. Alternatively, because of comparatively low affinity for the cytochrome P450s, formation of TCA metabolites (especially trichloroethanol and trichloroacetic acid) may be reduced due to enzymatic inhibition caused by co-exposure with other chemicals.

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