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

3.1 TOXICOKINETICS

- Chlorobenzene is readily absorbed from the respiratory and gastrointestinal tracts.
- Chlorobenzene is widely distributed in the blood, but may accumulate to some extent in adipose tissue due to its lipophilicity.
- Most chlorobenzene is metabolized via a chlorobenzene 3,4-epoxide pathway to ultimate urinary glucuronide or sulfate conjugates.
- Urinary excretion of chlorobenzene metabolites is the major route of excretion.

3.1.1 Absorption

Limited information was located regarding absorption of inhaled chlorobenzene. Absorption from the respiratory tract of two workers exposed to airborne chlorobenzene concentrations in the range of 0.5–0.84 ppm was estimated to have been 70% (Ogata and Shimada 1983). In other human studies that involved occupational exposure to chlorobenzene, the detection of chlorobenzene metabolites in blood and urine provides unquantified demonstration that chlorobenzene is absorbed from the respiratory tract (Knecht and Woitowitz 2000; Kumagai and Matsunaga 1994; Kusters and Lauwerys 1990; Ogata et al. 1991; Yoshida et al. 1986). Rats were reported to readily absorb ^{14}C-labeled chlorobenzene at airborne concentrations up to 700 ppm (Sullivan et al. 1983). Shimada (1981, 1988) evaluated distribution and urinary excretion of chlorobenzene and its metabolites in laboratory animals exposed to chlorobenzene by inhalation, thus demonstrating that inhaled chlorobenzene is absorbed.

Chlorobenzene is readily absorbed from the gastrointestinal tract. Ogata and Shimada (1983) reported at least 31% absorption of chlorobenzene orally administered to a single volunteer. In the same study, rats administered chlorobenzene absorbed at least 18% of the administered dose. Lindsay Smith et al. (1972) administered [^{14}C]chlorobenzene to two rabbits orally at approximately 500 mg/rabbit, twice per day for 4 days and measured radioactivity in urine and feces of both rabbits and tissues of one rabbit. Recovered radioactivity was 19.6% in the urine, 1.05–1.55% in the feces, and 0.05% in tissues; thus, approximately 20% of the administered dose was absorbed. The absorption of orally-administered chlorobenzene from the gastrointestinal tract was demonstrated in a variety of oral animal studies that were designed to evaluate chlorobenzene metabolites in urine (e.g., Azouz et al. 1952; Gillham and Young 1968; Krewet et al. 1989; Parke and Williams 1953; Lindsay Smith et al. 1950, 1972; Spencer and Williams 1950).

3.1.2 Distribution

Limited information was located regarding distribution of absorbed chlorobenzene in humans. Knecht and Woitowitz (2000) exposed eight volunteers to Germany's maximum workplace concentration (MAK) of 10 ppm chlorobenzene 8 hours/day for 5 days. There was no apparent tendency for chlorobenzene or its metabolites to accumulate in blood or urine with prolonged exposure. Blood levels reached a steady state (mean, 197.0±9.7 µg/L) after the first hour of exposure. The mean concentration of chlorobenzene in blood in five subjects exposed during physical exercise (75 W, 10 minutes/hour on a bicycle) was 217 µg/L. The mean chlorobenzene blood concentrations were 133 µg/L in two subjects exposed during mild exercise (50 W, 10 minutes/hour on a bicycle) and 78 µg/L in one subject exposed while at rest.

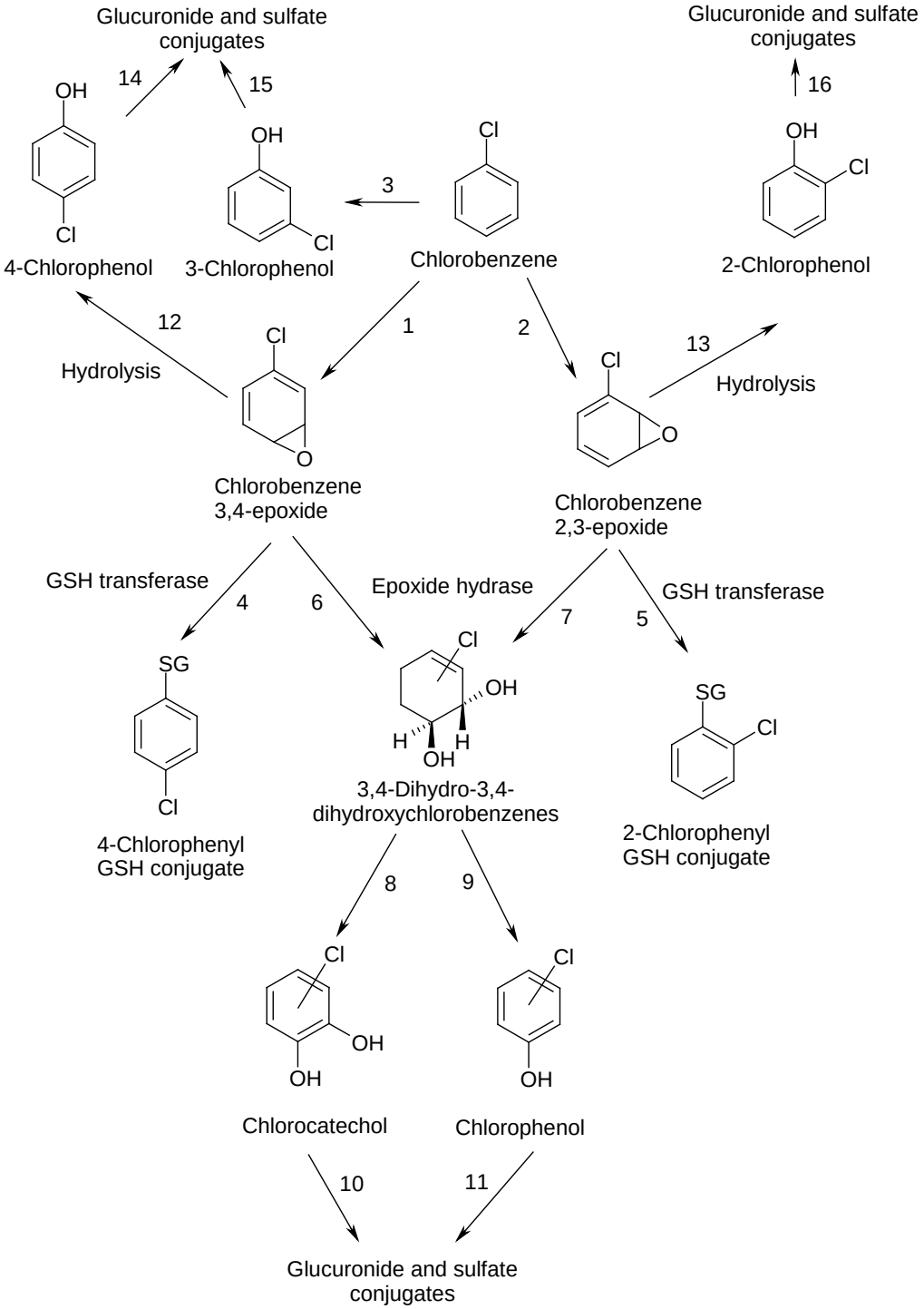
Studies in laboratory animals indicate that chlorobenzene is widely distributed and may accumulate in adipose tissue (Shimada 1988; Sullivan et al. 1983). Accumulation in adipose tissue is related to the lipophilicity of chlorobenzene and likely depends on the species-specific lipid distribution in various organs.

3.1.3 Metabolism

A proposed metabolic pathway of chlorobenzene (Chadwick et al. 1984) is shown in Figure 3-1. The numbers 1–16 in Figure 3-1 correspond to the numbers in the following text that presents the various metabolic processes.

According to the proposed metabolic pathway, chlorobenzene undergoes CYP450 catalyzed oxidation to form chemically-reactive chlorobenzene 3,4-epoxide (1), relatively nontoxic chlorobenzene 2,3-epoxide (2) to a lesser extent, and 3 chlorophenol (3). Both epoxides can be formed in liver and lung (and other tissues such as kidney and adrenal cortex) and are capable of covalently binding to DNA, RNA, and proteins. The chlorobenzene epoxides can be further metabolized by three separate pathways. One pathway for 3,4- and 2,3-chlorobenzene epoxides involves the GSH transferase-catalyzed formation of glutathione conjugates of 4-chlorophenyl (4) and 2-chlorophenyl (5), respectively, followed by conversion to mercapturic acid derivatives. Another metabolic pathway for the 3,4- and 2,3-epoxides is the enzymatic (epoxide hydrase) conversion to 3,4-dihydro-3,4-dihydroxychlorobenzene (6 and 7, respectively) which is enzymatically converted to chlorocatechol (8) or chlorophenol (9). Both chlorocatechol and chlorophenol can form glucuronide and sulfate conjugates (10 and 11, respectively).

Figure 3-1. Mammalian Metabolism of Chlorobenzene to Phenols, Dihydrodiols, Catechols, and Glutathione Conjugates



Source: Chadwick et al. 1984; reprinted from Pesticide Biochemistry and Physiology 21:148-161 (1984) with permission from Elsevier.

The 3,4- and 2-3-epoxides can also undergo hydrolysis to form 4-chlorophenol (12) and 2-chlorophenol (13), respectively. The chlorophenols (4-chlorophenol, 3-chlorophenol, and 2-chlorophenol) can form glucuronide and sulfate conjugates (14, 15, and 16, respectively).

Chlorobenzene metabolites that have been detected in the urine of a variety of animal species include 2-, 3-, and 4-chlorophenyl-mercapturic acid, chlorophenols and chlorocatechols and their glucuronide and sulfate conjugates, and 3,4-dihydro-3,4-dihydroxychlorobenzene. Chlorocatechol and 2-chlorophenyl-mercapturic acid were detected in the urine of humans who received chlorobenzene orally or by inhalation (Ogata and Shimada 1983). Chlorobenzene metabolites that have been detected in the urine of chlorobenzene-exposed humans include 4-chlorocatechol, 4-chlorophenol, and 2-chlorophenyl-mercapturic acid (Kusters and Lauwerys 1990; Ogata and Shimada 1983; Ogata et al. 1991; Yoshida et al. 1986).

Cytochrome P-450 2E1 is the main enzyme involved in the oxidation of chlorobenzene in mice, rats, and humans. Cytochrome P-450 3A also appears to play a role in the generation of reactive metabolites in mice, rats, and humans. It is important to note, however, that, compared to mice and rats, the rate of metabolism of chlorobenzene to soluble metabolites is higher in humans, and the formation of covalently bound products is lower (Nedelcheva et al. 1998). In addition, there is up to a 10-fold difference in the rate of metabolism of chlorobenzene in different human livers. There are also significant species and sex differences in the metabolism of chlorobenzene with markedly higher rates of oxidation in male mice than in male rats and female mice.

Co-treatment of rats with chlorobenzene and an epoxide hydrase inhibitor (cyclohexane oxide) resulted in decreases in chlorobenzene metabolism and its necrotic toxicity on the liver, suggesting that the metabolism of chlorobenzene is partially responsible for its liver toxicity (Oesch et al. 1973).

3.1.4 Excretion

Knecht and Woitowitz (2000) exposed eight volunteers to Germany’s MAK of 10 ppm chlorobenzene 8 hours/day for 5 days. Half-lives of elimination of chlorobenzene from blood were 53 minutes in the first hour after cessation of exposure and 150 minutes thereafter. The major urinary metabolite of chlorobenzene was 4-chlorocatechol (74%), with the remainder comprised of chlorophenol isomers of which 4-chlorophenol (13%) was the most abundant. Urinary 4-chlorophenol was useful as a biomarker

of exposure due to its half-life of approximately 12 hours. The elimination half-life of urinary 4-chlorocatechol was 6.4 hours (Knecht and Woitowitz 2000).

Ogata and Shimada (1983) reported that in two workers exposed by inhalation to 0.84 and 0.5 ppm of chlorobenzene, the excretion of 4-chlorophenylmercapturic acid was markedly lower than that of 4-chlorocatechol. Ogata and Shimada (1983) also assayed the urinary metabolites of chlorobenzene of a 57-year-old male volunteer given an oral dose of 0.3 mmol/kg of chlorobenzene. Two urinary metabolites, 4-chlorophenylmercapturic acid and 4-chlorocatechol, were detected. As in the case of inhalation exposure, the excretion of 4-chlorophenylmercapturic acid was reported to be markedly lower than that of 4-chlorocatechol. However, the ratio of mercapturic-acid to 4-chlorocatechol in the urine of human subject receiving oral chlorobenzene was similar to that of the two workers inhaling chlorobenzene.

Linear correlations between urinary 4-chlorocatechol excretion and airborne exposure of workers to chlorobenzene were established by Yoshida et al. (1986) after monitoring end-of-shift urinary metabolites in healthy male workers in two chemical factories where chlorobenzene was used as a solvent. The primary urinary metabolites were 4-chlorocatechol (mean 76.9%) and 4-chlorophenol (mean 12.4%). In factories A and B, average chlorobenzene concentrations in air were 3.16 ppm (range 1.72–5.78 ppm) and 3.14 ppm (range 2.68–3.68 ppm), respectively. These levels of exposure in factories A and B corresponded, respectively, to mean 4-chlorocatechol levels of 0.362 µmoles/mg creatinine (range 0.166–0.787 µmoles/mg creatinine) and 0.482 µmoles/mg creatinine (range 0.354–0.655 µmoles/mg creatinine) in urine (Yoshida et al. 1986).

Assessing 44 maintenance workers in a diphenylmethane 4,4'-diisocyanate plant for chlorobenzene exposure, Kusters and Lauwerys (1990) also found that the main urinary metabolites at the end of shift were 4-chlorophenol and 4-chlorocatechol, with the latter being 3 times more abundant than the former. The time-weighted average exposure to chlorobenzene in air (mean 1.2 ppm, range 0.05–106 ppm) was less than the current German MAK value of 10 ppm established in 1995. More than 80% of the metabolites were eliminated within 16 hours after the end of exposure, and there was no tendency for an increase in concentration during the working week.

Ogata et al. (1991) reported that, in order of abundance, the main urinary metabolites of chlorobenzene in exposed workers were 4-chlorocatechol and 2-chlorophenylmercapturic acid. The concentrations of chlorobenzene in blood and metabolites in urine (e.g., 4-chlorocatechol, approximately 26% of exposure)

were both proportional to the concentration of chlorobenzene in air. The molar ratio of urinary chlorocatechol to inhaled chlorobenzene was estimated to be approximately 26%, and the mean slope of regression line for chlorobenzene in air versus blood was 4.6±1.15 µg/L for 1 ppm chlorobenzene. The measured biological half-time of 4-chlorocatechol was 2.9 hours.

Rats were exposed to ^{14}C-chlorobenzene vapor at concentrations of 100, 400, and 700 ppm for 8 hours (Sullivan et al. 1983). The plasma concentration-time profile for chlorobenzene on cessation of exposure, as estimated by respiratory elimination of radioactivity, indicated a two-compartment elimination. Increase in exposure by a factor of 7 (100–700 ppm) increased the total uptake of radioactivity by a factor of about 13. This increase in body burden was associated with a decrease in total body clearance, as indicated by an approximate 4-fold increase in the half-life of the central compartment. The proportion of the dose excreted via the lungs (which may be presumed to be largely, if not entirely, unchanged chlorobenzene) increased nonlinearly and the proportion eliminated by hepatic metabolism decreased. Increase in the dose of chlorobenzene was associated with a decrease in the proportion cleared as the mercapturic acid derivative. Of interest, the half-life of chlorobenzene was shorter at the 700 ppm exposure level when the animals were subjected to repeated exposure daily for 5 days, as compared with that of the single 700 ppm exposure animals, raising the possibility of induction of metabolic clearance. In agreement with this possibility, the proportion cleared by metabolism in the multi-exposed animals was increased, and the proportion excreted unchanged via the lung was decreased, as compared with the 700 ppm-single exposure animals.

In the repeated-dose oral study of rabbits administered [^{14}C]chlorobenzene (Lindsay Smith et al. 1972), total recovery of radioactivity from the urine was approximately 20% of the administered dose. The contributions of the various metabolites in the urine were 33.88% for ethereal sulfates, 33.57% for glucuronides, 23.8% for mercapturic acids, 4.17% for diphenols, 2.84% for monophenols, and 0.57% for 3,4-dihydro-3,4-dihydroxychlorobenzene. It was concluded that the remaining radiolabel was excreted in the expired air. The major urinary metabolites were 4-chlorophenylmercapturic acid and conjugates of 4-chlorocatechol. Other identified urinary metabolites included quinol, 3-chlorocatechol, and 2- and 3-chlorophenylmercapturic acid. Ogata and Shimada (1983) reported that the primary urinary metabolite in rats was 4-chlorophenylmercapturic acid and that 4-chlorocatechol was a minor metabolite.

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.

Thrall et al. (2004) developed a rat PBPK model for chlorobenzene in air using metabolic data derived from groups of F344 male rats exposed to chlorobenzene levels ranging from 82 to 6,750 ppm in air. Physiological values (e.g., breathing rate, organ volumes, etc.) were taken from the literature, and partition coefficients were determined from *in vitro* experiments with rat tissues and blood samples. The finished model was evaluated by using it to predict the chlorobenzene levels in exhaled breath of rats exposed by corn oil gavage (127 mg/kg) or intraperitoneal injection (131 mg/kg).

A PBPK model was developed to estimate the amount of 19 different VOCs that a nursing infant would receive from its occupationally-exposed mother (Fisher et al. 1997). In a simulation of a lactating woman exposed to the threshold limit value (TLV) concentration of chlorobenzene in air at the workplace, the amount of chlorobenzene transferred to a nursing infant from mother's milk was calculated to be 0.229 mg for a 10-kg infant.

3.1.6 Animal-to-Human Extrapolations

A number of differences between humans and various laboratory animal species preclude meaningful extrapolation from animals to humans. Compared to mice and rats, the rate of metabolism of chlorobenzene to soluble metabolites is higher in humans, and the formation of covalently bound products is lower (Nedelcheva et al. 1998). In addition, there is up to a 10-fold difference in the rate of metabolism of chlorobenzene in different human livers. There are also significant species and sex differences in the metabolism of chlorobenzene with markedly higher rates of oxidation in male mice than in male rats and female mice.

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 chlorobenzene are discussed in Section 5.7, Populations with Potentially High Exposures.

No information was located regarding potential differences in susceptibility to chlorobenzene.

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 (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 (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 chlorobenzene 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 chlorobenzene 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 (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 chlorobenzene 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

Levels of chlorobenzene and its metabolites have been measured in blood, urine, and exhaled air. Levels of 0.05–17 mg/L in the blood and 25–120 µg/L in the urine were detected in samples from residents living near a former toxic chemical dump, while trace amounts were found in exhaled air (Barkley et al. 1980). Yoshida et al. (1986) demonstrated linear correlations between urinary 4-chlorocatechol excretion and airborne exposure of workers to chlorobenzene. These authors suggested that the former might be an effective biomarker of exposure in humans.

Kumagai and Matsunaga (1994, 1995) also found that the major urinary metabolites of chlorobenzene in humans, including 4-chlorocatechol (especially) and 4-chlorophenol, are good biomarkers of recent exposure in workers. The slopes of the regression line for urinary metabolite concentration versus inhalation exposure concentration do appear to vary somewhat between studies, probably because of differences in workloads (active versus at rest) and patterns of exposure (acute versus chronic). Nevertheless, controlled chamber studies with workers have demonstrated that the concentrations of both major urinary metabolites of chlorobenzene correlate well with workers' 8-hour time-weighted average exposure to chlorobenzene and reflect variations in workplace exposure to chlorobenzene (Kumagai and Matsunaga 1995).

In an occupational study by Knecht and Woitowitz (2000), the major urinary metabolite of chlorobenzene was 4-chlorocatechol (74%). The remainder consisted of chlorophenol isomers of which 4-chlorophenol (13%) was the most abundant. In spite of its being <20% as abundant as 4-chlorocatechol, urinary 4-chlorophenol was still considered to be potentially useful as a biomarker of exposure due to its longer half-life (approximately 12 hours). The elimination half-life of urinary 4-chlorocatechol was 6.4 hours.

3.3.2 Biomarkers of Effect

There are no known biomarkers of effect that are specific to chlorobenzene exposure.

3.4 INTERACTIONS WITH OTHER CHEMICALS

In an attempt to identify the proposed epoxide intermediate of chlorobenzene, Oesch et al. (1973) co-administered the epoxide hydrase inhibitor, cyclohexane oxide, together with chlorobenzene to rats. Instead of increasing the toxicity of chlorobenzene as expected, through the inhibition of epoxide hydrase, cyclohexane oxide actually decreased the metabolism of chlorobenzene and its necrotic toxicity on the liver, suggesting that the metabolism of chlorobenzene is partially responsible for its liver toxicity.

In a mechanistic rat study, a chlorobenzene oral dose of 0.04 mL/180 g (approximately 246 mg/kg) caused extensive liver necrosis in rats pretreated with phenobarbital, but little or none in rats that were that were not pretreated with phenobarbital (Brodie et al. 1971). In another study, the severity of chlorobenzene-induced necrosis was decreased by pretreatment with the microsomal enzyme inhibitor, SKF 525A. The authors concluded that reactive metabolites of chlorobenzene that were formed in the liver may have subsequently reacted with tissue macromolecules (Brodie et al. 1971).