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

3.1 TOXICOKINETICS

No studies were located regarding the toxicokinetics of hexachlorobutadiene in humans, but there are limited data from studies in animals. These data are summarized below.

- Based on health effect and excretion data, hexachlorobutadiene is absorbed following inhalation, oral, or dermal exposure.
- Absorbed hexachlorobutadiene is distributed throughout the body with the highest concentrations found in the kidney, liver, and fat.
- The predominant pathway for hexachlorobutadiene metabolism is conjugation with glutathione in the liver and subsequent metabolism to form cysteine conjugates. The cysteine conjugates can be further metabolized by β-lyase to form reactive intermediates.
- The major route of excretion of hexachlorobutadiene is expiration of the parent compound or metabolite (carbon dioxide) through the lung, urinary excretion of metabolites and parent compound, and fecal excretion of parent compound and metabolites.

3.1.1 Absorption

No studies were located regarding absorption in humans or animals after inhalation exposure to hexachlorobutadiene. The occurrence of effects after exposure (de Ceaurriz et al. 1988; Gage 1970) indicate that absorption does occur.

No studies were located regarding absorption in humans after oral exposure to hexachlorobutadiene. There have also been no direct studies of absorption in animals although data on excretion and distribution provide information that suggests that absorption does occur from the gastrointestinal tract (Reichert et al. 1985). In animals, absorption is rapid and virtually complete at low doses of hexachlorobutadiene (1 mg/kg). At a higher dose (50 mg/kg), unmetabolized hexachlorobutadiene is found in the fecal matter (Reichert et al. 1985).

When Alderley Park rats were given a single dose of 200 mg/kg of radiolabeled hexachlorobutadiene and sacrificed at 2, 4, 8, and 16 hours, an autoradiogram of longitudinal sections of whole animals sacrificed 4 hours after dosing demonstrated that the label was concentrated in the intestines. The intestinal label was determined to be 85% unmodified, unabsorbed hexachlorobutadiene. At 8 hours, the intestinal

concentration of the label was no longer apparent as hexachlorobutadiene was absorbed and distributed to the tissues (Nash et al. 1984).

Most of the data pertaining to oral administration of hexachlorobutadiene utilized triglycerides (corn oil or tricaprylin) as a gavage dosing medium. Because of its high lipophilicity and low water solubility, it is likely that the absorption of hexachlorobutadiene from an aqueous solution would differ from that from a triglyceride media. When 1 mg/kg hexachlorobutadiene in tricaprylin was administered to female Wistar rats, 30.61% was excreted in the urine over 72 hours (Reichert et al. 1985), while when the same dose in aqueous polyethylene glycol solution was given to male Sprague Dawley rats, only 18% was in the urine (Payan et al. 1991). These data suggest that absorption from the lipid solvent was greater than that with the aqueous solvent.

No studies were located regarding absorption in humans after dermal exposure to hexachlorobutadiene. In animals, pure hexachlorobutadiene (388–1,550 mg/kg) applied to the skin of rabbits was completely absorbed in 8 hours (Duprat and Gradiski 1978).

3.1.2 Distribution

Hexachlorobutadiene has been identified in samples of human adipose tissue (Mes et al. 1985). The tissue samples were obtained from cadavers and, thus, no data were available pertaining to exposure.

No studies were located regarding distribution in humans after oral exposure to hexachlorobutadiene. In animals, 5–14 % of ^{14}C-hexachlorobutadiene was retained in the tissues and carcass 72 hours after compound administration (Dekant et al. 1988a; Reichert et al. 1985). The kidney (outer medulla), liver, and adipose tissue appeared to concentrate hexachlorobutadiene label when single doses of up to 200 mg/kg ^{14}C-hexachlorobutadiene in corn oil were administered by gavage (Dekant et al. 1988a; Nash et al. 1984; Reichert et al. 1985). In one report, the brain was also determined to contain a relatively high concentration of label 72 hours after exposure (Reichert et al. 1985). Label in the kidney 72 hours after exposure was more extensively covalently bound to proteins than that in the liver (Reichert et al. 1985).

Levels of label in the liver, kidney, and plasma were determined for the donor and recipient rats when secretions from bile duct cannulated donor rats given a dose of 100 mg/kg hexachlorobutadiene were infused directly into the bile duct of nonexposed recipient rats, and thereby into their intestines (Payan et al. 1991). In the donor rats, after 30 hours, the kidney contained 0.26% of the dose, the liver contained

0.11%, and the plasma contained 0.013% from the intestinally absorbed material. In the recipient rats, the kidney contained 0.15% of the dose, the liver contained 0.97%, and the plasma contained 0.009% from the resorbed biliary metabolites. For each tissue, the level of label from resorbed metabolites was about two-thirds of that from the original dose. The kidneys contained more of the label than the liver in both instances, clearly identifying the kidneys as a target organ for distribution. This is consistent with studies showing that the kidney is the most sensitive organ of hexachlorobutadiene toxicity.

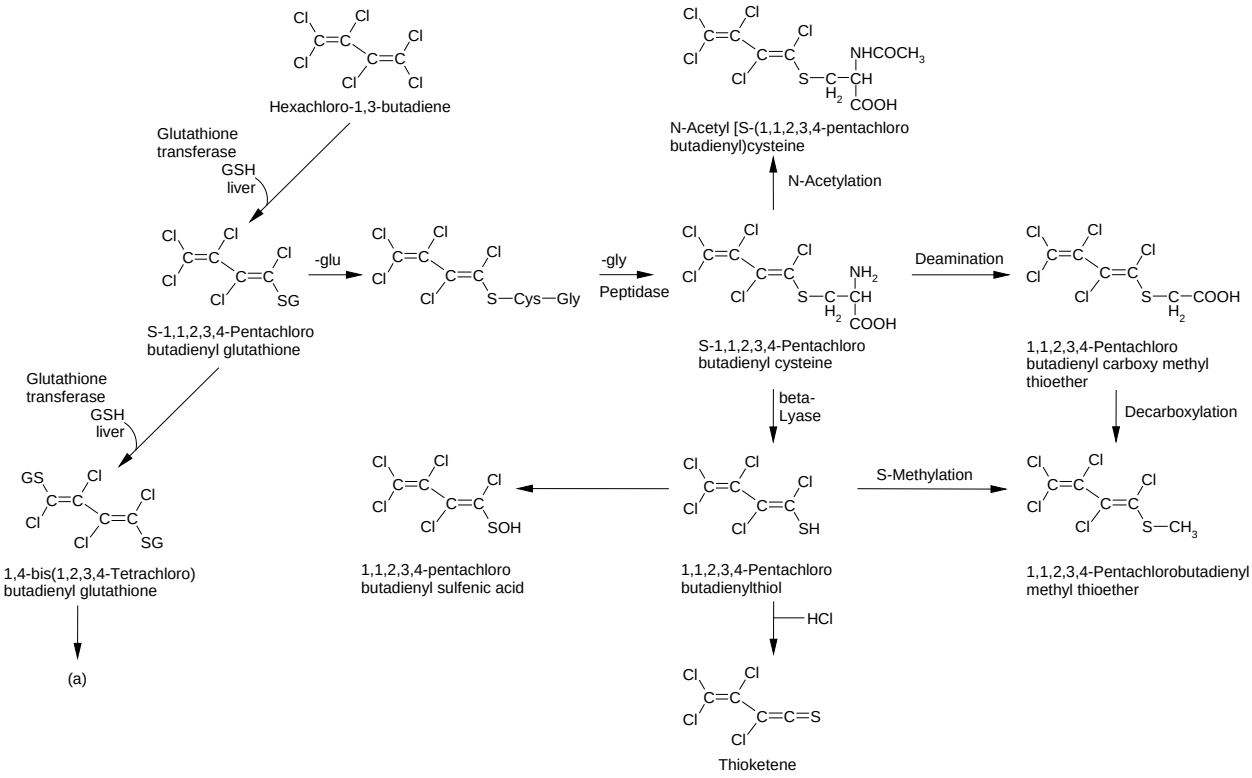
In a study using doses of 0.1 and 300 mg/kg intraperitoneally-administered radiolabeled hexachlorobutadiene, the label was found in the liver, kidney, and adipose tissue. Very little of the label was found in the brain, lung, heart, and muscle tissue at 48 hours after dosing (Davis et al. 1980). The reported levels in the brain in this study differ from those reported at 72 hours following oral administration (Reichert et al. 1985). This may indicate that there is a gradual deposition of labeled hexachlorobutadiene and/or its metabolites in the brain lipids over time.

3.1.3 Metabolism

There is a considerable amount of information available concerning the metabolism of hexachlorobutadiene in animals. Figure 3-1 presents a proposed metabolic pathway for hexachlorobutadiene. This pathway is based on the metabolites identified in urine and bile using chromatographic techniques.

Most of the absorbed hexachlorobutadiene is transported via the portal circulation to the liver where it is conjugated with glutathione (Garle and Fry 1989). In rat livers, both mono- and di-substituted conjugates have been identified (Jones et al. 1985), whereas mice appear to produce only the monosubstituted conjugate (Dekant et al. 1988a). There was a dose-related decrease in hepatic levels of glutathione following exposure to hexachlorobutadiene, and pretreatment of experimental animals with agents that interfere with glutathione synthesis or conjugation reactions decreased the amount of glutathione conjugate that can be synthesized (Gietl and Anders 1991). There appears to be no oxidation of the hexachlorobutadiene by the mixed function oxidase system enzymes prior to conjugation (Garle and Fry 1989).

Figure 3-1. Proposed Pathways for Hexachlorobutadiene Metabolism



(a) = metabolism parallels that for the monosubstituted compound; glu = glutamic acid; gly = glycine; GSH = glutathione

Sources: Dekant et al. 1991; Jaffe et al. 1983; Jones et al. 1985; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985; Wild et al. 1986; Wolf et al. 1984

The glutathione conjugate (S-(1,2,3,4,4-pentachlorobutadienyl)glutathione, PCBG) is excreted with the bile into the intestinal tract. A portion of the material is hydrolyzed with the removal of glutamate or glutamate and glycine from the glutathione tripeptide to form the cysteine derivative [S-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine, PCBC] or the cysteinylglycine derivative (Gietl and Anders 1991; Gietl et al. 1991; Nash et al. 1984). In one study, the glutathione conjugate accounted for 40% of the label in the bile and the cysteine derivative for 15% of the label. Another 45% of the label was present as unidentified compounds (Nash et al. 1984).

The conversion of the glutathione conjugate to its cysteinyl derivative is mediated, at least in part, by enzymes in the intestinal epithelial cells. PCBG and PCBC are partially reabsorbed from the intestines and transported to the liver and subsequently to the body tissues (Gietl et al. 1991). Only a portion of the reabsorbed material is taken up by the liver for additional metabolism. When liver uptake of the glutathione conjugate was measured using perfused rat livers, the maximum uptake observed was 39%

(Koob and Dekant 1992). A portion of this material was re-excreted in bile without any metabolic modification.

The cysteine conjugate, acetylated cysteine conjugate, and six bis-substituted metabolites were synthesized from the glutathione conjugate and excreted in bile. Two of the bis-substituted metabolites were identified as the bis-1,4-glutathione conjugate and the bis-1,4-cysteine conjugate. The cysteine conjugate was taken up by the liver to a greater extent than the glutathione conjugate (Koob and Dekant 1992). Up to 79% of the cysteine conjugate was absorbed, but this metabolite appeared to be toxic to the liver and caused decreased bile flow within 20 minutes. There were only small portions of the cysteine derivative and acetylated cysteine derivative in the bile. Bis-substituted derivatives, including the 1-cysteinyl-4-glutathionyl tetrachlorobutadiene, bis-1,4-cysteinyl tetrachlorobutadiene, and 1-cysteinyl-4-cysteinyl glycine tetrachlorobutadiene, were formed.

Additional processing of the hexachlorobutadiene metabolites produces the compounds identified in the urine (1,1,2,3-tetrachlorobutenoic acid, 1,1,2,3,4-pentachloro-1:3-butadienyl sulfenic acid, N-acetyl-S-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine, S-1,1,2,3,4-pentachlorobutadienylmercaptoacetic acid, 1,1,2,3,4-pentachlorobutadiene methylthioether, and 1,1,2,3,4-pentachlorobutadiene carboxymethylthio-ether) (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). Birner et al. (1995) also demonstrated that N-acetyl-S-1, 1,2,3,4-pentachlorobutadienyl-L-cysteine could be further metabolized to form N-acetyl-S-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine sulfoxide, but that may only occur in male rats; the metabolite was not detected in female rat urine.

A very small portion of the absorbed hexachlorobutadiene is oxidized to carbon dioxide. This pathway can be saturated since an increase in the hexachlorobutadiene dose does not cause a corresponding increase in excretion of labeled carbon dioxide (Dekant et al. 1988b; Payan et al. 1991; Reichert et al. 1985).

Green et al. (2003) compared the *in vitro* metabolism of hexachlorobutadiene in human and rat tissues and found higher metabolic rates and affinity constants in rat tissues than in human tissues (Table 3-1). The ratios (rat:human) of the V_{max} values ranged from 2.4 to 73.6. The investigators developed a physiologically based pharmacokinetic (PBPK) model using these data, as well as other published pharmacokinetic modeling parameters (see Section 3.1.5 for more information on the model); the model predicted that humans would need to be exposed to a 10-fold higher hexachlorobutadiene air concentration than rats to obtain the same β-lyase metabolite levels as found in rats.

Table 3-1. Metabolic Rate (V_{max}) and Affinity Constant (K_m) of Liver and Kidney Enzymes Measured in Human and Rat Tissues (*In Vitro*)

	V_{max} (nmol/minute/mg)			K_m (mM)		
Enzyme	Rat	Human[a]	Ratio	Rat	Human[a]	Ratio
Glutathione S-transferase (liver microsomes)	1.23	0.25	4.9	0.21	0.16	1.3
β-lyase (kidney cytosol)	1.13	<0.05[b]	22.6[c]	0.3	–	–
β-lyase (kidney mitochondria)	4.17	1.76	2.4	0.12	0.25	0.48
Cysteine N-acetyl transferase (kidney microsomes)	144.9	37.6	3.8	0.39	0.19	2.0
Acylase (kidney cytosol)	7.36	<0.10[b]	73.6[c]	1.43	–	–

[a]Mean of results from 3–6 tissue samples.
[b]No measurable rate, limit of detection shown.
[c]Calculated using the limit of detection for humans.

Source: Green et al. 2003

3.1.4 Excretion

In animals, hexachlorobutadiene and its metabolites are excreted in exhaled air, urine, and feces. In studies where radiolabeled (^{14}C) hexachlorobutadiene was administered at doses of 1, 30, 50, or 100 mg/kg, 4–8% of the dose was removed from the body in the exhaled air as unmetabolized hexachlorobutadiene and carbon dioxide within the 72 hours after compound administration (Dekant et al. 1988b; Payan et al. 1991; Reichert et al. 1985). In another study, 1.1 and 2.0% of ^{14}C-hexachlorobutadiene was excreted in exhaled air 48 hours after a single dose of 200 mg/kg in male and female rats, respectively (Birner et al. 1995); 0.02 and 0.03% of the dose was exhaled as carbon dioxide.

With single doses ranging from 1 to 200 mg/kg ^{14}C-hexachlorobutadiene, the percent of the label in the urine ranged from 3.1 to 30.6%, with the highest percentage associated with the lowest dose (Birner et al. 1995; Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). These data suggest that the absorption or excretion may be saturable. At the higher doses, urinary excretion values of 5–10% were common (Nash et al. 1984; Reichert and Schutz 1986). Some of the hexachlorobutadiene label excreted in the urine originates from the biliary metabolites reabsorbed from the intestinal tract and processed by the kidneys for excretion. A study of bile duct cannulated rats and noncannulated rats estimated the contribution of reabsorbed biliary metabolites to urinary excretion. When a dose of 1 mg/kg hexachlorobutadiene in polyethylene glycol solution was given to bile duct cannulated male rats,

the urine contained 11% of the label after 72 hours; in noncannulated rats given the same dose, it contained 18 % of the label (Payan et al. 1991). When a dose of 100 mg/kg was given, the urine of the cannulated rats contained 7% of the label and the urine of the noncannulated rats contained 9% after 72 hours.

Metabolites identified in the urine include: S-(1,1,2,3,4-pentachlorobutadienyl)glutathione; S-(1,1,2,3,4-pentachlorobutadienyl) cysteine; 1,1,2,3-tetrachlorobutenoic acid; 1,1,2,3,4-pentachloro-1: 3-butadienyl sulfenic acid; N-acetyl-S- 1,1,2,3,4-pentachlorobutadienyl)-L-cysteine; S-pentachloro-butadienylmercaptoacetic acid; 1,1,2,3,4-pentachlorobutadiene methylthioether; and 1,1,2,3,4-penta-chlorobutadiene carboxymethylthioether (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). Birner et al. (1995) found sex-related differences in the identification and quantification of urinary metabolites between male and female rats administered a single 200 mg/kg gavage dose of hexachlorobutadiene. In females, the primary urinary metabolite was N-ac-PCBC; radiolabel was also detected as part of the minor urinary metabolite, PCBC. In males, N-ac-PCBC and PCBC were minor metabolites and the radiolabel was primarily detected as unchanged hexachloro-butadiene and as N-acetyl-S-(pentachlorobutadienyl)-L-cysteine sulfoxide.

Fecal excretions contained unmetabolized, unabsorbed hexachlorobutadiene plus a portion of the hepatic metabolites excreted with the bile. At the lower doses, almost all of the label in the feces originated with the biliary metabolites, whereas at the higher doses, there was also some unabsorbed hexachlorobutadiene in the fecal matter (Dekant et al. 1988b). In rats given 200 mg/kg, feces collected during the 5-day period contained a total of 39% of the dose. Only 5% was excreted in the first 2 days after dosing. In another study, the feces and contents of the gastrointestinal tract contained 62% of a 1 mg/kg dose and 72% of a 100 mg/kg dose (Payan et al. 1991). A third study found that 15.6 and 11.1% of ^{14}C-hexachlorobutadiene label was excreted in the feces during the 2-day period following a gavage dose of 200 mg/kg in male and female rats (Birner et al. 1995). The only metabolite that had been identified in the feces was S-(1,1,2,3,4-pentachlorobutadienyl) glutathione (Dekant et al. 1988b), although unidentified metabolites were also present and most likely included the cysteine derivatives. Similarly, Birner et al. (1995) reported the radiolabel in the feces represented both unmetabolized hexachlorobutadiene and S-conjugates.

In one study where a single 200 mg/kg dose was given to rats by gavage, 35% of the label was found in the bile in the first 2 days after dosing. The biliary label was equally distributed over the 2 days of collection. In a different study, 66% of a 1 mg/kg dose was excreted in the bile of bile duct cannulated

rats in 72 hours and 58% of a 100 mg/kg dose (Payan et al. 1991). Secretions from bile duct cannulated rats given a dose of 100 mg/kg hexachlorobutadiene were infused directly into the bile duct of nonexposed rats (Payan et al. 1991). The levels of label in the urine, bile, and feces of both the donor and recipient rats were measured 30 hours after dosing. The label in the urine and bile of the recipient rats represented label that was reabsorbed from the gastrointestinal tract. It was determined that 80% of the biliary metabolites were reabsorbed and only 20% remained in the feces and gastrointestinal tract.

The distribution of radiolabel in excreta was measured in male rats for the 72-hour period after intravenous administration of doses of 1 or 100 mg/kg (Payan et al. 1991). At both doses, about 8% of the radiolabel was exhaled. The amount of label in the urine was 21% of the low dose and 9% of the high dose; the amount in the feces was 59% of the low dose and 72% of the high dose. In a parallel study, the fecal, urinary, and biliary excretions were measured for rats with cannulated bile ducts. The urine contained 6–7 % of the dose and the feces <0.5 % for both doses. The bile contained 89% of the 1 mg/kg dose and 72% of the 100 mg/kg dose.

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.

Green et al. (2003) developed a PBPK model for hexachlorobutadiene based on an inhalation PBPK model for styrene with an added kidney compartment. The model was designed to estimate an inhalation concentration that would result in the same body burden as an oral dose of 0.2 mg/kg/day (NOAEL for renal toxicity). The renal concentration of β-lyase metabolites was then estimated for the inhalation concentration. Lastly, an inhalation concentration that would result in the same concentration of β-lyase metabolites in humans was estimated. The model used published partition coefficients and physiological parameters, and enzyme metabolic rates and affinity constants were calculated using *in vitro* human and rat liver and kidney samples. The model predicted that the level of β-lyase metabolites associated with an

oral dose of 0.2 mg/kg/day (137.7 mg/L) would be equivalent to an inhalation concentration of 0.07 ppm for rats. In humans, an inhalation concentration of 1.41 ppm would be needed to obtain 137.7 mg/L of β-lyase metabolites. The results of this model should be interpreted cautiously since the model was constructed using limited available data and has not been validated in humans or rats.

3.1.6 Animal-to-Human Extrapolations

The results of an *in vitro* study suggest differences in hexachlorobutadiene metabolism between rats and humans (Green et al. 2003) and predicted that 10 times less β-lyase metabolites are formed in humans than rats at a given air concentration. However, the study did not investigate whether this would result in differences in hexachlorobutadiene toxicity between the species. The limited available toxicity data in humans do not allow for a comparison with experimental animal species. Based on the toxicity studies presented in Table 2-2, rats and mice appear to have similar targets of toxicity, with the kidney being the most sensitive target. Although direct comparisons of dose-response curves in rats and mice are not possible due to differences in exposure durations, subroutes of exposure (gavage versus diet), and range of doses tested, the available data do not appear to suggest large differences in the sensitivity of rats and mice to the renal toxicity of hexachlorobutadiene.

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

Studies in animals revealed that hexachlorobutadiene causes damage to the proximal tubules of the kidney. Accordingly, people with preexisting kidney damage may have compromised organ functions and are expected to be more vulnerable to chemical insult than people with normal kidney function. This is supported by a study that found that compromised glomerular function increased the renal toxicity of hexachlorobutadiene (Kirby and Bach 1995). Pre-treatment with Adriamycin, which induces glomerular damage, resulted in an increase in the severity of the hexachlorobutadiene-induced damage to the proximal tubules.

Several studies have evaluated potential age-related differences in the toxicity of hexachlorobutadiene. Kociba et al. (1977) reported much lower LD_{50} values in weanling rats (65 mg/kg in males) as compared to adult rats (580 mg/kg in males). Another study which examined rats aged 1, 3, 6, 9, and 12 months did not find any differences in the renal response to an intraperitoneal injection of 100 mg/kg hexachlorobutadiene (Zanetti et al. 2010). Studies examining the developmental toxicity of hexachlorobutadiene reported decreases in fetal or pup body weights (Harleman and Seinen 1979; Saillenfait et al. 1989; Schwetz et al. 1977), but did not find increases in anomalies or malformations.

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 hexachlorobutadiene 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 hexachlorobutadiene 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 hexachlorobutadiene 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

Human exposure to hexachlorobutadiene can be determined by measuring the parent compound in blood and adipose tissue (Bristol et al. 1982; Mes et al. 1985). Data in animals are limited, but do suggest that hexachlorobutadiene can be detected in urine and exhaled air. Approximately 4–31% of the administered radioactivity was detected in the urine of mice or rats within 72 hours following the administration of single oral doses of ^{14}C-hexachlorobutadiene (1–200 mg/kg) (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). No information was located on how long before it can no longer be detected. Unmetabolized hexachlorobutadiene was detected in exhaled air after animals were given doses of 1–100 mg/kg (Dekant et al. 1988b; Payan et al. 1991; Reichert et al. 1985). Cysteine conjugates of hexachlorobutadiene are converted to thio derivatives (e.g., 1,1,2,3,4-pentachlorobutadiene methylthioether and 1,1,2,3,4-pentachlorobutadiene carboxy methylthioether), which have been detected in urine (Reichert et al. 1985). Accordingly, tests to determine concentrations of these sulfur derivatives in urine may be useful in determining if exposure to hexachlorobutadiene has occurred.

3.3.2 Biomarkers of Effect

Data are sparse regarding biomarkers of the effects of hexachlorobutadiene in humans. Workers chronically exposed to the compound (along with carbon tetrachloride and perchloroethylene) had increased serum bile acids (Driscoll et al. 1992). Because the workers were also exposed to other chemicals, effects reported cannot be attributed to hexachlorobutadiene alone.

As discussed in Chapter 2, renal damage is the primary toxic effect associated with exposure to hexachlorobutadiene in animals (Harleman and Seinen 1979; Kociba et al. 1971, 1977; NTP 1991; Schwetz et al. 1977). Several studies have investigated the reliability of biomarkers to assess hexachlorobutadiene-induced renal toxicity in rats receiving a single intraperitoneal injection of hexachlorobutadiene (Maguire et al. 2013; Swain et al. 2011, 2012). These biomarkers are not specific to hexachlorobutadiene and may be altered due other causes of renal damage. These studies identify urinary α-glutathione S-transferase (α-GST) and albumin as the most sensitive biomarkers of early renal damage. In a study testing multiple dose levels (Swain et al. 2012), the lowest dose causing minimal proximal tubular damage also resulted in significant increases in urinary α-GST levels (approximately 300% higher than controls). The urinary α-GST level increase followed a dose-related pattern; at the highest dose tested (90 mg/kg), which caused very marked degeneration, α-GST levels were approximately 65,000 times higher than in controls. Albumin levels were not increased at the lowest adverse dose level; however, the magnitude of the increase at higher doses was greater than other biomarkers examined. In addition to α-GST and albumin, urinary levels of glucose, kidney molecule-1 (KIM-1), β-hydroxy-butyrate, osteopontin, and clusterin were found to be sensitive biomarkers of renal damage (Maguire et al. 2013; Swain et al. 2012); with the exception of β-hydroxybutyrate, increases in these biomarkers were not observed at doses resulting in minimal proximal tubular damage (Swain et al. 2012). β-Hydroxybutyrate levels were increased at the lowest dose tested, which did not result in histological alterations (Swain et al. 2012). KIM-1 and clusterin levels appeared to be reliable biomarkers of the regeneration and repair that occurred several days after exposure (Maguire et al. 2013; Swain et al. 2011, 2012). In contrast to the urinary biomarkers, plasma biomarkers appeared to be relatively insensitive. Increases in plasma creatinine and urea levels were only observed at doses resulting in marked tubular degeneration (Swain et al. 2012).

3.4 INTERACTIONS WITH OTHER CHEMICALS

Several studies have been conducted to assess factors which influence the toxicity of hexachloro butadiene. Most of these studies have involved effects of mixed function oxidase activity (MFO) on renal toxicity. The administration of MFO inhibitors, including SKF-525A (Lock and Ishmael 1981) and piperonyl butoxide (Davis 1984; Hook et al. 1982), did not alter hexachlorobutadiene-induced renal damage. Similar results were reported in tests evaluating MFO inducers such as phenobarbital (Lock and Ishmael 1981), β-naphthoflavone, isosafrole, and Aroclor 1254 (Hook et al. 1982). Renal toxicity was not exacerbated by prior exposure to ketonic solvents (Hewitt and Brown 1984).

There are reports of interactions of hexachlorobutadiene with other chemicals. Combined administration of minimally toxic doses of hexachlorobutadiene with mercuric chloride and potassium dichromate for 24 hours caused synergistic effects as evident by marked increases in urinary (6–24 hours) alkaline phosphatase, lactate dehydrogenase, and NAG activities, as well as more severe tubular necrosis than caused by treatment with hexachlorobutadiene alone (Jonker et al. 1993a). Antagonistic effects were evident as characterized by smaller increases in urinary γ-glutamyl transferase activity compared to treatment with hexachlorobutadiene alone. Combined administration of the same chemicals did not cause additive interactions regarding biochemical parameters or histopathological changes in the kidney (Jonker et al. 1993a). An additional study revealed that when animals are treated for 4 weeks with minimally toxic doses of hexachlorobutadiene in combination with other chemicals (mercuric chloride, δ-limonene, and lysinoalanine), there is an increase in growth retardation and renal toxicity (renal weight, urine concentrating ability, and renal structure) in male rats, but not in female rats (Jonker et al. 1993b).