CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1   TOXICOKINETICS

Limited data on 1,1,2-trichloroethane in humans and animals are available. These data are summarized below.

- 1,1,2-Trichloroethane is rapidly absorbed through the respiratory tract in humans. In animals, 1,1,2-tricholoethane is rapidly absorb through the skin and is well-absorbed from the gastrointestinal tract.

- In animals and presumably humans, absorbed 1,1,2-trichloroethane is distributed throughout the body with the highest concentrations found in the fat, liver, and brain.

- The primary metabolites of 1,1,2-trichloroethane are chloroacetic acid (formed by cytochrome P-450), and S-carboxymethylcysteine and thiodiacetic acid (formed following conjugation with glutathione).

- The major route of excretion after oral exposure is via metabolites in the urine; smaller amounts of 1,1,2-trichloroethane are excreted in exhaled air and feces. Little 1,1,2-trichloroethane was detected in the urine following inhalation exposure in humans. The half-life of 1,1,2-trichloro-ethane in animals exposed via inhalation exposure was 49 minutes.

3.1.1   Absorption

Studies in humans indicate that 1,1,2-trichloroethane is absorbed rapidly after inhalation exposure (Morgan et al. 1970, 1972). In one of the studies (Morgan et al. 1970), a volunteer took one breath of radiolabeled 1,1,2-trichloroethane and expired 2–3% of the inspired dose in the alveolar air after 12 seconds and about 0.5% after 40 seconds of breath-holding. More than 90% of the administered dose was retained in the body after 50 minutes. These data indicate that 1,1,2-trichloroethane was extensively absorbed into the bloodstream, supported by a blood:air partition coefficient (K_D) of 44.2. Gargas et al. (1989) determined a blood:air partition coefficient in humans of 35.7.

Data on absorption of 1,1,2-trichloroethane following inhalation exposure in animals were generated in an effort to determine time courses for repeat exposure and to validate physiologically-based pharmacokinetic (PBPK) models. Rats and mice exposed to 1,1,2-trichloroethane under closed chamber conditions at 100 ppm 6 hours/day, 5 days/week for 4 weeks showed significant concentrations of 1,1,2-trichloroethane in the blood, indicating that 1,1,2-trichloroethane is extensively absorbed in both species (The Sapphire Group 2003). This is supported by the identification of a partition coefficient for

rats of 58.0 (Gargas et al. 1989). A supporting study (using few numbers of animals/time point and evaluating blood levels at a limited number of sampling intervals) found that the maximal blood concentrations after 4 weeks exposure were 2.2 µg/mL in rats and 2.1 µg/mL in mice (Poet 2003). The only other absorption information comes from the assumption that an administered chemical has been absorbed by the body if it can be shown to affect physiological processes. For example, following inhalation exposure to 1,1,2-trichloroethane under closed chamber conditions, exhalation of acetone was altered. This provides indirect evidence that 1,1,2-trichloroethane is absorbed following inhalation exposure.

In an effort to determine time courses for repeat exposure and to validate PBPK models, rats were administered 1,1,2-trichloroethane via gavage in corn oil at 92 mg/kg/day or in water at 1.7 mg/kg/day for 5 days. Similarly, mice were treated at 390 mg/kg/day in corn oil or 10 mg/kg/day in water. Significant concentrations of 1,1,2-trichloroethane were detected in the blood, indicating that 1,1,2-trichloroethane is well absorbed in both species. Maximal blood concentrations were observed in rats on day 1 (up to 17 µg/g) and in mice on days 3 and 5 (8.5 to 25 µg/g) (Poet 2003; The Sapphire Group 2003). The only other data available in animals showed that oral doses near the maximum tolerated dose in mice (300 mg/kg) or rats (70 mg/kg) were 81% metabolized, indicating that at least this amount was absorbed (Mitoma et al. 1985). This suggests that 1,1,2-trichloroethane, like other structurally related halocarbons, is well absorbed from the gastrointestinal tract of animals, and probably humans as well.

Two studies in animals indicate that 1,1,2-trichloroethane is easily absorbed through the skin. In the guinea pig, blood concentration of 1,1,2-trichloroethane peaked at ≈3.7 µg/mL within a half-hour following 1.0 mL 1,1,2-trichloroethane single epicutaneous application to the skin (Jakobson et al. 1977). Following the peak, the blood level declined to ≈2.5 µg/L at 1 hour, remained at this level until ≈4 hours, and then rose to ≈3.7 µg/L at 6 hours. The authors suggested that this complex dermal absorption of 1,1,2-trichloroethane in guinea pigs may be due to an initial increased barrier function of the skin after 1 hour, which led to decreased absorption. Subsequent absorption during the next few hours may represent an overcoming of the barrier. However, it is also possible that the prolonged time-course for dermal absorption could reflect retention of 1,1,2-trichloroethane in skin lipids. In mice, 15 minutes after application of 0.5 mL of 1,1,2-trichloroethane, 99.7% was retained in the body and 0.3% was expired in the breath (Tsuruta 1975). The absorption rate was calculated to be 130 nmoles/minute/cm^2 of skin. The rapid absorption through the skin may well be due to the highly lipid soluble character of 1,1,2-trichloro-ethane (Kronevi et al. 1977).

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3.1.2   Distribution

After an inhalation exposure of 1,000 ppm for 1 hour in mice, 1,1,2-trichloroethane was distributed in the following manner: approximately 600 µg/g in fats, 80 µg/g in the kidney and liver, 45–60 µg/g in the blood and brain, and 20–35 µg/g in the heart, spleen, and lung (Takahara 1986a). Examination of partition coefficients showed that 1,1,2-trichloroethane had a moderate degree of lipid solubility compared to other hydrocarbons but was still quite lipid soluble (Gargas et al. 1989; Morgan et al. 1972; Sato and Nakajima 1979). Poet (2003) measured partition coefficients in rat brain and spleen and in mouse blood. Partition coefficients for 1,1,2-trichloroethane were 43 for rat spleen:air, 56 for rat brain:air, and 71 for mouse blood:air. Rat liver:air and fat:air partition coefficients of 73.1 and 1,438, respectively, were determined by Gargas et al. (1989). This indicates that 1,1,2-trichloroethane could be easily distributed and retained in fat, liver, and brain in both animals and humans.

Limited information is available on the distribution of 1,1,2-trichloroethane following oral exposure. One study showed that 1,1,2-trichloroethane was distributed to the liver following oral exposure in animals (Mitoma et al. 1985). In this study, 1,1,2-trichloroethane was extensively metabolized (presumably by the liver), and was also found to bind to hepatic protein. It is likely that 1,1,2-trichloroethane is also distributed to the liver in humans.

3.1.3   Metabolism

The primary metabolites identified by high-performance liquid chromatography in rats and mice given 1,1,2-trichloroethane by gavage were chloroacetic acid, S-carboxymethylcysteine, and thiodiacetic acid (Mitoma et al. 1985). An earlier study reported these three compounds to be the primary metabolites of 1,1,2-trichloroethane following intraperitoneal injection in mice (Yllner 1971). S-Carboxymethylcysteine and thiodiacetic acid are formed from 1,1,2-trichloroethane following conjugation with glutathione (Yllner 1971). Chloroacetic acid is formed by hepatic cytochrome P-450 (Ivanetich and Van Den Honert 1981). This reaction is thought to proceed via oxidative dechlorination of 1,1,2-trichloroethane. Cytochrome P-450 can also apparently produce free radicals from 1,1,2-trichloroethane (Mazzullo et al. 1986). These proposed pathways are shown in Figure 3-1. Acyl chlorides and free radicals are reactive metabolites that can bind to proteins and nucleic acids, and are suspected of being cytotoxic, mutagenic, and carcinogenic (Ivanetich and Van Den Honert 1981; Mazzullo et al. 1986). Acyl chlorides conjugated with GSH generate S-carboxymethylcysteine and thiodiacetic acid, which are major urinary metabolites of 1,1,2-trichloroethane (Yllner 1971). Other metabolites, found only in trace amounts in mice and rats following exposure to 1,1,2-trichloroethane, included trichloroacetic acid and trichloroethanol (Ikeda and

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Ohtsuji 1972; Takahara 1986c; Yllner 1971). It is not clear how these compounds were formed; it was suggested by Yllner (1971) that they might be derived from impurities in the 1,1,2-trichloroethane samples used.

Figure 3-1. Proposed Metabolic Pathway of 1,1,2-Trichloroethane*

- - - supposed pathway
----- proven pathway
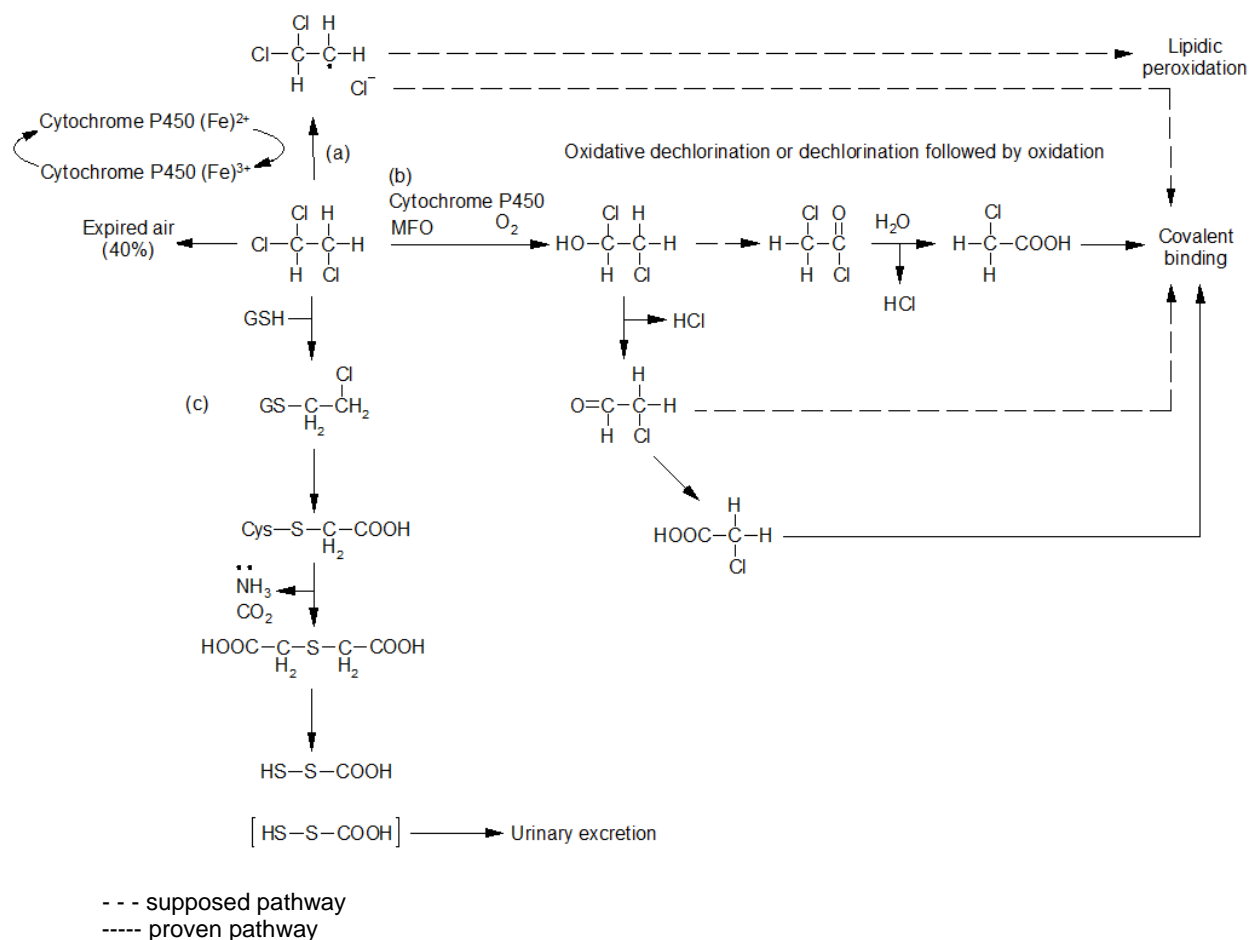
*(a) one-electron oxidation; (b) two-electron oxidation; (c) detoxification step

Source: Mazzullo et al. 1986 (based on studies in rats and mice)

Although percent of the orally-administered dose metabolized was identical in rats and mice (81%), the actual amount of 1,1,2-trichloroethane metabolized was much higher in mice (Mitoma et al. 1985). The chemical was given to each species at the maximum tolerated dose, which was 4.3 times greater in mice; mice experienced a higher body burden than rats but were able to metabolize the same percentage of it. The inherent ability of mice to metabolize 1,1,2-trichloroethane at a higher rate than rats may contribute to the greater susceptibility of mice to 1,1,2-trichloroethane cytotoxicity and carcinogenicity (Ivanetich

and Van Den Honert 1981; Mazzullo et al. 1986). It is not known how the rate of 1,1,2-trichloroethane metabolism in humans compares to that in mice and rats. However, metabolism in humans is likely to be qualitatively similar to that in animals.

3.1.4 Excretion

The excretion rate of inhaled 1,1,2-trichloroethane in humans was measured in the breath and urine of humans (Morgan et al. 1970). Excretion in the breath after 1 hour was 2.9% of the administered dose; the slope of the retention curve was 0.006. The excretion rate in the urine was <0.01%/minute of administered radioactivity. From these data, the half- life for urinary excretion was estimated to be about 70 minutes.

Poet (2003) performed breath analysis in female mice exposed to 1,1,2-trichloroethane at 250 and 500 ppm for 4–6 hours. The half-life following 1-hour inhalation exposure to 1,005 ppm of 1,1,2-tri-chloroethane in mice was determined to be 625 minutes in the heart, 203 minutes in the fat, 147 minutes in the brain, 127 minutes in the spleen, 122 minutes in the lungs, 43 minutes in the kidney, 39 minutes in the blood, and 19 minutes in the liver (Takahara 1986a). The half-life in the whole body was calculated to be 49.3 minutes. The presence of 1,1,2-trichloroethane in tissue samples was determined by gas chromatography.

The excretion routes were shown to be similar in rats and mice, regardless of whether the chemical was given orally (Mitoma et al. 1985) or intraperitoneally (Yllner et al. 1971). Following a dose of radiolabeled compound, about 7–10% of 1,1,2-trichloroethane was exhaled unchanged in the breath, 3–7% was exhaled as CO_2, 72–87% was found as metabolites in the urine, about 1% was in the feces, and 1–3% remained in the carcasses of rats and mice after 48 hours. It is not known how excretion of 1,1,2-trichloroethane in humans compares to that in mice and rats, but (in the absence of additional information), it is likely that excretion in humans is primarily via metabolites in the urine.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test

species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

PBPK models were developed to extrapolate from the oral route of exposure to the inhalation route of exposure. The Sapphire Group (2003) determined K_m and V_{max} and, utilizing data from pharmacokinetic experiments by Poet (2001), developed and validated a PBPK model to predict the disposition of 1,1,2-trichloroethane in mice and rats for inhalation and ingestion. The optimized model included suicide inhibition (i.e., enzyme destruction via inactivation by bound, reactive intermediate) as a mechanism by which a reduction in V_{max} was seen in female mice after exposure. The initial PBPK model (The Sapphire Group 2003) was expanded and refined to model the following endpoints: immunotoxicity in mice (The Sapphire Group 2004a), acute neurotoxicity in rats (The Sapphire Group 2004b), subchronic neurotoxicity in rats (The Sapphire Group 2005a), reproduction in rats (The Sapphire Group 2006), development in rats (The Sapphire Group 2005b), and carcinogenicity in rats and mice (The Sapphire Group 2004c). These PBPK models are designed to predict inhalation exposure levels equivalent to those used in oral toxicity studies in rodents (based on selection of a critical endpoint and using the most appropriate internal dose measure) and are not applicable to humans.

3.1.6 Animal-to-Human Extrapolations

There are no data available that evaluate the sensitivity of humans to 1,1,2-trichloroethane-induced toxicity relative to animals. Lethality data in animals do not suggest a high degree of interspecies variability; however, available information suggests that species differences in metabolism (among rats and mice) may affect susceptibility. In both species, lethality was observed following inhalation exposures >400 ppm and following oral exposures >300 mg/kg, with effects being seen at slightly lower levels of exposure in mice than rats. Based on data from one study, the absorption of 1,1,2-trichloro-ethane is expected to be similar in rats and mice; similar concentrations of 1,1,2-trichloroethane were detected in the blood of rats and mice after 4 weeks of inhalation exposure to 1,1,2-trichloroethane (Poet 2003). In another study, the primary metabolites in rats and mice administered 1,1,2-trichloroethane by gavage were identified as chloroacetic acid, S-carboxymethylcysteine, and thiodiacetic acid (Mitoma et al. 1985). The metabolites of 1,1,2-trichloroethane, rather than the parent compound, is thought to be responsible for most of its health effects, owing to greater reactivity of the metabolites. Data from the study by Mitoma et al. (1985) also suggest that mice may have an inherent ability to metabolize 1,1,2-tri-chloroethane at a higher rate than rats; this may contribute to the greater susceptibility of mice to the

toxicity of 1,1,2-trichloroethane than rats. In support, Mazzullo et al. (1986) showed increased binding of 1,1,2-trichloroethane to DNA from murine organs (liver, lung, stomach, and kidney) relative to rat organs. Although the metabolism of 1,1,2-trichloroethane is expected to be similar to that seen in animals, it is not known how the rate of metabolism in humans compares to that in mice and rats. Data from studies of other compounds, such as trichloroethylene (TCE) or perchloroethylene (PCE), suggest that humans absorb and metabolize less of these halocarbons than rats (Bernauer et al. 1996; Green et al. 1997; NAS 2009; Volkel et al. 1998); however, specific data comparing the metabolism of 1,1,2-trichloroethane in humans and rats were not identified. Owing to the increased surface area of the olfactory epithelium of rats relative to humans, rats may be a particularly sensitive model for chemically-induced injury to these tissues.

3.2   CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to 1,1,2-trichloroethane are discussed in Section 5.7, Populations with Potentially High Exposures.

The results from two intermediate-duration oral studies in rats suggest that immature rats were not more susceptible to the toxic effects of 1,1,2-trichloroethane than mature rats. In a two-generation study, decreased body weight gain during gestation was observed in P1 and F1 females administered 1,1,2-trichloroethane in drinking water at 82 mg/kg/day; effects in pups (decreased weights on postnatal days 4 to 21) were observed at the same dose (Mylchreest 2006). In a developmental study, female rats treated at 111 mg/kg/day in drinking water on days 6–20 of gestation showed decreased body weight

gain; this effect occurred in the absence of significant, treatment-related effects on fetal weight, sex, or the incidences of external, visceral, or skeletal abnormalities (Wilson 2005). However, few data are available to assess the susceptibility of children to 1,1,2-trichloroethane .

Persons with diabetes (Hanasono et al. 1975), with prior exposure to polybrominated biphenyls (PBBs) (Kluwe et al. 1978), or with prior exposure to isopropyl or ethyl alcohol or acetone (Traiger and Plaa 1974) may be more susceptible to the hepatotoxic effects of 1,1,2-trichloroethane. It is possible that prior exposure to drugs or chemicals that induce enzyme activity (including cytochrome P-450) could have the same effect.

3.3   BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (IOM 2010; NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (IOM 2010; NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to 1,1,2-trichloroethane are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for 1,1,2-trichloroethane from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (IOM 2010; NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by 1,1,2-trichloroethane are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Currently, biomarkers for 1,1,2-trichloroethane do not adequately capture exposure, possibly due to 1,1,2-trichloroethane's short half-life. No epidemiology studies were conducted that assessed exposure by measuring 1,1,2-trichloroethane levels in the blood or urine and associated health effects. 1,1,2-Trichloroethane and its metabolites have been detected in blood, urine, expired air, and tissues of humans and animals (Jakobson et al. 1977; Mitoma et al. 1985; Morgan et al. 1970; Poet 2003; Takahara 1986a; The Sapphire Group 2003; Tsuruta 1975). The parent compound was measured in the blood and expired air; its primary metabolites (chloroacetic acid, S-carboxymethylcysteine, and thiodiacetic acid) have been detected in the urine (Mitoma et al. 1985). Based on data from 2003–2004, 2005–2006, 2007–2008, 2009–2010, and 2011–2012 NHANES, levels of blood 1,1,2-trichloroethane were less than the limit of detection (CDC 2017). Levels below the limit of detection or only trace amounts of 1,1,2-trichloroethane have been reported in exhaled air (Wallace et al. 1984). Low levels of 1,1,2-trichloroethane were likewise detected in the tissues of humans soon after they were exposed primarily via inhalation (Bauer 1981a, 1981b), exemplifying that it is difficult to assess lower levels of exposure to 1,1,2-trichloroethane with current methodologies.

3.3.2 Biomarkers of Effect

No studies were located that identified biomarkers specific for 1,1,2-trichloroethane-induced disease states. None of the available epidemiological studies (Brender et al. 2014; Dosemeci et al. 1999) identified alterations in blood chemistry indices or other pathological endpoints that could be used to identify the disease state. Biomarkers for diagnosis of target organ toxicity (e.g., AST for liver damage) may be considered a biomarker of effect if it is known that exposure to 1,1,2-trichloroethane occurred.

3.4   INTERACTIONS WITH OTHER CHEMICALS

PBBs were shown to increase the renal toxicity of 1,1,2-trichloroethane as measured by decreases in p-aminohippurate accumulation in renal cortical slices (Kluwe et al. 1978). PBBs are known to increase the activities of microsomal mixed-function oxygenases in the kidney and liver, so increased metabolism of 1,1,2-trichloroethane and the increased presence of metabolites more toxic than the parent compound itself are likely responsible for the increased toxicity of 1,1,2-trichloroethane in the kidney. However, the study also showed that PBBs did not increase the hepatotoxic effects of 1,1,2-trichloroethane in mice, as indicated by relative liver weight or AST levels.

Phenobarbital, another microsomal enzyme inducing agent, was found to potentiate liver toxicity, as indicated by increases in AST and ALT in rats that were exposed to 1,1,2-trichloroethane vapor (Carlson 1973). Guinea pigs treated with pentobarbital as an anesthetic following dermal application of 1,1,2-trichloroethane were shown to produce reduced glycogen levels and hydropic changes in the liver (Kronevi et al. 1977). Liver effects were not found in anesthetized "control" animals or animals that were treated with 1,1,2-trichloroethane, but not anesthetized. The authors suggest that the liver effects they observed were produced by the interaction of pentobarbital and 1,1,2-trichloroethane. The lack of untreated controls makes this claim difficult to evaluate, however. Potentiation is usually seen only after pretreatment with the inducer, since time is required for enzyme induction. It may be that dermal absorption of 1,1,2-trichloroethane was slow enough, compared to intraperitoneal absorption of pentobarbital, for this to occur.

Pretreatment with lower, but not higher, doses of acetone (MacDonald et al. 1982) potentiated the hepatotoxicity of 1,1,2-trichloroethane in rats as indicated by a rise in ALT and a decrease in hepatic GSH levels. Acetone also potentiated the 1,1,2-trichloroethane-induced elevation of ALT in mice (Traiger and Plaa 1974).

Pretreatment with isopropyl alcohol (Traiger and Plaa 1974) or ethanol (Klaassen and Plaa 1966) potentiated the 1,1,2-trichloroethane-induced elevation of ALT activity in mice. Pretreatment with ethanol did not alter bromosulfophthalein retention (Klaassen and Plaa 1966).

Pretreatment with alloxan, which induces a hyperglycemic state similar to that found in diabetic humans, also enhanced the hepatotoxic effects of 1,1,2-trichloroethane in rats as indicated by increased ALT

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activity and increased hepatic triglyceride concentration (Hanasono et al. 1975). The mechanism of this interaction is unknown.