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

3.1 TOXICOKINETICS

Limited data were found regarding the absorption, distribution, metabolism, and excretion of endrin in humans and animals after inhalation, oral, or dermal exposure. Available data are summarized below.

- Toxic effects following oral, inhalation, or dermal exposure indicate that the body absorbs endrin via all routes; however, data regarding absorption rates are very limited.
- Endrin is primarily distributed to fat.
- The major biotransformation product of endrin is anti-12-hydroxyendrin and the corresponding sulfate and glucuronide metabolites.
- Endrin is excreted in urine and feces.
- No studies were found that described the toxicokinetics of endrin aldehyde or endrin ketone.

3.1.1 Absorption

Quantitative data describing the rate of absorption of endrin following inhalation exposure were not available. Cases of occupational exposure reported by Hoogendam et al. (1965) and laboratory animal studies reported by Treon et al. (1955) indicate that when endrin is inhaled and absorbed, it can produce serious adverse biological effects.

Reported toxic effects in case studies indicate that humans absorb endrin following ingestion (Coble et al. 1967; Curley et al. 1970; Kintz et al. 1992; Rowley et al. 1987; Runhaar et al. 1985; Weeks 1967). Similarly, numerous animal studies reported serious adverse biological effects following oral exposure, indicating absorption. However, no studies have been located that report the rate or extent of absorption that occurs in orally exposed humans or animals.

No studies were located regarding absorption of endrin in humans after dermal exposure. Agricultural worker exposure studies demonstrated that dermal exposure (18.7 mg/hour without gloves) was significantly greater than respiratory exposure (0.41 mg/hour) and that workers exposed to endrin received about 0.2–1.5% of a toxic dose per hour of exposure (Wolfe et al. 1963).

Dermal exposure of rats and rabbits to endrin resulted in toxicity and death (Gaines 1960; Treon et al. 1955), indicating that percutaneous absorption of endrin occurs. Data describing the rate or extent of dermal absorption in animals were not located.

3.1.2 Distribution

No studies were located regarding distribution of endrin in humans or animals after inhalation exposure.

Measurable tissue concentrations of endrin have been observed in cases of acute oral poisoning. The time of sample collection is critical, as endrin residues in tissues decline rapidly after exposure has ceased (Coble et al. 1967). Evaluations shortly after exposure (<1 hour) have shown the highest concentrations in the gastrointestinal system and liver, followed by the kidneys, spleen, and heart (Coble et al. 1967; Curley et al. 1970; Kintz et al. 1992; Moriya and Hashimoto 1999; Tewari and Sharma 1978). Blood and bile concentrations were low compared to organ levels in all cases except those with rapid death (Kintz et al. 1992). Evaluations at a later time point show a different pattern; 11 days after a suicide attempt, the highest concentration was identified in the adipose tissue, followed by much lower levels in the liver, heart, brain, kidneys, and blood (Runhaar et al. 1985). In the general population, low levels of endrin in the adipose tissue of Jordanian men and woman generally increased with age (Alawi et al. 1999); however, studies in the United States and Canada did not find measurable levels of endrin in adipose tissue of the general population (EPA 1986b; Williams et al. 1984).

Endrin tends to bioaccumulate in fat because of its high lipid solubility. Three days after an acute oral dose of 2.5 mg/kg of radio-labeled endrin, the percentages of the administered dose in male rat organs were 1.2% in liver, 0.6% in kidneys, 1.7% in fat, 2.3% in skin, and 12.2% in the carcass. Female rats retained higher concentrations in tissues: 2% of the dose in liver, 0.35% in kidneys, 8% in fat, 4% in skin, and 28.2% in carcass (Hutson et al. 1975). Richardson et al. (1970) also reported an unidentified metabolite of endrin in the brain, liver, and adipose tissue of male rats following a single oral exposure. Following administration of radiolabeled endrin to lactating cows, the highest tissue concentrations were in the fat (about 8% of the total dose). Residues in liver, muscle, kidneys, and fat primarily contained unchanged endrin (Baldwin et al. 1976). Endrin and 12-ketoendrin were detected in the maternal liver and fetal tissue of rats and hamsters administered endrin during gestation (Chernoff et al. 1979; Kavlock et al. 1981). Concentrations of endrin in fetal tissue ranged from 2 to 8% of those measured in maternal livers, indicating that endrin can cross the placenta.

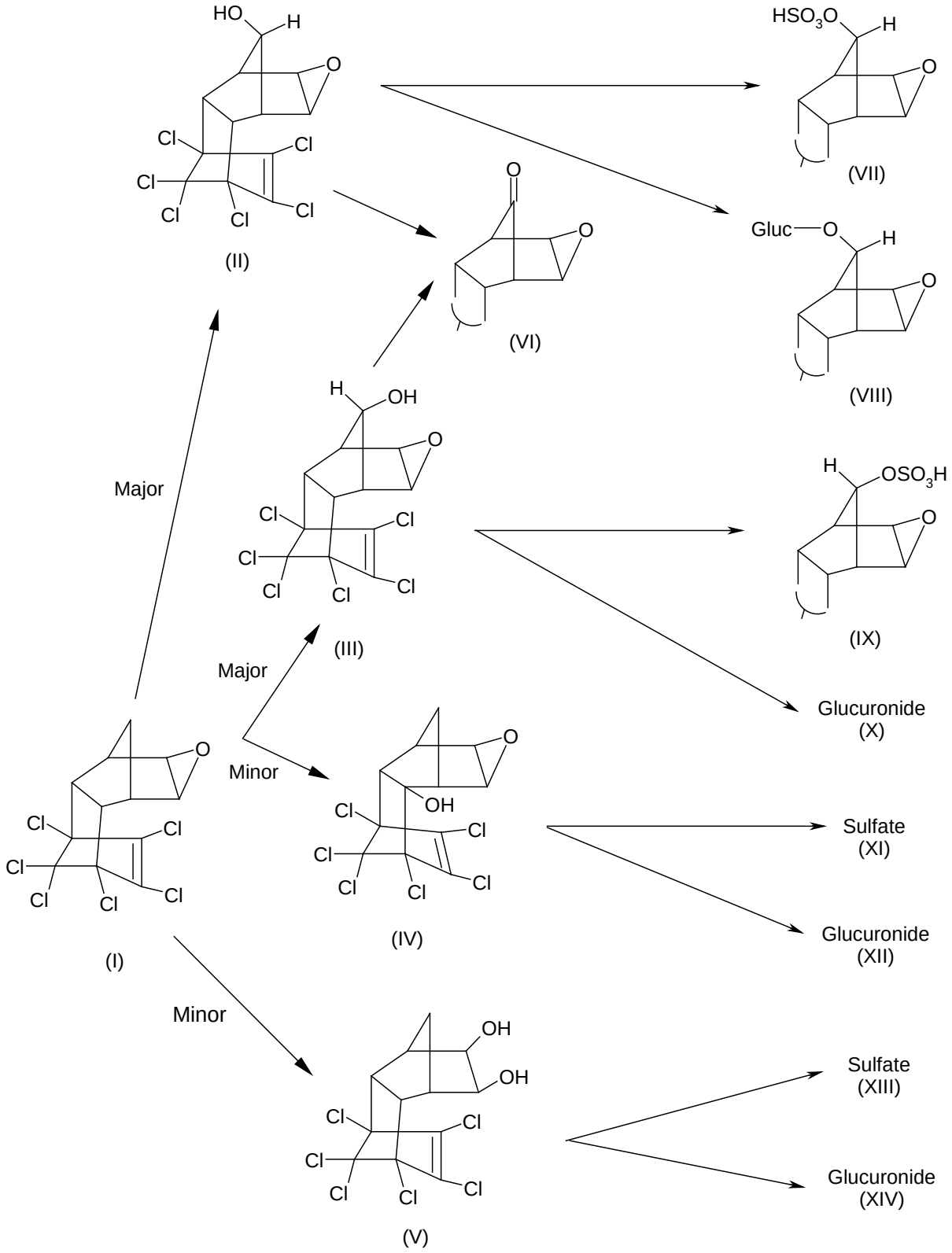
In Beagle dogs that had died after apparent ingestion of endrin-containing bait, the stomach contents contained 34–5,000 mg/kg endrin (Quick et al. 1989). The highest tissue concentrations were found in the fat (5.4–40 mg/kg), followed by liver (0.82–4.5 mg/kg) and brain (0.34–2.7 mg/kg). Lower concentrations were found in lungs and muscles. Following administration of 0.1 mg/kg/day in the feed for 128 days, concentrations of endrin in the blood of Beagle dogs showed no accumulation over time (Richardson et al. 1967). At termination, there was no correlation between the concentration of endrin in blood with that in heart, pancreas, liver, kidneys, spleen, and lungs, although a trend of high concentrations in fat (250–760 ppb) and high concentrations in blood (1–8 ppb) were noted. The highest tissue concentrations of endrin were generally found in fat, followed by muscle (120–310 ppb), heart (125–170 ppb), pancreas (87–280), liver (77–84 ppb), kidneys (38–82 ppb), and lungs (17–33 ppb). Concentrations in the spleen were highly variable (7–2,620 ppb). Results from this study may be somewhat confounded by a potential feeding error, as dieldrin (being fed to a concurrent group) was detected in the blood and tissues of the three endrin-treated dogs.

No studies were located regarding distribution of endrin in humans or animals after dermal exposure.

3.1.3 Metabolism

The metabolism of endrin varies among species, regardless of the route of exposure. In all species, oxidation of the methylene bridge in endrin (Compound I in Figure 3-1) to syn-, but mostly anti-12-hydroxyendrin occurs (Compounds II and III), followed by dehydrogenation to 12-ketoendrin (Compound VI). Minor independent pathways involve the hydrolysis of the epoxide to a transdiol (Compound V in Figure 3-1) and hydroxylation of the C-3 position (Compound IV) (Bedford et al. 1975b; Hutson 1981; Petrella et al. 1977). Hydroxylation at C-3 and C-4 is inhibited by the presence of the bulky hexachlorinated fragment (Hutson 1981). In rats, both anti-12-hydroxyendrin and 12-ketoendrin are produced at higher rates in the male rat, with higher formation of anti-12-hydroxy-endrin O-sulphate in female rats (Hutson et al. 1975). Richardson et al. (1970) also reported sex differences in the proportion of fecal metabolites in rats; however, metabolites were identified as “1” and “2” and not further characterized except to state that they were not ketone rearrangement products of endrin. In mice, strains resistant to acute endrin toxicity produce anti-12-hydroxyendrin at higher rates (~2-fold) compared with strains that are susceptible to acute endrin toxicity (Petrella et al. 1977).

Figure 3-1. Proposed Metabolism Scheme for Endrin in Mammals



Hydroxylated metabolites are conjugated as glucuronides and sulfates. The balance of products in this last step and their distribution between urine and feces distinguishes the metabolism between humans, rats, and rabbits (Baldwin and Hutson 1980; Bedford et al. 1975b; Hutson 1981; Hutson et al. 1975). Similarly, studies in lactating cows ingesting radiolabeled endrin in the diet for 21 days suggest metabolic pathways similar to those in rats and rabbits with apparent differences between the three species attributed more to differences in biliary versus renal excretion (Baldwin et al. 1976).

In workers in pesticide manufacturing plants, anti-12-hydroxyendrin as the glucuronide and 12-ketoendrin were found in both urine and feces (three of seven workers) (Baldwin and Hutson 1980).

Anti- and syn-12-hydroxyendrin and 12-ketoendrin are more toxic in the rat than endrin itself. The hydroxyendrins are rapidly converted to the more toxic 12-ketoendrin, and this latter metabolite is most likely the toxic entity of endrin (Bedford et al. 1975a; Hutson et al. 1975).

3.1.4 Excretion

Measurements of human serum concentrations of endrin following incidents of acute poisoning indicate rapid decline in concentration after exposure, suggesting rapid excretion (Coble et al. 1967; Rowley et al. 1987). Anti-12-hydroxyendrin and 12-ketoendrin were detected in the feces of pesticide manufacturing workers and its glucuronide conjugate and 12-ketoendrin have been detected in the urine (Baldwin and Hutson 1980). In another study, the levels of anti-12-hydroxyendrin increased accompanied by a sharp rise in D-glucaric acid levels in 29 workers after 7 days of exposure (Ottevanger and Van Sittert 1979; Vrij-Standhardt et al. 1979). Endrin has also been detected in human breast milk, cord blood, and placental tissues in several studies worldwide (Alawi et al. 1992; Bedi et al. 2013; Bordet et al. 1993; Fujii et al. 2012; Gladen et al. 1999; Guillette et al. 1998; Lopez-Espinosa et al. 2007; Polanco-Rodriguez 2017; Romero et al. 2000; Schaalan et al. 2012). Endrin, endrin aldehyde, and endrin ketone may also be excreted in humans via sweat (Genuis et al. 2016).

In rats, the bulk of endrin metabolites excreted by rats are in the bile as glucuronides (Hutson et al. 1975). Rabbits excrete ^{14}C-endrin in the urine as sulfates (Bedford et al. 1975b). Studies in lactating cows ingesting endrin in the diet for 21 days show that ^{14}C-endrin is readily excreted as unchanged endrin in the milk, accounting for 2.5–4.3% of the total dose (Baldwin et al. 1976). Due to its lipophilic nature (partition coefficient [Logow]: 5.6), endrin was contained in the lipid portion of the milk.

In rats, the major route of elimination is the feces, with a smaller percentage eliminated in the urine, and there are apparent sex differences. Twenty-four hours after oral exposure to 0.5–2.5 mg/kg, 55–57% of ^{14}C-endrin was metabolized in the bile; the predominant metabolite was the glucuronide of anti-12-hydroxyendrin (Hutson et al. 1975). Other minor components (<10%) were the glucuronides of 3-hydroxy- and 12-ketoendrin. Male rats eliminated 69% of the radioactive label within 3 days and females eliminated 45%. Another study in male rats reported elimination of 49% of the radioactive label within 3 days following oral exposure (Richardson et al. 1970). In studies with isolated perfused livers, ^{14}C-endrin was excreted in the bile of livers from male rats at a rate 2–12 times higher than that for females (Klevay 1971). In urine, the major metabolites in males and females were 12-ketoendrin and 12-hydroxyendrin-O-sulfate, respectively, following oral exposure (Hutson et al. 1975). Baldwin et al. (1970) also detected 9-ketoendrin in the urine of rats.

In rabbits administered radiolabeled endrin, 50% of the radioactivity was excreted in the urine over a 50-day period (Bedford et al. 1975b). Excretion of the label was 87% complete within 13 days. The major compounds detected in urine were anti-12-hydroxyendrin sulfate and 3-hydroxyendrin sulfate (14%).

No studies were located concerning excretion of endrin in animals after inhalation or dermal exposure.

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.

No chemical-specific PBPK models have been developed for endrin.

3.1.6 Animal-to-Human Extrapolations

No studies were identified that could evaluate potential differences in the toxicity or toxicokinetics of endrin between humans and animals. However, the primary toxicity target (nervous system) is consistent between exposed humans and animals. Some species differences were observed between different laboratory species; however, the targets of toxicity appear to be similar. Available mechanistic data are inadequate to evaluate potential species differences.

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

Persons with a history of convulsive disorders are expected to be at increased risk of nervous system effects if exposed to endrin. Data are inadequate to determine if children may be more sensitive than adults to the acute toxic effects of endrin. In an endrin poisoning episode in Pakistan, children 1–9 years old represented about 70% of the cases of convulsions (Rowley et al. 1987). The causative factor responsible for the outbreak was not identified, however, so it is unclear whether the age distribution of cases is due to increased susceptibility in children or age-specific exposure situations. In general, following oral administration, female animals appear to be more susceptible to endrin toxicity than males (Gaines 1960; Treon et al. 1955). The difference may be due to the more rapid excretion of endrin by

male versus female rats (Hutson et al. 1975; Klevay 1971; Korte et al. 1970). A sex-related difference in toxicity was not apparent following dermal exposure (Gaines 1960, 1969). No sex-based differences in endrin-related human toxicity have been documented. For example, an equal number of male and female patients were affected in the endrin poisoning episode in Pakistan (Rowley et al. 1987). Genetic differences in metabolism may also alter susceptibility to endrin toxicity as well, as a heritable resistance in pine mice to endrin raises the LD50 from as low as 1.37 mg/kg in sensitive strains to as high as 36.4 mg/kg in resistant strains (Webb et al. 1973). This trait is correlated with the higher amounts of detoxifying enzymes in resistant mice.

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 endrin 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 endrin 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 endrin are discussed in Section 3.3.2.

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

3.3.1 Biomarkers of Exposure

Levels of endrin or endrin metabolites can be measured in tissue and excreta, thereby serving as biomarkers of exposure. Further, measurements of endrin in blood are best suited for detecting recent exposures because endrin is cleared rapidly from blood. The lack of persistence of endrin in human tissues and blood seen in the study of Coble et al. (1967) indicates a brief half-life for endrin on the order of 1–2 days. Sera levels of endrin (time to sample not specified) in Pakistani patients who were poisoned with endrin ranged from 0.3 to 254 ppb (0.3–254 µg/L); survivors had sera levels that ranged from 1.3 to 17.4 ppb (1.3–17.4 µg/L) (Rowley et al. 1987). An endrin concentration of 0.3 ppb was detected in the cerebrospinal fluid. Hair may be a useful biomarker for prior exposure to endrin. Smith-Baker and Saleh (2011) demonstrated a sensitive method of endrin detection in hair that accurately distinguished occupationally exposed workers from the general public.

Measurements of endrin metabolites can also be useful in monitoring endrin exposure. The glucuronides of anti-12-hydroxyendrin and 12-ketoendrin have been detected in feces and urine (Baldwin and Hutson 1980). The anti-12-hydroxyendrin glucuronide marker is the most sensitive and specific urinary marker. D-glucaric acid is a nonspecific marker that may indicate prior exposure to endrin (Hunter et al. 1972; Ottevanger and Van Sittert 1979; Vrij-Standhardt et al. 1979). High levels of D-glucaric acid were detected in workers for up to 6 weeks, after which levels returned to normal ranges (Ottevanger and Van Sittert 1979).

Endrin levels in fat tissues may only be a useful biomarker after high occupational exposure, as endrin has only been detected in the adipose tissue of workers after very high exposures and not in the general population (EPA 1986b; Williams et al. 1988). Endrin has been detected in the milk of lactating women (0.02–6.24 mg/kg milk fat) (Alawi et al. 1992; Bordet et al. 1993).

3.3.2 Biomarkers of Effect

Changes in the nervous system are the most common effects associated with human exposure to endrin. Various signs and symptoms of exposure include twitching of muscles, dizziness, mental confusion, and epileptiform seizures (Carbajal-Rodriquez et al. 1990; Coble et al. 1967; Curley et al. 1970; Davies and Lewis 1956; Hoogendam et al. 1962, 1965; Rowley et al. 1987; Runhaar et al. 1985; Waller et al. 1992; Weeks 1967). However, these effects also occur following exposure to other organochlorine pesticides and other drugs, and are not specific to endrin.

3.4 INTERACTIONS WITH OTHER CHEMICALS

The toxicity of endrin may be influenced by interactions with other chemicals and physical agents, particularly other organochlorine pesticides.

Quails treated with endrin and chlordane had significantly lower endrin residues in brain tissue ($p<0.025$) than birds treated with endrin alone (Ludke 1976). It is not clear how co-administration of chlordane altered tissue distribution of endrin; however, the authors attributed this difference to the presence of one or more metabolites of chlordane in the nervous system. However, despite differences in endrin residues, toxicity of endrin was not altered by prior exposure to chlordane; 15/20 animals died within 10 days of daily endrin exposure and 14/20 animals died within 10 days of daily endrin exposure following 10 weeks of chlordane exposure (Ludke 1976).

Keplinger and Deichmann (1967) evaluated potential interactions between endrin and several other pesticides based on observed versus expected LD_{50} values in mice. After determining LD_{50} values for each compound, the expected LD_{50} value of a mixture was calculated and compared to the observed LD_{50} value of the mixture. The study authors considered ratios between 0.79 and 1.27 essentially additive, with higher ratios indicating greater-than-additive effects, and lower ratios indicating less-than-additive effects. Greater-than-additive effects were noted for endrin and chlordane (ratio of 2.22) and for endrin and aldrin (ratio of 1.83). The interactions observed for endrin plus dieldrin, diazinon, toxaphene, or malathion were additive, and the interactions observed for endrin plus parathion, DDT, and Delnav were less than additive (ratios of 0.65, 0.53, and 0.44, respectively). When a mixture of aldrin, chlordane, and endrin was administered, observed effects were considered additive (ratio of 1.27).

In an *in vitro* estrogenic potential assay, binary mixtures of organochlorine compounds (DDT, DDD, DDE, aldrin, dieldrin, endrin) did not increase estrogenic activity compared with estradiol; additional

testing with individual compounds also found no significant dose-related estrogenic activity (Mumtaz et al. 2002; Tully et al. 2000). More studies are needed to better identify and characterize potential synergistic effects of endrin with other compounds.

Pohl and Tylenda (2000) conducted binary weight-of-evidence (WOE) determinations of the potential for joint toxic action between endrin and other organochlorine pesticides. Based on their analyses, there is direct mechanistic data indicating a synergistic effect between endrin and the following organochlorines: DDT, aldrin, dieldrin, chlordane, hexachlorobenzene (HCB), and α-, β-, and δ-hexachlorocyclohexane (HCH). For γ-HCH, there is direct mechanistic data indicating an antagonistic effect on endrin. Demonstrated toxicological significance was only available for chlordane (see Ludke 1976 above); for the remaining compounds, Pohl and Tylenda (2000) inferred toxicological significance.