

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

### 3.1 TOXICOKINETICS

**Mirex.** Mirex is absorbed from the digestive tract of animals. Following exposure to mirex, an initial rapid excretion of the majority of the ingested mirex occurs via the feces within the first 48 hours postdosing. This fecal mirex represents unabsorbed compound. Once absorbed, mirex is widely distributed throughout the body, but is sequestered in the fat. It has a long retention time in the body. Mirex is not metabolized in humans, rodents, cows, or minipigs. The parent compound is the only radiolabeled compound that has been found in the plasma, fat, and feces. In animals, mirex is excreted unchanged mainly in the feces; urinary excretion is negligible. Mirex is also excreted in human milk. Only a very limited number of studies were located regarding the toxicokinetics of mirex via inhalation or dermal routes. Limited data indicate that mirex is absorbed by rats following exposure to the compound in cigarette smoke.

**Chlordecone.** Occupational studies indicate that chlordecone is absorbed via the inhalation and oral routes. Chlordecone is readily absorbed from the gastrointestinal tract of humans and animals. Chlordecone is widely distributed throughout the body and concentrates in the liver of humans and animals. It has a long retention time in the body. Chlordecone is metabolized to chlordecone alcohol in humans, gerbils, and pigs. Rats, guinea pigs, and hamsters cannot convert chlordecone to chlordecone alcohol. Chlordecone, chlordecone alcohol, and their glucuronide conjugates are slowly excreted in the bile and eliminated in the feces. However, a substantial enterohepatic recirculation of chlordecone exists that curtails its excretion in the feces. Chlordecone is also excreted in saliva and mother's milk. Only a very limited number of studies were located regarding the toxicokinetics of chlordecone via inhalation or dermal routes. Occupational studies indicate that chlordecone can be absorbed via inhalation and oral routes. Limited animal data indicate that dermal absorption of chlordecone is low.

#### 3.1.1 Absorption

**Mirex.** Very limited data show that inhaled mirex can be rapidly absorbed into the blood of rats (Atallah and Dorough 1975; Dorough and Atallah 1975). The fate of [<sup>14</sup>C] mirex in cigarette smoke was assessed in rats with the aid of a smoking device (Atallah and Dorough 1975; Dorough and Atallah 1975). Eight 5-mL puffs were administered to the trachea of rats at 15-second intervals. At 2–4 minutes after

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inhalation, 47% of the radiolabel was exhaled, 36% was found in the lung, 11% was found in the blood, and 1% was found in the heart.

Several studies in rats indicate that mirex is absorbed from the digestive tract (Byrd et al. 1982; Gibson et al. 1972; Mehendale et al. 1972). Experiments with rats given single oral doses of mirex ranging from 0.2 to 10 mg/kg showed that an initial rapid excretion of mirex occurs in the feces within the first 48 hours post-dosing (Byrd et al. 1982; Gibson et al. 1972; Mehendale et al. 1972). The excretion of mirex in the feces within this time period is attributed to unabsorbed mirex. A majority (85–94%) of the total quantity excreted after 7 days is eliminated in this first rapid excretion phase (Gibson et al. 1972; Mehendale et al. 1972). Other data provided an absorption estimate of 69%, which occurred with female rats given a single oral dose of 10 mg/kg (Byrd et al. 1982). Similarly, most of the fecal mirex was recovered within the first 48 hours. This was attributed to the elimination of unabsorbed mirex (Byrd et al. 1982). Intestinal absorption of mirex was slightly decreased by the presence of an existing body burden (Gibson et al. 1972). For example, rats fed 12.5 mg/kg of unlabeled mirex before administration of a single dose (0.2 mg/kg) of mirex excreted 25% of the administered dose in the feces, as compared with 18% excretion for the animals given only a single dose (Gibson et al. 1972).

Mirex is rapidly absorbed by rats and monkeys. Peak plasma concentrations of <sup>14</sup>C-mirex occurred within 4–7 hours after female rats were given a single oral dose of 10 mg/kg (Byrd et al. 1982) and within 2 hours after male rats were administered a single oral dose of 100 mg/kg (Brown and Yarbrough 1988). <sup>14</sup>C-Mirex levels in plasma peaked 5 hours after oral administration of 1 mg/kg to a female rhesus monkey (Wiener et al. 1976). Thereafter, the decline in plasma <sup>14</sup>C concentration continued at a much slower rate and paralleled that in the intravenously-dosed monkeys (Wiener et al. 1976).

Mirex rapidly entered the maternal bloodstream of pregnant rats dosed orally with 5 mg/kg on gestation days 15, 18, or 20 (Kavlock et al. 1980). Four hours after oral dosing on gestation day 15, the plasma concentration of mirex was 13 ppm. Mirex plasma concentrations were significantly affected by both the time of administration and the hour of observation. Higher plasma concentrations were found at older gestation ages (13 ppm on gestation day 15, compared to 23 ppm on gestation day 20; measured 4 hours after administration). Plasma concentrations declined with time after dosing (Kavlock et al. 1980).

Mirex concentrations in plasma of pregnant goats fed daily doses of 1 mg/kg for 61 weeks stabilized after 15 weeks (Smrek et al. 1977). An increase in the dose from 1 to 10 mg/kg at the end of the study resulted in an increase in the plasma level of mirex. Females dosed for 18 weeks starting at the first day

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postpartum had plasma levels that were similar to females that were started on mirex in early pregnancy (Smrek et al. 1977).

**Chlordecone.** Chlordecone is absorbed after occupational exposure; however, due to extremely poor workplace hygiene in available sources of human data, relative contributions from inhalation, oral, and dermal exposure routes are not available (Cannon et al. 1978; Cohn et al. 1978; Taylor 1982, 1985). Mean blood levels of workers exposed to chlordecone at a manufacturing plant in Hopewell, Virginia were 2.53 ppm for workers manifesting illness (nervousness or unfounded anxiety; pleuritic chest pain; weight loss of up to 60 pounds in 4 months; visual difficulties; skin rashes of an erythematous, macropapular nature) and 0.6 ppm for workers with no illness (Cannon et al. 1978). Two months following cessation of exposure, blood levels in workers were in excess of 2 ppm (Taylor 1982, 1985). Following exposure in humans, mean half-lives of 96 days (range of 63–148 days) (Adir et al. 1978) and 165 days (Cohn et al. 1978) in blood have been reported for chlordecone. This relatively long half-life may be due to the high degree of lipid solubility and limited metabolism of chlordecone.

Chlordecone is readily absorbed (90%) from the gastrointestinal tract of rodents and has a long half-life (Egle et al. 1978). In rats exposed to a single oral dose of 40 mg/kg chlordecone, the blood half-lives at 4, 8, and 14 weeks posttreatment were 8.5, 24, and 45 days, respectively (Egle et al. 1978). Chlordecone is also rapidly absorbed by pregnant rats (Kavlock et al. 1980). Four hours after dosing (5 mg/kg) on gestation day 15, the plasma concentration of chlordecone was 6 ppm.

Chlordecone is absorbed to a limited extent following dermal exposure in rats (Hall et al. 1988; Shah et al. 1987). The percent of dose absorbed was determined by dividing the radioactivity in the body (carcass) and in the excreta by the total radioactivity recovered (in carcass, excreta, treated skin, and washes of the application materials). The results showed that fractional absorption decreased as the dose of chlordecone increased. At 72 hours after exposure to 0.29, 0.54, or 2.68  $\mu\text{mol } ^{14}\text{C}$ -chlordecone/cm<sup>2</sup>, skin penetration of chlordecone in young rats was 10.17, 7.23, and 1.93%, respectively, of the applied dose. Skin penetration of chlordecone in adult rats at 72 hours was 9.2, 5.96, and 1.03% for the low-, middle-, and high-dose groups, respectively. The area of application when expressed as the percentage of the total surface area ( $\approx 2.3\%$ ) was the same in both young and adult rats. The actual amount of chlordecone absorbed (0.03 pmol/cm<sup>2</sup>) was similar for all dose groups, suggesting that saturation occurred at the low dose. No significant age-dependent differences in dermal absorption were seen.

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**3.1.2 Distribution**

**Mirex.** Mirex has been detected in a variety of human samples. Mirex levels of 0.16–5.94 and 0.3–1.13 ppm (males and females, respectively) were found in adipose tissue samples taken either from postmortem examinations or during surgery (Kutz et al. 1974). The adipose tissue samples came from individuals who lived in areas in which mirex was used extensively in a program to control fire ants. Adipose tissue levels of mirex ranging from 0.03 to 3.72 ppm have been found in residents living near a dump site in Tennessee (Burse et al. 1989). Mirex has also been detected in human serum samples (e.g., Butler Walker et al. 2003; Fenster et al. 2006; Greizerstein et al. 1999; Schell et al. 2003; van Oostdam et al. 2004), milk samples from lactating women (Fitzgerald et al. 2001; Greizerstein et al. 1999; Mes et al. 1978; Newsome and Ryan 1999; Newsome et al. 1995), and placental tissue and umbilical cord blood (Butler Walker et al. 2003; Lopez-Espinosa et al. 2007).

Only very limited animal data were located regarding the distribution of absorbed mirex following inhalation exposure. Mirex was found in the lungs (36%), blood (11%), and hearts (1%) of rats exposed to mirex in cigarette smoke (Atallah and Dorough 1975; Dorough and Atallah 1975).

Following oral dosing in animals, mirex is distributed to various tissues and sequestered in fat. Females generally accumulated greater amounts than males. Mirex demonstrated an affinity for lipids in male and female rats given a single oral dose of mirex (0.2 mg/kg); highest concentrations were found in fat (Chambers et al. 1982; Gibson et al. 1972). The levels in fat of females were approximately 2 times higher than levels in fat of males (Chambers et al. 1982). For females, mirex levels in the fat ranged from 338 to 944 ng/g at 7 days and increased to 483–1,043 ng/g at 14 days. For males, mirex levels in fat ranged from 161 to 479 ng/g at 7 days and from 419 to 530 ng/g at 14 days. Mirex also accumulated in nervous tissue, with females accumulating higher amounts than males (Chambers et al. 1982). Mirex concentrations in the nervous tissue in males and females at 7 days posttreatment were 13.228 ng/g and 40–59 ng/g, respectively; concentrations declined during posttreatment days 7–14. Mirex accumulated in various other tissues of both males and females, including gastrointestinal tract, liver, lung, heart, kidney, adrenals, brain, skeletal muscle, spleen, and thymus (Chambers et al. 1982; Gibson et al. 1972).

Seven days after a single administration of mirex (6 mg/kg) to rats, 34% of the total dose was retained in the tissues and organs; 27.8% was stored in the fat, 3.2% was stored in the muscle, and 1.75% was stored in the liver (Mehendale et al. 1972). The remaining tissues each retained <1% of the total dose. No metabolite of mirex was detected in the tissues. The repetitive administration of 10 mg/kg mirex to rats

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resulted in an accumulation of mirex in several tissues (plasma, liver, kidney, fat), with more accumulating in fat tissue (Plaa et al. 1987). Following oral administration of 1 mg/kg <sup>14</sup>C-mirex to a female rhesus monkey, the highest tissue levels of radioactivity were found in fat, followed by large intestine, adrenal glands, liver, ovaries, and peripheral nerves (Wiener et al. 1976). The administered dose of radioactivity was distributed as follows: 55.3% was recovered in fat and ≤2% was recovered in the remaining tissues. Mirex was the only labeled compound identified in fat. Mirex fed to minipigs for 7 consecutive days (3–4.5 mg/kg/day) was distributed to backfat (41.5 ppm), liver (1.24 ppm), kidney (0.44 ppm), plasma (0.04 ppm), and red blood cells (0.01 ppm) at 9 days after dosing (Morgan et al. 1979).

Mirex was detected in brains from male rats within 0.5–2 hours after a single oral dose of 100 mg/kg mirex (Brown and Yarbrough 1988). By 96 hours, the following concentrations (in μmol <sup>14</sup>C-mirex/g) were measured in the brain regions: cerebral cortex (0.47), cerebellum (0.50), brain stem (0.73), and spinal cord (0.75). Mirex was also distributed to the liver, kidneys, testes, and omental fat. Peak tissue concentrations of mirex in the kidneys, testes, liver, and omental fat occurred 12, 48, 48, and 96 hours postdosing, respectively. Following a single oral dose of 50 mg/kg mirex to mice, mirex was distributed to the brain; mirex levels in the striatum and medulla/pons were significantly higher than in the cortex, midbrain, or cerebellum at 48 hours postdosing (Fujimori et al. 1982a). However, at 6, 12, and 96 hours postdosing, discrete brain area levels of mirex did not differ significantly. Mirex levels in whole brain and plasma were 3–40 times lower than levels found in chlordecone-treated mice, and mirex showed less-specific distribution in discrete areas of the brain than did chlordecone (Fujimori et al. 1982a). Samples of brain tissue from rats fed 0, 0.089, or 0.89 mg mirex/kg/day for 34–49 days showed that mirex accumulates in rat brain tissue in a dose-dependent manner; mirex levels in brain tissue were 7–8 times higher in the high-dose group than in the low-dose group (Thorne et al. 1978).

Mirex accumulates in maternal tissues, readily crosses the placenta of animals, and accumulates in fetal tissues. Maximum concentrations of mirex found in the placenta of rats ranged from 3.5 to 4 ppm at 4 hours postdosing (Kavlock et al. 1980). Mirex levels in the placenta at 48 hours postdosing were <50% of the 4-hour level. The uptake of mirex by fetal organs was in the order of liver > brain = heart > kidney in a ratio of 3:2:2:1. Fetal mirex concentrations remained low at 4 hours postdosing, increased slightly at 24 hours, and decreased thereafter. The decline noted in the second 24-hour period was due to both organ growth and mirex elimination. Mirex accumulated in maternal and fetal tissues at all dose levels (1.5, 3, 6, 12.5 mg/kg given on gestation days 6–15) (Khera et al. 1976). At the 12.5 mg/kg/day dose level, fetal brain levels were >3 times higher (31.5 ppm) than mean maternal brain levels (8.87 ppm). All other mean

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fetal tissue values were lower than mean maternal values. The highest maternal levels of mirex were found in the fat, indicating the potential for long-term sequestering of the compound.

In a study in which dams were dosed with 1 or 10 mg/kg of mirex on days 2–5 postpartum, mirex was found in the stomach milk of pups (Kavlock et al. 1980). Mirex appeared in the milk in direct proportion to the dose. Mirex was also distributed to the liver, brain, and eyes of the pups in the approximate ratio of 40:4:1. Mirex tissue levels paralleled milk levels.

Mirex concentrations in adipose tissues of goats fed daily doses of 1 mg/kg did not reach a steady state, but continued to increase throughout a 61-week exposure period and did not seem to be affected by pregnancy or lactation (Smrek et al. 1977). When the dose was increased from 1 to 10 mg/kg, the adipose tissue levels did not increase dramatically. Twenty-eight days postdosing, the following residue levels were found in tissues of lactating cows given daily doses of 0.005 mg/kg/day for 28 days: 0.21 ppm in fat, 0.03 ppm in liver, and 0.02 ppm in kidney (Dorough and Ivie 1974). Muscle and brain contained no detectable residues. Mirex was the only compound identified in the fat. Analyses of the composition of residues in liver and kidney were not performed.

There was a dose-related increase in the levels of mirex in fat of rats fed 0.02, 0.2, or 1.5 mg/kg/day for 16 months (Ivie et al. 1974b). Mirex levels in fat were 120-fold higher than corresponding dietary intakes. Mirex levels increased in tissues throughout the exposure period, with fat accumulating the highest amounts of mirex. No plateau of residue accumulation occurred in any tissue during the feeding period. Removal of animals from treatment after 6 months resulted in a decline of residue levels in all tissues.

Mirex is rapidly absorbed and distributes to plasma and liver after intraperitoneal injection. Peak concentrations were seen at 3 hours postdosing in plasma and 6 hours postdosing in the liver following single or multiple doses of mirex (4 mg/kg) injected into mice (Charles et al. 1985). Significant amounts were rapidly taken up by the liver (21–29%) within the first 3–6 hours postdosing. Plasma-to-liver ratios were low (<1), indicating an increased influx of the chemical into tissue. Mirex decay curves for plasma and liver during 72 hours postdosing showed a biphasic pattern that consisted of a rapid phase (up to 24 hours) and a slow phase (24–72 hours). For plasma, the half-lives were 9.2 and 62.8 hours for the rapid and slow phases, respectively. For liver, the half-lives for the rapid and slow phases were 12.1 and 62.4 hours, respectively (Charles et al. 1985).

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Mirex was rapidly cleared from the blood of rats following intravenous injection of 10 mg/kg (Byrd et al. 1982). Mirex blood levels at 8 hours postinjection were <4% of the levels seen 2 minutes postinjection. Pharmacokinetic modeling predicted that intravenously administered mirex was quickly cleared from the blood into a rapidly equilibrating compartment. Over the next several weeks, mirex was redistributed to a slowly equilibrating compartment, which acted as a depot for mirex storage (Byrd et al. 1982). The biological half-life of mirex was estimated to be 435 days (Byrd et al. 1982).

Following a single intravenous dose of 1 mg/kg to female rhesus monkeys, 86–87% of the administered dose was recovered in fat, 3.7–10% in skin, 0.6–1.7% in skeletal muscle, and <0.5% in other tissues (Wiener et al. 1976). Mirex was the only compound identified in fat.

***Chlordecone.*** In humans, chlordecone is absorbed and distributed to various tissues and has a long retention time in the body (Cannon et al. 1978; Cohn et al. 1978; Taylor 1982, 1985). Chlordecone was eliminated slowly from the blood (half-life of 165 days) and fat (half-life of 125 days) of industrial workers (Cohn et al. 1978). Tissue-to-blood ratios for liver, fat, muscle, and gallbladder bile were 15, 6.7, 2.9, and 2.5, respectively (Guzelian et al. 1981). The relatively higher partition of chlordecone to blood (fat-to-blood concentration ratio of 1:7) compared to that of other organochlorine pesticides (e.g., DDT with a fat-to-blood concentration ratio of 300:1) may be explained by the fact that chlordecone is bound specifically by the proteins in plasma, particularly high-density lipoproteins (HDLs), unlike most organochlorine pesticides, which distribute among tissues in direct proportion to the concentration of tissue fat (Guzelian et al. 1981).

In rats, chlordecone was absorbed and distributed to various tissues, with the highest concentrations in liver (Egle et al. 1978; Hewitt et al. 1986b; Plaa et al. 1987). Chlordecone was detected in liver (125.8 mg/kg), adipose tissue (27.3 mg/kg), kidney (25.2 mg/kg), and plasma (4.9 mg/L) of rats 8 days following a single oral dose of 50 mg/kg (Hewitt et al. 1986b). Chlordecone was detected in liver, kidney, and fat of rats following single or repetitive dosing (0.5, 1, 2, 2.5, 5, 10, or 25 mg/kg) (Plaa et al. 1987). For all dose groups, the liver contained the highest concentration, followed by kidney, then fat. The ratios of tissue levels in animals that received multiple doses to levels in animals that received single doses were as follows: 4.27 (plasma), 3.27 (liver), 3.74 (kidney), and 3.42 (fat). These ratios show an even accumulation of chlordecone in the tissues. Rats given four daily doses of 10 mg/kg chlordecone had tissue-to-blood distribution ratios for fat, liver, muscle, and skin of 15, 55, 5, and 6, respectively (Bungay et al. 1981).

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Studies show that pretreatment with an inducer (phenobarbital) or inhibitor (SKF-525A) of CYP450 causes an alteration in the distribution of chlordecone in rats (Aldous et al. 1983). Following a single oral dose of chlordecone alone, the liver had the highest chlordecone level, followed by adrenal gland, lung, kidney, and spinal cord (Aldous et al. 1983). Pretreatment with phenobarbital (particularly with multiple phenobarbital doses) caused an increase in the accumulation of chlordecone in the liver compared to animals given no pretreatment. This hepatic increase resulted in a significant decrease of chlordecone levels in other tissue (e.g., brain, kidney, muscle) as well as significantly reduced excretion. Pretreatment with SKF-525A caused a nonsignificant reduction in chlordecone levels in the liver and significant increases in digestive system tissues. The results of the chlordecone distribution following SKF-525A pre-dosing must be interpreted with caution, since the effects may have resulted partly from SKF-525A-mediated decreases in absorption of chlordecone (Aldous et al. 1983).

Following a single oral dose of 50 mg/kg chlordecone to male mice, chlordecone was distributed to the brain (Fujimori et al. 1982a; Wang et al. 1981). The results showed that the striatum and medulla/pons had significantly higher levels of chlordecone than the cortex, midbrain, or cerebellum (Fujimori et al. 1982a). Mice similarly treated with mirex did not exhibit marked differences in distribution among these brain areas. Chlordecone levels were 3–40 times higher than mirex levels in plasma and brain. Following repeated oral doses of chlordecone (10 mg/kg/day) for 12 days, the compound was rapidly absorbed and distributed to the brain (Wang et al. 1981). Plasma levels of chlordecone increased during the 12-day treatment period. Brain levels of chlordecone increased linearly for the first 8 days and reached a plateau of 90 µg/g on the 10th day (Wang et al. 1981).

Chlordecone is well distributed throughout the reproductive tract of male rats and appears in the ejaculate. In rats given a single oral dose of 40 mg/kg chlordecone, the descending order of concentration was vas deferens (81.6) > seminal vesicular fluid (19.7) > unwashed sperm (14.6) > prostate (11.3) > seminal vesicle (6.2) > washed sperm (1.97). This relationship persisted as levels declined over the 21-day observation period (Simon et al. 1986).

Chlordecone accumulates in maternal tissues, readily crosses the placenta of rats, and accumulates in fetal tissues (Chernoff et al. 1979a; Kavlock et al. 1980). Four hours following a single oral dose of 5 mg/kg, maximal concentrations of chlordecone in the placenta ranged from 3.5 to 4 ppm (Kavlock et al. 1980). Concentrations of chlordecone in the placenta remained steady for up to 48 hours postdosing. Chlordecone levels in the fetus were generally highest in the liver, followed by the brain, heart, and kidney. Concentrations increased during the first 24 hours after dosing and declined in the second

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24-hour period, regardless of gestation age at the time of dosing (Kavlock et al. 1980). Chlordecone levels found in maternal and fetal tissues were slightly higher than the levels of mirex following administration of equal doses (Kavlock et al. 1980). The livers of weanling rats fed diets of 0.05 mg/kg chlordecone or mirex for 28 days accumulated higher levels of chlordecone (6.1 ppm) than mirex (0.89 ppm) (Chu et al. 1980a). Possible explanations for this are that mirex is more poorly absorbed from the feed than is chlordecone or that the absorbed dose of mirex accumulates in the liver to a lesser extent than absorbed chlordecone (Chu et al. 1980a).

In a study in which lactating rat dams were dosed with 1 or 10 mg/kg chlordecone on days 2–5 postpartum, chlordecone was found in the stomach milk of pups (Kavlock et al. 1980). Chlordecone appeared in the milk in direct proportion to the dose. Chlordecone was distributed to the liver, brain, and eyes of the pups in the approximate ratio of 16:4:1 (Kavlock et al. 1980).

### 3.1.3 Metabolism

**Mirex.** Radiolabeling experiments showed that mirex is not metabolized in humans, rodents, cows, or minipigs; the parent compound was the only radiolabeled compound present in the plasma, fat, and feces (Dorough and Ivie 1974; Gibson et al. 1972; Kutz et al. 1974; Mehendale et al. 1972; Morgan et al. 1979). However, a monohydro derivative of mirex was identified in the feces, but not the fat or plasma, of rhesus monkeys given an oral or intravenous dose of mirex (Pittman et al. 1976; Stein et al. 1976; Wiener et al. 1976). It is believed that the suspected metabolite may have arisen as a result of bacterial action in the lower gut or feces (Stein et al. 1976).

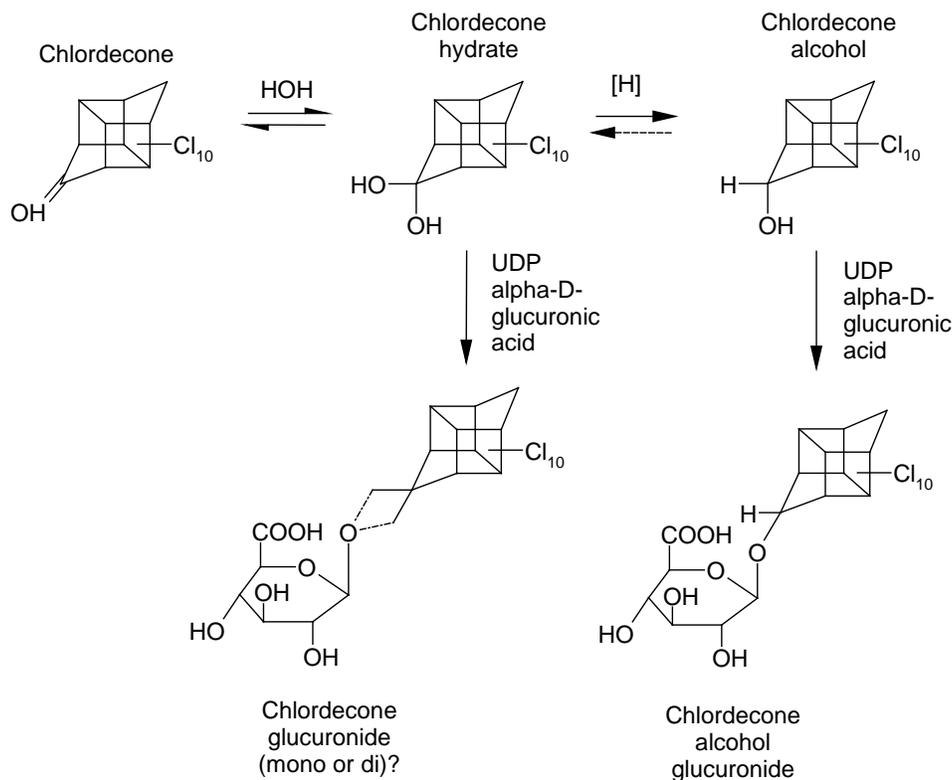
The potential for *in vivo* conversion of mirex to chlordecone was also examined (Morgan et al. 1979). Mirex was found in a variety of tissues from minipigs administered mirex in the feed for 7 days; however, chlordecone was not detected in any tissues (Morgan et al. 1979). This result indicates that significant *in vivo* conversion of absorbed mirex to chlordecone is not likely.

**Chlordecone.** The fate of chlordecone in humans involves uptake by the liver, enzymatic reduction to chlordecone alcohol, conjugation with glucuronic acid, partial conversion to unidentified polar forms, and excretion of these metabolites mainly as glucuronide conjugates into bile (Fariss et al. 1980; Guzelian et al. 1981) (see Figure 3-1). Of the total chlordecone measured in bile of occupationally exposed workers, the predominant portion (72%) was unconjugated, with only a small portion conjugated with glucuronic acid or sulfate (9%) (Fariss et al. 1980). The remaining fraction (19%) of total chlordecone measured in

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the bile was stable polar metabolites, which were resistant to  $\beta$ -glucuronidase. Following treatment of bile with  $\beta$ -glucuronidase plus sulfatase, the ratio of total chlordane to total chlordane alcohol was 1:3 in human bile (Fariss et al. 1980). Bioreduction of chlordane to chlordane alcohol is species-specific since rats treated orally or intraperitoneally with chlordane produced no chlordane alcohol in the feces, bile, or liver (Fariss et al. 1980; Guzelian et al. 1981; Houston et al. 1981). Following treatment of bile with  $\beta$ -glucuronidase plus sulfatase, the ratio of total chlordane to total chlordane alcohol in rat bile was in excess of 150:1 for orally exposed rats (Fariss et al. 1980; Guzelian et al. 1981). Guinea pigs and hamsters given an intraperitoneal dose of 20 mg/kg chlordane also did not convert chlordane to chlordane alcohol, as indicated by the fact that no chlordane alcohol was detected in the feces, bile, or liver (Houston et al. 1981). Therefore, rats, guinea pigs, and hamsters are not good animal models for predicting chlordane metabolism in humans because they do not convert chlordane to chlordane alcohol. Gerbils were found to be the most suitable animal model of chlordane metabolism in humans because only gerbils converted chlordane to its alcohol (Houston et al. 1981). Reduction of chlordane is catalyzed in gerbil liver by a species-specific reductase, chlordane reductase. This chlordane reductase was characterized in gerbil liver cytosol *in vitro* and determined to be of the "aldo-keto reductase" family (Molowa et al. 1986b). It is specific to gerbils and humans (Molowa et al. 1986b). Like humans, chlordane-treated gerbils excreted chlordane alcohol exclusively in the stool and not in the urine (Houston et al. 1981). Following intraperitoneal dosing of 20 mg/kg  $^{14}\text{C}$ -chlordane, the ratio of chlordane to chlordane alcohol in the bile of gerbils was approximately 2.5:1. No quantitative estimate of the extent to which chlordane was metabolized was given. Following treatment of bile with  $\beta$ -glucuronidase plus acid hydrolysis, the ratio of chlordane to chlordane alcohol in the bile was 1:2, indicating that chlordane is present in the bile largely in the form of its glucuronide conjugate (Houston et al. 1981). Incubation of chlordane with the cytosolic fraction of gerbil liver homogenate in the presence of NADPH produced chlordane alcohol (Houston et al. 1981). Intraperitoneally-injected chlordane was biotransformed in pigs to conjugated chlordane, chlordane alcohol, and conjugated chlordane alcohol, which were excreted in the bile and eliminated in the feces (Soine et al. 1983). Relatively high levels of chlordane alcohol and conjugated chlordane alcohol in the bile and the absence of these metabolites in the plasma and liver suggest that chlordane alcohol is formed and conjugated in the liver and excreted into the bile (Soine et al. 1983).

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**Figure 3-1. Proposed Metabolic Pathways for Chlordane**

Source: Fariss et al. 1980

### 3.1.4 Excretion

**Mirex.** Available information regarding mirex-related excretion in humans is limited. Mirex was detected in milk samples from lactating women (Fitzgerald et al. 2001; Greizerstein et al. 1999; Mes et al. 1978; Newsome and Ryan 1999; Newsome et al. 1995).

In animals, mirex is excreted unchanged mainly in the feces; urinary excretion is negligible (Byrd et al. 1982; Chambers et al. 1982; Gibson et al. 1972; Ivie et al. 1974b). Female rats receiving a single oral dose of  $^{14}\text{C}$ -mirex (0.2 mg/kg) excreted 18% of the total administered dose in the feces during a 7-day posttreatment period; very little was excreted in the urine (0.3% of the total dose) (Gibson et al. 1972). Of the total quantity eliminated, 85% was excreted in the feces within the first 48 hours. This percentage represents unabsorbed material. The virtual lack of urinary excretion and the fact that fecal excretion was only about 3% of the administered dose after the initial 48 hours suggest that mirex is not metabolized in rats and that the absorbed portion is only slowly excreted. In female rats administered a single oral dose

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of 10 mg/kg mirex, cumulative fecal excretion of mirex during 21 days posttreatment was 18–45% (Byrd et al. 1982). Most of the fecal mirex was excreted within 48 hours and represented unabsorbed mirex. A biological half-life of mirex was estimated to be 460 days by a model developed to simulate mirex pharmacokinetics after oral administration (Byrd et al. 1982). Male rats receiving a single oral dose of mirex at 6 mg/kg excreted 58.5% of the administered dose in the feces during 7 days posttreatment (Mehendale et al. 1972). Fifty-five percent of the administered dose was excreted in the feces within the first 48 hours post-dosing and probably represented unabsorbed dose from the gut. Only 0.69% of the administered dose was excreted in the urine. Mirex was the only treatment-related compound identified in the urine or feces. A half-life of 38 hours was estimated based on the first rapid elimination. A second half-life was projected to be >100 days, indicating a very slow rate of elimination from the body.

Following oral administration of 1 mg/kg <sup>14</sup>C-mirex to a female rhesus monkey, 25% of the radioactivity was recovered in the feces within 48 hours, with a cumulative excretion of 26.5% over 23 days. Less than 1% was recovered in the urine over 23 days (Wiener et al. 1976). A monohydro derivative of mirex was identified in the feces of rhesus monkeys given daily doses of 1 mg/kg mirex; the exact duration of dosing was not specified (Stein and Pittman 1977).

The secretion of mirex in milk was a major route of elimination for nursing rat dams given either 1 or 10 mg/kg/day of mirex via gavage on postpartum days 2–5 (Kavlock et al. 1980). Mirex entered the milk supply more quickly than chlordecone. Greater amounts of mirex were excreted via the milk as compared with chlordecone because of the octanol-water partition coefficient. Mirex was also excreted in the milk of lactating goats given daily doses of 1 mg/kg for 18 or 61 weeks followed by daily doses of 10 mg/kg for 4 weeks (Smrek et al. 1977). The concentration of mirex in colostrum fat ranged from 16 to 20 ppm. Colostrum, which is fluid secreted for the first few days after parturition, is characterized by high protein and antibody content. Over 8 weeks, the levels of mirex in milk fat decreased to less than half the amount excreted in colostrum immediately after birth of the kids. The goats eliminated more mirex in colostrum than in regular milk. A lactating Jersey cow given a daily dose equivalent to 0.005 mg/kg/day in the diet for 28 days, excreted 50% of the administered dose in the feces during the 28-day exposure period (Dorough and Ivie 1974). Only approximately 3% of the administered dose of mirex was excreted in the feces in the 28 days after treatment ended. These results show that the radioactivity in the feces represents unabsorbed mirex, and that the turnover rate of mirex stored in the tissues is very low. In this study, mirex was also found in cow's milk. About 10% of the administered dose was excreted in the milk 10 days after treatment began. Cumulative excretion in the milk was 13% after 28 days of exposure. Only 2% of the administered dose was excreted in the milk during the entire 28-day post-treatment

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period. The levels of mirex in milk equilibrated after 1 week of treatment, with the concentration in whole milk being 0.058 ppm. One week after treatment ended, the residues in the milk dropped to 0.006 ppm and then declined to 0.002 ppm after 28 days (Dorough and Ivie 1974). Mirex was the only treatment-related compound identified in the feces and cow's milk.

Mirex has a long retention time in the body and is excreted slowly. Cumulative fecal excretion was 7% of the administered dose 21 days following intravenous dosing of 10 mg/kg in rats (Byrd et al. 1982). Cumulative urinary excretion was <1% of the administered dose (Byrd et al. 1982). The biological half-life of mirex was estimated to be 435 days (Byrd et al. 1982). Cumulative fecal excretion was 4.69 and 6.91% of the dose after 106 and 388 days, respectively, following a single intravenous dose of 1 mg/kg to female monkeys (Wiener et al. 1976). Cumulative urinary excretion accounted for 0.18–0.37% of the administered dose by the end of 1 week. Mirex was the only labeled compound identified in the feces. An unidentified substance found in the feces was thought to be a decomposition product of mirex, not a metabolite (Wiener et al. 1976). Mirex and an unidentified metabolite, a nonpolar derivative, were found in the feces of rhesus monkeys given an intravenous dose of 1 mg/kg of mirex (Stein et al. 1976). It is believed that the suspected metabolite may have arisen as a result of bacterial action in the lower gut or feces (Stein et al. 1976).

***Chlordecone.*** Chlordecone, chlordecone alcohol, and their glucuronide conjugates were excreted in the bile and eliminated via the feces of humans occupationally exposed to chlordecone (Blanke et al. 1978; Boylan et al. 1979; Cohn et al. 1978; Guzelian et al. 1981). In the study of Guzelian et al. (1981), most of the total chlordecone measured in bile was unconjugated (72%), a small amount (9%) was conjugated with glucuronic acid, and the final portion (19%) was present as an uncharacterized “acid releasable” form. Only a minor amount of chlordecone alcohol (<10%) was present in bile as the free metabolite; the remainder was conjugated with glucuronide. A substantial enterohepatic recirculation of chlordecone exists that curtails its excretion (Boylan et al. 1979; Cohn et al. 1978; Guzelian et al. 1981). Only 5–10% of the biliary chlordecone entering the lumen of the duodenum appeared in the feces (Cohn et al. 1978; Guzelian et al. 1981). Similarly, the rate of chlordecone excretion in the bile was, on average, 19 times greater than the rate of elimination of chlordecone in the stool (Cohn et al. 1978). Chlordecone was not detected in the sweat and was detected in only minor quantities in urine, saliva, and gastric juice (Cohn et al. 1978). Similarly, stool contained 11–34% of the quantities excreted in bile for workers exposed for 6 months (Boylan et al. 1979). When biliary contents were diverted, fecal excretion of chlordecone alcohol fell to low or undetectable levels; however, chlordecone excretion in feces persisted, suggesting a nonbiliary mechanism for the excretion of chlordecone into the intestine and feces (Boylan et al. 1979).

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Analogous experiments with rats gave similar results (Boylan et al. 1979). With no bile in the gut, the average amount of chlordecone in the human stool in two 72-hour collections was eight times as great as with the biliary circuit intact (Boylan et al. 1979). This suggests that bile may suppress nonbiliary excretion of chlordecone. When bile was completely diverted from the intestines of rats, however, fecal excretion of radiolabel was unchanged (Boylan et al. 1979).

In rats, chlordecone is slowly eliminated in the feces (Egle et al. 1978). Rats given a single oral dose of 40 mg/kg  $^{14}\text{C}$ -chlordecone excreted 65.5% of the administered dose in the feces and 1.6% of the dose in the urine by 84 days (Egle et al. 1978). Less than 1% of the administered dose was expired as radiolabeled carbon dioxide ( $^{14}\text{C}$ - $\text{CO}_2$ ) (Egle et al. 1978). Rats fed  $^{14}\text{C}$ -chlordecone (0.2 mg/kg/day for 3 days) excreted 52.16% of the radioactivity in the feces and 0.52% in the urine 25 days postdosing (Richter et al. 1979).

Chlordecone was excreted in the saliva of rats following administration of 50 mg/kg (Borzelleca and Skalsky 1980; Skalsky et al 1980). Peak levels of chlordecone in saliva were reached 6–24 hours postdosing (Borzelleca and Skalsky 1980; Skalsky et al. 1980). The saliva-to-plasma ratios were <1 throughout the study period, indicating that chlordecone is not actively concentrated by the salivary glands (Borzelleca and Skalsky 1980). Thus, chlordecone enters the salivary tissue (submaxillary, parotid, and sublingual tissues) and saliva by passive diffusion (Borzelleca and Skalsky 1980; Skalsky et al. 1980).

Chlordecone is also excreted in the milk of nursing rats (Kavlock et al. 1980). When compared with mirex-treated rats, chlordecone entered the milk supply more slowly than mirex. More mirex was excreted via the milk than chlordecone because of a higher octanol-water partition coefficient.

Chlordecone was detected in the bile and feces of rats, guinea pigs, hamsters, gerbils, and pigs given intraperitoneal doses of 20 mg/kg chlordecone (Houston et al. 1981; Soine et al. 1983). Rats given intraperitoneal injections of chlordecone had a fecal excretion half-life of 40 days (Pore 1984).

Chlordecone alcohol was detected in the bile and feces of gerbils and pigs only (Houston et al. 1981; Soine et al. 1983).

Chlordecone appeared in the bile within 1–3 hours after intravenous dosing of rats (0.1, 1, or 10 mg/kg) (Bungay et al. 1981). The average concentration of chlordecone in the bile varied linearly with dose: 0.051, 0.50, and 5  $\mu\text{g/g}$  in the low-, middle-, and high-dose groups, respectively (Bungay et al. 1981).

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Rats given a single intravenous dose of 1 mg/kg had a chlordecone excretion rate in the bile of 0.22% of the dose per hour (Bungay et al. 1981).

### 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 for mirex have not been developed. Several models were developed for chlordecone. Bungay et al. (1979) developed a model to predict the kinetics of chlordecone in the gastrointestinal tract by comparing excretion following oral administration to intact rats and intravenous administration to bile-cannulated rats. Heatherington et al. (1998) used experimental data from chlordecone-treated young and adult rats to predict percutaneous absorption and disposition. El-Masri et al. (1995) evaluated interactions between chlordecone and carbon tetrachloride in the rat liver using pharmacokinetic and pharmacodynamic modeling. Belfiore et al. (2007) developed a model to describe sequestration of chlordecone in the rat liver. None of the models are useful for predicting the toxicokinetic behavior or target concentrations of chlordecone in humans.

### 3.1.6 Animal-to-Human Extrapolations

The toxicokinetics of mirex has been widely studied in experimental animals (Atallah and Dorough 1975; Brown and Yarbrough 1988; Byrd et al. 1982; Chambers et al. 1982; Dorough and Atallah 1975; Gibson et al. 1972; Ivie et al. 1974b; Kavlock et al. 1980; Mehendale et al. 1972; Morgan et al. 1979; Plaa et al. 1987; Smrek et al. 1977; Wiener et al. 1976). Available data demonstrate that mirex accumulates in tissues (particularly fat), is not metabolized, and is slowly excreted in feces. Most animal studies were conducted using rats. A few studies using monkeys, goats, or cows yielded results generally similar to those reported for rats. Limited human data have not identified or quantified the toxicokinetics of mirex (Burse et al. 1989; Kutz et al. 1974; Mes et al. 1978). No information was located to indicate that the

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toxicokinetics of mirex in humans would be significantly different from that observed in experimental animals.

Toxicokinetic studies have been performed using multiple animal species; the data indicate that rats, guinea pigs, and hamsters may not represent appropriate models for extrapolation to humans because these animal species do not convert chlordecone to chlordecone alcohol (Fariss et al. 1980; Guzelian et al. 1981; Houston et al. 1981). Gerbils and pigs may be more appropriate species to study animal-to-human extrapolation because these species convert chlordecone to chlordecone alcohol (Houston et al. 1981; Soine et al. 1983). Limited human toxicokinetic data are available for chlordecone (Adir et al. 1978; Blanke et al. 1978; Boylan et al. 1978; Cannon et al. 1978; Cohn et al. 1978; Guzelian et al. 1981; Taylor 1982, 1985). It does not appear that sufficient data exist to provide meaningful extrapolation from animals to humans with respect to chlordecone toxicokinetics.

#### **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 mirex or chlordecone are discussed in Section 5.7, Populations with Potentially High Exposures.

Review of the literature regarding toxic effects of mirex and chlordecone did not reveal any human populations that are known to be unusually sensitive to mirex or chlordecone. However, based on knowledge of the toxicities of mirex and chlordecone, some populations can be identified that may demonstrate unusual sensitivity to these chemicals. Those with potentially high sensitivity to mirex

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include the very young. Those with potentially high sensitivity to chlordecone include juvenile and elderly persons as well as persons being treated with some antidepressants or the anticonvulsant, diphenylhydantoin.

In experimental animals, mirex administered within the week after birth causes a high incidence of cataracts and other lesions of the lens (Chernoff et al. 1979b; Gaines and Kimbrough 1970; Rogers and Grabowski 1984; Scotti et al. 1981). These effects were observed whether the neonatal animals received mirex through the milk of lactating dams or directly by gavage. Although it is unclear whether the lens of humans also undergoes a similar period of susceptibility, the possibility exists that newborn children may also develop cataracts if exposed to mirex shortly after birth.

Studies in rats have demonstrated that certain treatments exacerbate the tremors associated with chlordecone exposure. These include pretreatment with the anticonvulsant, diphenylhydantoin (Hong et al. 1986; Tilson et al. 1985, 1986b), and treatment with the non-selective serotonergic receptor agonist, quipazine (Gerhart et al. 1983). Therefore, persons being treated with diphenylhydantoin for epilepsy or quipazine for depression may be likely to experience more severe tremors upon exposure to high levels of chlordecone. Extrapolating from the effects seen in animals with quipazine, it might be likely that persons taking the prescription drug Prozac<sup>®</sup>, a selective serotonin reuptake inhibitor (SSRI) used to treat depression, may also experience more severe tremors. Furthermore, the elderly may be a susceptible population because serotonin metabolism is increased during aging (Walker and Fishman 1991).

Studies in animals have also shown that juvenile animals experience a higher death rate than adults following exposure to chlordecone at equivalent mg/kg doses (Huber 1965). No explanation was given for these findings, but similar sensitivities may exist in children. Furthermore, although inhibition of Na<sup>+</sup>K<sup>+</sup>ATPase, Mg<sup>2+</sup>ATPase, and Ca<sup>2+</sup>ATPase activities have not been definitively shown to be the mechanism underlying chlordecone toxicity, sufficient evidence exists to suggest that their inhibition may be involved in a number of adverse effects. Neonatal rats have shown a greater inhibition of these enzymes than adult rats (Jinna et al. 1989). This provides additional support for the suggestion that infants and young children may represent a susceptible population to the toxic effects of chlordecone.

In contrast, a recent study of developing postnatal rats has shown that the young may be less susceptible to at least one of the toxic effects of chlordecone. Young and adolescent rats show less potentiation of carbon tetrachloride toxicity than adult rats (Cai and Mehendale 1993). This may be due to a combination of incomplete development of the microsomal enzyme systems and a higher level of hepatic regenerating

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activity in the very young rats. In adolescent rats (35 and 45 days old), the microsomal enzyme activity is comparable to adult levels, but the level of damage is still less than in adult rats (60 days old). This may be due to that fact that hepatic regenerating activity remained higher in the adolescents than in the adults.

Several studies (Dalu and Mehendale 1996; Dalu et al. 1995, 1998; Murali et al. 2004) provide additional insight to earlier findings of age-related differences in the lethality and hepatotoxicity induced by exposure of rats to nontoxic levels of chlordecone and subsequent exposure to otherwise nonlethal levels of carbon tetrachloride. Results of Blain et al. (1999) indicate both sex- and age-dependent influences on chlordecone-carbon-tetrachloride-induced hepatotoxicity in rats.

In studies performed by Sobel and coworkers (Sobel et al. 2005, 2006; Wang et al. 2008), chronic exposure of systemic lupus erythematosus-prone female (NZB x NZW) F<sub>1</sub> mice to chlordecone via subcutaneously-implanted pellets significantly shortened the time to onset of elevated autoantibody titers and renal disease in a dose-related manner. These effects were not seen in nonlupus-prone BALB/c mice. These results indicate that humans with lupus may be particularly sensitive to chlordecone toxicity.

### 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 mirex or chlordecone 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 mirex or chlordecone 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

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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 mirex or chlordecone 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

The primary biomarkers of exposure to mirex include mirex concentrations in blood (Butler Walker et al. 2003; Byrd et al. 1982; Fenster et al. 2006; Greizerstein et al. 1999; Kavlock et al. 1980; Schell et al. 2003; Smrek et al. 1977; van Oostdam et al. 2004; Wiener et al. 1976), fat (Burse et al. 1989; Kutz et al. 1974), feces (Byrd et al. 1982; Chambers et al. 1982; Gibson et al. 1972; Ivie et al. 1974b), or breast milk (Dorough and Ivie 1974; Fitzgerald et al. 2001; Greizerstein et al. 1999; Kavlock et al. 1980; Mes et al. 1978; Newsome and Ryan 1999; Newsome et al. 1995; Smrek et al. 1977). Since mirex is not metabolized, it is the only biomarker to be measured in these biological media. Since mirex is retained in the body for long periods of time and only slowly excreted, its measurement is useful as a biomarker of acute-, intermediate-, or chronic-duration exposures to both low and high levels.

The biomarkers of exposure to chlordecone include blood or saliva concentrations of chlordecone, and fecal or bile concentrations of chlordecone, chlordecone alcohol, and/or their glucuronide conjugates. Blood samples are the most useful tool for epidemiological studies of exposure to chlordecone (Guzelian et al. 1981). The unusually high concentration of chlordecone in blood compared with its concentration in fat (1:7 in humans), which is due to chlordecone's association with plasma proteins, and its long half-life, make chlordecone in blood (a readily sampled tissue) a good biomarker of exposure. The blood concentration of chlordecone serves as an accurate reflection of total body content of chlordecone. Blood is the best biological material to monitor and to use for determining acute, intermediate, and chronic exposures to both low and high levels of chlordecone.

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Blood is a better indicator of exposure to chlordecone than saliva (Borzelleca and Skalsky 1980; Skalsky et al. 1980). Chlordecone has been detected in saliva of humans only in trace amounts and in rats at concentrations 3–4 times lower than in blood (Guzelian et al. 1981; Skalsky et al. 1980). Peak chlordecone concentrations occurred within the first 24 hours of exposure; therefore, the period of utility of saliva as a biomarker is limited. The movement of chlordecone from blood into saliva is one of passive diffusion and is not concentration dependent (Borzelleca and Skalsky 1980; Skalsky et al. 1980). Thus, blood is a better biological material than saliva for monitoring chlordecone exposure.

Other biomarkers of exposure include tissue concentrations of chlordecone (Bungay et al. 1981; Cannon et al. 1978; Cohn et al. 1978; Egle et al. 1978; Hewitt et al. 1986b; Plaa et al. 1987; Taylor 1982, 1985) and fecal or bile concentrations of chlordecone, chlordecone alcohol, and their glucuronide conjugates (Blanke et al. 1978; Boylan et al. 1979; Cohn et al. 1978; Guzelian et al. 1981). These can be measured and are reliable indicators of exposure to chlordecone.

### 3.3.2 Biomarkers of Effect

Microsomal enzyme induction has been shown to be increased by both mirex and chlordecone in humans and/or experimental animals. Serum levels of chlordecone associated with enzyme induction in exposed workers were estimated to range from 100 to 500 µg/L (Guzelian 1985). Urinary D-glucaric acid levels have been shown to be a sensitive indicator of microsomal enzyme induction in workers exposed to chlordecone (Guzelian 1985). However, other substances such as barbiturates, phenytoin, chlorbutanol, aminopyrine, phenylbutazone, and contraceptive steroids as well as other organochlorinated pesticides also cause microsomal enzyme induction and cause changes in urinary D-glucaric acid (Morgan and Roan 1974).

Studies in experimental animals suggest that biliary excretion of chemicals from the liver may be impaired by mirex or chlordecone (Berman et al. 1986; Curtis and Hoyt 1984; Curtis and Mehendale 1979; Curtis et al. 1979b, 1981; Davison et al. 1976; Mehendale 1976, 1977b, 1977c, 1981b; Teo and Vore 1991). Measurement of serum bile acid levels may provide information regarding biliary excretory function.

Studies in experimental animals have also shown increased urinary protein accompanied or unaccompanied by histopathological changes of the kidneys following exposure to mirex (NTP 1990) or

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chlordecone (Larson et al. 1979b). Although these changes are not specific for mirex or chlordecone, measurement of these parameters may provide information about renal damage in exposed populations.

Chlordecone causes a number of neurotoxic responses in humans and animals exposed to sufficiently high levels. Tremor accentuated by intentional acts, sustained postural movement, anxiety, and/or fatigue have been observed in workers exposed to high levels of chlordecone. Tremorograms have been used to objectively assess tremors associated with chlordecone exposure in humans (Taylor et al. 1978). An infrared reflection technique and oculography have been used to assess oculomotor disturbances caused by chlordecone (Taylor et al. 1978). Standard tests for memory and intelligence can be used to determine the presence of encephalopathy, but in the absence of baseline preexposure levels for individuals, subtle changes may be difficult to detect.

Decreased sperm count has been observed following exposure to mirex or chlordecone in humans and/or experimental animals. Clinically, the most straightforward biomarker would be examination of sperm in the ejaculate. However, testicular biopsies may also be helpful. Both procedures have been used to assess the male reproductive toxicity of chlordecone in exposed persons (Taylor et al. 1978).

#### 3.4 INTERACTIONS WITH OTHER CHEMICALS

Limited data are available regarding interactions with other chemicals that affect the toxicity of mirex or chlordecone. Selected agents have been shown to exacerbate or suppress chlordecone-induced tremors in laboratory animals. Pretreatment of rats with diphenylhydantoin resulted in exacerbation of chlordecone-induced tremors (Hong et al. 1986; Tilson et al. 1985, 1986b). The mechanism for the exacerbation of the tremors is unknown. Therefore, if persons receiving diphenylhydantoin treatment for epilepsy were exposed to sufficiently high concentrations of chlordecone, increased tremor severity may be likely to occur. Treatment with quipazine (a nonselective serotonergic receptor agonist) was shown to potentiate chlordecone-induced tremors in rats (Gerhart et al. 1983). Therefore, it is possible that persons being treated for depression with quipazine or with SSRIs such as Prozac<sup>®</sup> may experience enhanced tremors.

A number of pharmacological agents have been shown to decrease the tremors produced by chlordecone in rats (Gerhart et al. 1983, 1985; Herr et al. 1987). Agents shown to be effective in at least one study include yohimbe or phenoxybenzamine ( $\alpha$ -noradrenergic antagonists), mecamlamine (a nicotinic antagonist), chlordiazepoxide ( $\alpha$  benzodiazepine), muscimol (a GABA agonist), and mephenesin (a

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centrally acting muscle relaxant). Persons being treated therapeutically with any of these drugs are likely to experience diminished tremors following exposure to chlordecone.

Pretreatment of rats with difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase, prior to exposure to a tremorgenic dose of chlordecone, also resulted in inhibition of the tremor (Tilson et al. 1986b). DFMO was more effective if given 5 hours prior to the chlordecone than if given 24 hours prior to exposure. The DFMO was ineffective if given 19 hours after chlordecone exposure. These results suggest an interaction of the polyamine synthetic pathway with tremors produced by chlordecone. The mechanism of the interaction is unclear, but may involve effects of polyamines on intracellular calcium homeostasis. Persons being treated with DFMO for cancer or protozoal infections would be likely to have reduced tremor severity after exposure to chlordecone.

Cholestyramine, a chelating agent, binds chlordecone present in the gastrointestinal tract and limits its enterohepatic recirculation (Boylan et al. 1978; Cohn et al. 1978). This interaction leads to increased excretion of chlordecone and decreased toxicity. Thus, persons being treated with cholestyramine to lower plasma cholesterol may experience increased excretion of chlordecone and decreased toxicity.

A number of animal studies have focused on effects of chlordecone on toxicity produced by other agents. Although these studies do not address the issue of interactions that affect chlordecone toxicity, results are summarized below.

By far, the most extensively studied interaction of mirex or chlordecone is the ability of chlordecone to markedly potentiate the hepatotoxicity of halomethanes such as carbon tetrachloride (Agarwal and Mehendale 1983c; Bell and Mehendale 1985; Chaudhury and Mehendale 1991; Curtis et al. 1979b, 1981; Davis and Mehendale 1980; Klingensmith and Mehendale 1981, 1982b, 1983a, 1983b; Klingensmith et al. 1983; Kodavanti et al. 1989a, 1990a, 1991; Lockard et al. 1983a, 1983b; Mehendale and Klingensmith 1988; Soni and Mehendale 1993; Tabet et al. 2016), bromotrichloromethane (Agarwal and Mehendale 1982; Faroon and Mehendale 1990; Faroon et al. 1991; Klingensmith and Mehendale 1981), and chloroform (Cianflone et al. 1980; Hewitt et al. 1979, 1983, 1986a, 1986b, 1990; Iijima et al. 1983; Mehendale et al. 1989; Purushotham et al. 1988). For example, pretreatment of rats with 5 mg/kg chlordecone resulted in a 67-fold increase in carbon tetrachloride-induced lethality due to liver failure (Klingensmith and Mehendale 1982b). The increase in hepatotoxicity is characterized by increased serum enzymes, extensive necrosis, increased destruction of CYP450 isozymes, and decreased biliary function. The potentiation of hepatotoxicity does not appear to be due solely to increased metabolism of the

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haloalkanes to toxic intermediates ( $\text{CCl}_3$ , free radical and phosgene) and, as such, is distinct from the potentiation of halomethane toxicity by phenobarbital (Agarwal and Mehendale 1984a, 1984d; Bell and Mehendale 1987; Klingensmith and Mehendale 1983a, 1983b; Mehendale and Klingensmith 1988; Mehendale et al. 1990) or mirex (Bell and Mehendale 1985; Cianflone et al. 1980; Hewitt et al. 1979, 1986a; Mehendale and Klingensmith 1988; Mehendale et al. 1989; Purushotham et al. 1988).

Several studies (Dalu and Mehendale 1996; Dalu et al. 1995, 1998; Murali et al. 2004) provide additional insight to findings of age-related differences in the lethality and hepatotoxicity induced by exposure of rats to nontoxic levels of chlordecone and subsequent exposure to otherwise nonlethal levels of carbon tetrachloride (Cai and Mehendale 1993). Results of Blain et al. (1999) indicate both sex- and age-dependent influences on chlordecone-carbon tetrachloride induced hepatotoxicity in rats.

The primary mechanism for potentiation of hepatotoxicity may be the suppression of the early tissue regenerative response normally seen in livers of rats and mice exposed to low doses of halomethanes (Mehendale 1992, 1994). The dramatic increase in mitotic activity that normally occurs soon after halomethane exposure does not occur in chlordecone-pretreated animals (Faroon and Mehendale 1990; Lockard et al. 1983b). Gerbils, which do not exhibit early hepatocellular regeneration following halomethane exposure (and thus are more susceptible to the toxic and lethal effects of halomethanes), do not exhibit potentiation following chlordecone pretreatment (Cai and Mehendale 1990, 1991b). Experiments performed with partially hepatectomized animals provide further evidence for the role of suppressed regeneration following carbon tetrachloride exposure (Cai and Mehendale 1991a). Partial hepatectomy, which stimulates tissue regeneration, afforded partial protection from the potentiating effects of chlordecone in rats (Bell et al. 1988; Rao et al. 1989; Young and Mehendale 1989). Similarly, Cai and Mehendale (1993) have shown that young rats with greater hepatocellular regenerative activity than adult rats also experience less hepatocellular damage following exposure to both chlordecone and carbon tetrachloride. Cellular changes that may facilitate the chlordecone-induced suppression of regeneration include marked depletion of hepatocellular glycogen (Bell and Mehendale 1987; Faroon et al. 1991; Lockard et al. 1983a, 1983b), depletion of ATP (Faroon et al. 1991; Kodavanti et al. 1990a), and disruptions in the regulation of intracellular calcium (Agarwal and Mehendale 1984a, 1984c, 1984d, 1986; Hegarty et al. 1986; Kodavanti et al. 1991). It has been demonstrated that suppression of cell division due to glycogen depletion results in decreased ATP availability and, consequently, suppressed cellular regeneration (Soni and Mehendale 1993, 1994).

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

Both mirex and chlordecone are microsomal enzyme inducers, and as such, enhance the metabolism of compounds oxidized or reduced by the mixed function oxygenase system. For example, the metabolism of lindane was enhanced in rats previously exposed to chlordecone (Chadwick et al. 1979). For chemicals that undergo a loss of activity with metabolism, a decrease in effectiveness would be likely in mirex- or chlordecone-exposed persons. For example, pretreatment of rats with chlordecone reduced the cholinesterase inhibition produced by a subsequent dose of methyl parathion (Tvede et al. 1989). In this study, methyl parathion was apparently metabolized to its active metabolite, methyl paraoxon, and the methyl paraoxon was further metabolized to an inactive metabolite. For chemicals that undergo a transformation to an active or toxic metabolite, enhanced activity/toxicity would be likely in mirex- or chlordecone-exposed persons. An example of this type of interaction was shown in the enhancement of acetaminophen toxicity by 30 mg/kg of mirex or chlordecone (Fouse and Hodgson 1987).

Acetaminophen causes hepatic necrosis as the result of the binding of the reactive intermediate, postulated to be N-acetylquinoneimine, formed by the microsomal CYP450 dependent monooxygenase system. Mirex and chlordecone increased the activity of this system, and as a result, the toxicity of the acetaminophen was increased.