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

3.1 TOXICOKINETICS

Information on the toxicokinetics of disulfoton comes from studies in humans and animals. These data are summarized below.

- Disulfoton is readily and extensively absorbed by the gastrointestinal tract following oral exposure. It has been measured in the gastrointestinal tract up to 3 days after exposure in humans; however, the extent or rate of absorption in humans is unknown.
- Absorbed disulfoton is primarily distributed to the liver. It is also distributed to the kidneys, whole blood, red blood cells, plasma, fat, skin, muscles, brain, small intestine, pancreas, and bile.
- Three different pathways are associated with the metabolism of disulfoton: (1) oxidation of the thioether sulfur to produce sulfoxides and sulfones; (2) oxidation of the thiono sulfur to produce the oxygen analogs; and (3) hydrolysis of the P-S-C linkage to produce the corresponding phosphorothionate or phosphate.
- The major route of excretion of disulfoton is through urine, with smaller amounts being excreted in feces and expired air.

3.1.1 Absorption

Disulfoton and/or its metabolites have been detected in the blood and urine of humans who consumed unknown amounts of disulfoton (Futagami et al. 1995; Hattori et al. 1982; Yashiki et al. 1990). Di-Syston is a disulfoton mixture that is coated with surfactant and has a sandy and granular texture that may be easily absorbed into the gastric mucosa (Futagami et al. 1995). In one case, the concentration of disulfoton and the sum of its metabolites in the blood were 0.093 nmol/g (25.4 ng/g) and 4.92 nmol/g, respectively, about 2 hours after ingestion of Di-Syston (Yashiki et al. 1990). The 4.92 nmol/g blood concentration corresponded to 1.35 µg/g. Gastrointestinal absorption was not yet complete, since 3.3 mg of disulfoton was recovered from the stomach contents, which was collected about 2 hours after ingestion (Yashiki et al. 1990). In another case, total plasma phosphorodithioate sulfone (disulfoton and its metabolites, phosphorodithioate sulfoxide and its sulfone) in a patient who ingested an unknown amount of Di-Syston (5% disulfoton) was 1,095 ng/mL upon admission, and decreased to 505 ng/mL 20 hours after admission; however, there was a rebound increase on day 2 to 1,322 ng/mL (Futagami et al. 1995). Futagami et al. (1995) noted that the concentration at admission was about twice as high as reported in Yashiki et al. (1990). On day 2 the observed “rebound phenomenon” of the concentration in plasma was

partially attributed to the delayed, but prolonged absorption, of Di-Syston in the gastrointestinal tract. An odor of aspirated stomach fluid was also noted, suggesting the presence of Di-Syston in the gastrointestinal tract, up to day 3 of admission (Futagami et al. 1995). Contents from the stomach decreased following repeated gastrointestinal lavage. The concentration of metabolites in the urine was not quantitated in either the Yashiki et al. (1990) or Futagami et al. (1995) study. While these data indicate that disulfoton is absorbed from the gastrointestinal tract of humans, the data are not sufficient to estimate the extent or rate of absorption.

Male rats given a single acute dose (1.2 mg/kg) of [^{14}C]-disulfoton eliminated an average of 84.3, 6.1, and 9.2% of the dose in the urine, feces, and expired air, respectively, in the 10 days following exposure (Puhl and Fredrickson 1975). Female rats given 0.2 mg/kg eliminated 78.9, 7.8, and 9.2% of the administered radioactivity in the urine, feces, and expired air, respectively, in the same time period. The data indicate that at least 88–91% of the administered dose was absorbed over the 10-day period. Absorption rates were not determined; however, 50% of the administered dose was recovered in the urine during the first 4–6 hours after exposure in males and the first 30–32 hours after exposure in females. Although it was not possible to quantitatively determine the absorption rate in female rats, the data from the male rats suggest that absorption was almost complete within 12–24 hours of dosing. Female rats were given a lower dose than male rats, as they are more sensitive to the toxic effects of disulfoton. Nevertheless, it took longer for females to excrete 50% of the dose than males. Whether this sex difference is due to differences in absorption, metabolism, retention, excretion, or a combination of factors is not known.

In another study, rats received [^{14}C]-disulfoton at a single oral dose of 0.2 or 1.0 mg/kg or repeated oral doses 0.2 mg/kg/day for 14 days (Lee et al. 1985). In the rats given a single dose of 0.2 mg/kg, the respective percentages of administered radioactivity 72 hours later in females and males were: urine, 97.1 and 96.9%; feces, 1.1 and 1.4%; tissues, 0.1% in both sexes; carcass, 0.7% in both sexes; and cage rinses, 1.0 and 0.9%. In the rats given a single dose of 1.0 mg/kg, the respective percentages of administered radioactivity for females and males were: urine, 97.5 and 96.9%; feces, 1.7 and 1.9%; tissues, 0.1 and 0%; carcass, 0.5 and 0.4%; and cage rinse, 0.2 and 0.8%. In the rats given 14 daily doses of 0.2 mg/kg/day, the respective percentages in females and males were: urine, 97.1 and 98%; feces, 0.5 and 0.7%; tissues, 0.1 and 0.3%; carcass, 0.9 and 0.5%; and cage rinse, 1.4 and 0.5%. Based on the percentages of administered radioactivity in the urine, ≥97% of the administered dose was absorbed from the gastrointestinal tract within 72 hours. At least 90% of the administered dose was excreted in the urine in the first 24 hours, indicating rapid absorption. In a preliminary experiment, in which rats were given a

single oral dose of 0.2 mg/kg radioactive disulfoton, urinary excretion was essentially complete within 48 hours.

Gastrointestinal absorption of disulfoton was extensive following oral exposure of rats and guinea pigs, as evidenced by the small differences in the oral LD_{50} values versus the intraperitoneal LD_{50} values (Bombinski and DuBois 1958).

Zenzdian (2000) developed standard protocols for evaluating dermal penetration of pesticides in rats. The rate of dermal absorption for disulfoton at a dose of 3.1 nM/cm^2 ranged from 15.9% of the administered dose at 1 hour to 42.0% at 168 hours. Initially, absorption at higher doses was lower but it approached the same maximum after 168 hours.

3.1.2 Distribution

Analysis of tissues and blood for radioactivity at various time intervals after rats were dosed with [^{14}C]-disulfoton (1.2 mg/kg for males, 0.2 mg/kg for females) showed that peak levels occurred 6 hours after dosing (Puhl and Fredrickson 1975). The highest levels were found in the liver (peak was 3.6 mg/kg for males, 2.3 mg/kg for females). Peak levels in other tissues (kidneys, plasma, fat, whole blood, skin, muscles, and brain in descending order) also generally occurred at 6 hours. At 10 days after dosing, the levels of radioactivity in all tissues decreased; however, low levels were found in the heart at this sampling time. In Beagle dogs dosed with 0.5–1.5 mg/kg/day disulfoton in capsules for 2 years, disulfoton was detected in the kidneys (0.06 ppm), urine (0.06 ppm), liver (0.02 ppm), serum (0.04 ppm), and brain and spinal cord (0.01–0.02 ppm) (Hikita et al. 1973). Disulfoton and its metabolites (unidentified) were also detected in small intestine, pancreas, bile, fatty tissues, thymus, spleen, red blood cells, extraocular muscles, and muscles of the extremities and torso.

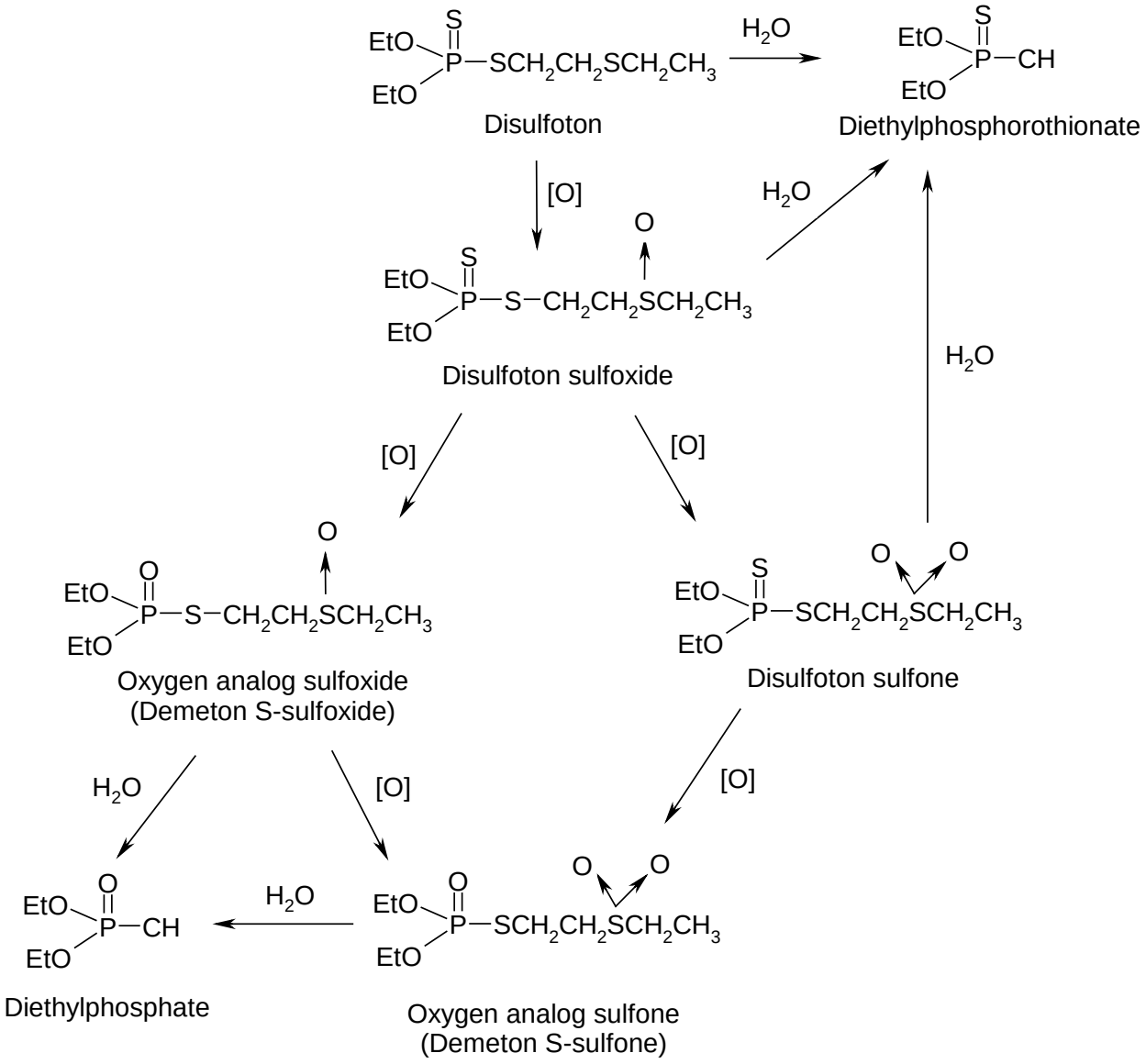
In rats, after 1 hour of dermal exposure to 3.1 nM/cm^2, the systemic distribution of disulfoton was 0.48% of the administered dose in the blood, and 4.84% in the carcass (Zenzdian 2000). These values reduced to 0.01 and 0.09% respectively, at 168 hours.

3.1.3 Metabolism

Three different pathways are associated with the metabolism of disulfoton: (1) oxidation of the thioether sulfur to produce sulfoxides and sulfones; (2) oxidation of the thiono sulfur to produce the oxygen

analogs; and (3) hydrolysis of the P-S-C linkage to produce the corresponding phosphorothionate or phosphate (WHO 1976) (see Figure 3-1). These pathways have been elucidated from data obtained in humans exposed to disulfoton and from *in vivo* and *in vitro* metabolism studies in rats and mice.

Figure 3-1. Metabolic Pathways for Disulfoton



The oxygen analog of disulfoton sulfoxide (demeton S-sulfoxide) and the oxygen analog of disulfoton sulfone (demeton S-sulfone) were identified in the urine from an 87-year-old man who accidentally drank an unknown amount of diluted disulfoton (Yashiki et al. 1990). Disulfoton sulfone and demeton S-sulfone were the only metabolites of disulfoton detected in the blood of this patient. The authors did not report whether they detected the products of disulfoton and/or sulfoxide/sulfone hydrolysis, DEP,

DETP, and DEDPT in the urine. From this case report, there is evidence of oxidation of the thioether and thiono sulfur, which produces sulfoxides or sulfones and oxygen analogs of disulfoton, respectively (Yashiki et al. 1990). In a 75-year-old woman who ingested Di-Syston granules, disulfoton and its metabolites phosphorodithioate sulfoxide and phosphorodithioate sulfone were detected in plasma (Futagami et al. 1995). Workers exposed mainly to disulfoton at a pesticide formulating plant excreted the metabolites DEP, DETP, DEDPT, and DEPTh in urine after dermal and possibly inhalation exposure to disulfoton (Brokopp et al. 1981).

Studies in rats and mice indicate pathways similar to humans. Unidentified urinary metabolites in mice injected intraperitoneally with 32-ρ disulfoton were described as hydrolysis products (March et al. 1957). The metabolites, disulfoton sulfoxide, disulfoton sulfone, demeton S-sulfoxide, and demeton S-sulfone were also identified as products of *in vitro* hepatic disulfoton metabolism. Disulfoton sulfoxide (11.3%), disulfoton sulfone (2.4%), demeton S-sulfoxide (26.7%), and demeton S-sulfone (59.6%) were identified in the livers of rats 30 minutes after intraperitoneal injection with disulfoton (Bull 1965). Disulfoton sulfone was the only one of these metabolites not recovered from the liver 120 minutes after exposure. DEP and DETP, formed from the hydrolysis of disulfoton and/or its oxidation products, were identified as the major urinary metabolites in rats dosed orally or intraperitoneally in several studies (Bull 1965; Puhl and Fredrickson 1975; Wolfe et al. 1978). The minor urinary metabolites included disulfoton sulfoxide, demeton S-sulfoxide, and demeton S-sulfone (Puhl and Fredrickson 1975). Although disulfoton sulfone was not detected in the urine in this study, it can be assumed that, subsequent to its formation, it was quickly oxidized to demeton S-sulfone or quickly hydrolyzed to DETP. In another study, disulfoton sulfone was found in the urine of rats after oral exposure to disulfoton (Lee et al. 1985). These findings are consistent with the pathways in Figure 3-1, whereby disulfoton metabolism proceeds via the sequential oxidation of thioether sulfur and/or oxidative desulfuration followed by hydrolysis of the ester. The data also suggest that a greater percentage of disulfoton sulfoxide is oxidized to demeton S-sulfoxide, rather than disulfoton sulfone, to form demeton S-sulfone (Bull 1965). The relative importance of each pathway, however, cannot be deduced from relative percentages of metabolites formed because the final urinary metabolites are common products of several of the intermediate metabolites. In addition, after a single dose of 0.2 mg/kg [^{14}C]-disulfoton, disulfoton sulfone, demeton S-sulfone, and demeton S-sulfoxide were found in urine of male rats, while only demeton S-sulfone was apparent in the urine of female rats (Lee et al. 1985). However, after dosing with 0.2 mg/kg/day for 14 days, the pattern in both sexes was reversed. This reversed pattern after repeated dosing was more likely due to metabolic rate differences than to a difference in pathway, since disulfoton sulfone and demeton S-sulfoxide are precursors to demeton S-sulfone.

The studies described above support the accepted theory that most thioether organophosphate insecticides, such as disulfoton, first undergo metabolic oxidation to sulfoxides, sulfones, and their respective oxygen analogs as part of the metabolic activation pathway (Eto 1974). These active metabolites bind to ubiquitous AChE and cause signs of disulfoton toxicity. In the detoxification pathway, these oxidation products and/or disulfoton subsequently undergo hydrolysis to more polar metabolites that are eliminated in the urine. Cytochrome P450 monooxygenase and Flavin adenine dinucleotide (FAD) monooxygenase are thought to be involved in the metabolic activation pathways.

Generally, organophosphates serve as substrates for the hepatic cytochrome P450 mixed-function oxidase (MFO) system. The components of the MFO system include cytochrome P450, the terminal oxidase, and NADPH, and NADPH-dependent cytochrome c reductase (Stevens and Greene 1974). Generally, anticholinesterase insecticides such as disulfoton bind to oxidized cytochrome P450 to form a disulfoton:cytochrome P450 complex (Stevens et al. 1973). An electron is then transferred from cytochrome c reductase to cytochrome P450 (Gillette et al. 1972), thereby reducing the disulfoton:cytochrome P450 complex. Molecular oxygen then binds to this complex to form a disulfoton:reduced cytochrome P450:02 complex (Gigon et al. 1969). A second electron from NADPH or reduced nicotinamide adenine dinucleotide (NADH) then reduces this complex to form an active oxygen intermediate that decomposes with the formation of the product and oxidized cytochrome P450 (Hildebrandt and Estabrook 1971).

Flavin monooxygenase specifically oxidizes sulfides to (R)-(+)-sulfoxide enantiomers, while cytochrome P450-dependent oxidations yield predominantly sulfoxides in the (S)-(-) configuration (Light et al. 1982; Waxman et al. 1982). Disulfoton has three sulfur atoms that can be oxidized: the thiophosphoryl or thiono, the thiol, and the thioether. It has been proposed that flavin monooxygenase I cannot catalyze P=S to P=O conversions (Hajjar and Hodgson 1980). The flavin monooxygenase enzymes metabolize thioether-containing organophosphates to sulfoxides only; that is, there is no evidence for the formation of any other products such as disulfoton sulfones in the presence of only FAD monooxygenase (Hajjar and Hodgson 1980). Sequential oxidations by both monooxygenases (FAD-dependent and cytochrome P450) may be required to form sulfones (Tynes and Hodgson 1985). Disulfoton interacted with cytochrome P450 to markedly inhibit the metabolism of p-nitroanisole and parathion, both of which have rather high affinities for cytochrome P450. These findings underscore the fact that cytochrome P450 and flavin monooxygenase both have the potential to participate in the oxidation of disulfoton (Tynes and Hodgson 1985).

FAD-dependent monooxygenase, purified from pig liver microsomes, oxidized disulfoton (Hajjar and Hodgson 1982). The product of this reaction was disulfoton sulfoxide. However, disulfoton sulfoxide was not a substrate for this enzyme, as disulfoton sulfone was not detected. Structure-activity relationships suggest that substitution by oxygen of either the thiono or thiol sulfur atoms decreases the activity of FAD-dependent monooxygenase and thus the rate of sulfoxidation. In addition, changes in the thioether sulfur have a similar effect. Structural changes on the thioether moiety may increase steric hindrance of the sulfur atom, affect enzyme-substrate binding, and decrease the rate of sulfoxidation. Thus, disulfoton sulfones were not formed, and further oxidation of the sulfoxides to sulfones did not involve FAD-dependent monooxygenase but rather another oxidase or nonenzymatic reaction. Sulfoxidation was not inhibited by n-octylamine, a known inhibitor of cytochrome P-450-dependent oxygenation (Hajjar and Hodgson 1982). This finding further suggests that FAD-dependent monooxygenase may play a greater role than cytochrome P-450 monooxygenase in the oxygenation of thioether organophosphates. Compared to most other thioether compounds, it was concluded that disulfoton is among the best known flavin monooxygenase substrates (Poulsen 1981). Compared to most other organophosphate insecticides (parathion, diazinon, ethion, phorate, azinophosmethyl, methyl parathion, and ronnel), disulfoton was more rapidly metabolized in the hepatic microsomal oxidative system involving NADPH from rats, guinea pigs, and monkeys (Rao and McKinley 1969).

The metabolism of disulfoton appears to be similar among mammalian species studied. For example, liver homogenates from rats, guinea pigs, and monkeys were generally more active in metabolizing disulfoton than liver homogenates from chickens (Rao and McKinley 1969). Flavin monooxygenase in pig liver also had a higher affinity (lower Km) than the mouse enzyme towards disulfoton (Smyser et al. 1985). Rat liver and lung microsomes have lower flavin monooxygenase activity towards disulfoton than liver or lung microsomes from the mouse or the rabbit (Tynes and Hodgson 1985). However, flavin monooxygenase activity was greater in rabbit and mouse lungs than in their respective livers. This disparity between lung and liver tissues was not observed in rats.

3.1.4 Excretion

No studies were located regarding the rate or extent of excretion in humans after exposure to disulfoton.

Male rats given a single acute dose (1.2 mg/kg) of [^{14}C]-disulfoton eliminated an average of 84.3, 6.1, and 9.2% of the dose in the urine, feces, and expired air, respectively, in the 10 days following exposure (Puhl

and Fredrickson 1975). Female rats given 0.2 mg/kg eliminated 78.9, 7.8, and 9.2% of the administered radioactivity in the urine, feces, and expired air, respectively, in the same time period. Male rats excreted 50% of the administered dose in the urine during the first 4–6 hours after exposure, while females required 30–32 hours to excrete 50% of the dose in the urine. The female rats were given a lower dose than the males because female rats are more sensitive than male rats to the toxic effects of disulfoton. Nevertheless, it took longer for females to excrete 50% of the dose. Whether this sex difference is due to differences in absorption, metabolism, retention, excretion, or a combination of factors is not known.

In another study, rats received [^{14}C]-disulfoton at a single oral dose of 0.2 or 1.0 mg/kg or repeated oral doses of 0.2 mg/kg/day for 14 days (Lee et al. 1985). In the rats given a single dose of 0.2 mg/kg, the respective percentages of administered radioactivity 72 hours later in females and males were 97.1 and 96.9% in urine and 1.1 and 1.4% in feces. In the rats given a single dose of 1.0 mg/kg, the respective percentages of administered radioactivity for females and males were 97.5 and 96.9% post-dosing in urine and 1.7 and 1.9% in feces. In the rats given 14 daily doses of 0.2 mg/kg/day, the respective percentages in females and males were 97.1 and 98% in urine and 0.5 and 0.7% in feces. Thus, the primary route of excretion in all dose groups was via the urine (at least 97% in each group), and excretion was essentially complete within 72 hours post-dosing, with at least 90% excreted in the first 24 hours. In a preliminary experiment in which rats were given a single oral dose of 0.2 mg/kg radioactive disulfoton, urinary excretion was essentially complete within 48 hours. Analysis of expired gases at 24-hour intervals for 144 hours post-dosing in the preliminary experiment indicated that only 0.5 and 0.2% of the radioactivity in females and males, respectively, was present in the expired air.

White rats given a single dose of radiolabeled disulfoton intraperitoneally eliminated the metabolites phosphoric acid (4.1%), DEP (61.2%), and DETP (24.8%) in urine as a percentage of excretory hydrolytic metabolites 12 hours after exposure (Bull 1965). Approximately 24 and 48 hours after exposure, 14.1 and 28.6%, respectively, of the administered dose was excreted in the urine. Excretion rates for disulfoton and its metabolites were not determined. Mice eliminated 30–60% of the radiolabeled intraperitoneal dose of disulfoton in the urine and 2–3% in the feces within 96 hours of exposure (March et al. 1957).

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 disulfoton.

3.1.6 Animal-to-Human Extrapolations

Metabolism of disulfoton in humans and animals appears to be qualitatively and quantitatively similar (Brokopp et al. 1981; Bull 1965; Puhl and Fredrickson 1975; Yashiki et al. 1990). The intermediary products of disulfoton metabolism, rather than disulfoton itself, are responsible for the signs of toxicity observed in humans and animals exposed to the pesticide. These metabolites (disulfoton sulfoxide, disulfoton sulfone) and the oxygen analogs (demeton S-sulfoxide and demeton S-sulfone) are oxidation products of disulfoton and are formed primarily in the liver (Bull 1965; March et al. 1957). In animal studies, females appear more sensitive to toxic effects of disulfoton following inhalation, oral, or dermal exposure (Bombinski and DuBois 1958; Crawford and Anderson 1974; Gaines 1969; Klaus 2006b; Mihail 1978; Pawar and Fawade 1978; Thyssen 1978). Males appear to excrete disulfoton faster (Lee et al. 1985; Puhl and Fredrickson 1975). It is unknown if these differences between the sexes are seen in humans.

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

No data were located that identify subpopulations of humans more susceptible to the toxic effects of disulfoton or data specific to children. Since significant cholinesterase activity inhibition has been seen in animals and humans following exposure to disulfoton (see Section 2.15), populations taking AChE inhibitors (anticholinesterases) may be further susceptible to toxic effects of disulfoton. Anticholinesterases, such as Galantamine and Donepezil, are used as pharmaceuticals most commonly to treat neurodegenerative disease such as Parkinson's disease, Alzheimer's disease, dementia, glaucoma, and myasthenia gravis (Khan et al. 2018; Knight et al. 2018; Moss 2020). Anticholinesterases are also used to reverse the effects of nondepolarizing muscle agents used during intubation in general anesthesia (Shaydenfish et al. 2020). Plasma cholinesterase activity may be lowered by liver disease, malnutrition, infection, and renal failure (Anderson et al. 2019).

Plasma cholinesterase is more often reduced by the present of certain genetic variants (Anderson et al. 2019). It is estimated that between 1:3000 and 1:10,000 individuals are homozygous for the genetic enzyme variant allele code for decreased cholinesterase activity or quantity (Anderson et al. 2019). Lockridge et al. (2016) also describes a genetic variant associated with decreased plasma butyrylcholinesterase activity and reduced enzyme concentration. Based on occupational and case studies, the study authors suspect that individuals with this genetic variant may likely be susceptible to further cholinesterase inhibition from exposure to organophosphates pesticides including disulfoton (Lockridge et al. 2016).

Depressed serum cholinesterase has been routinely reported in agricultural workers who are likely exposed to various carbamates and organophosphates in agropesticides (Ames et al. 1989; Neupane et al. 2014; Shentema et al. 2020; Thetkathuek et al. 2017). It is expected that workers with inhibited cholinesterase activity from previous or current exposures to other pesticides may be susceptible to further cholinesterase inhibition from exposure to disulfoton.

There is insufficient information to determine if effects in children would be similar to effects seen in adults after acute exposure or long-term, low-level exposure to disulfoton. It is unknown if disulfoton

affects the developing human fetus or the development of children, and if disulfoton or its metabolites cross the placental barrier. Animal studies suggest that younger animals are more susceptible to disulfoton toxicosis than older animals as mortality in weanling rats was seen at lower levels when compared to adult rats (Brodeur and Dubois 1963). The study proposed that the relatively slow rate of metabolic detoxification and/or incomplete development of detoxification enzymes in weanlings accounted for the difference in the effects. Also, calves were more sensitive to disulfoton than yearling cattle, as indicated by an increase in severe clinical signs and a greater depression of cholinesterase activity in calves (McCarty et al. 1969).

Animals and humans are expected to have similar metabolic pathways of disulfoton (see Section 3.1.3), and these are expected to be similar in children. However, there is insufficient information on the movement of disulfoton into the developing fetus or breast milk. There are no child, adult, or laboratory animal PBPK models, nor are there children-specific biomarkers.

Limited information is available from animal studies on potential effects of oral disulfoton exposure in the developing young, as discussed in Section 2.17. When pregnant rats were given disulfoton at 1.0 mg/kg/day, there were increased incidences of incomplete ossified parietal bones and sternebrae; however, no soft tissue, external, or skeletal malformations were observed in pups (Lamb and Hixson 1983). The authors considered this evidence of growth retardation due to maternal toxicity rather than specific fetotoxic effects. In another study, prior to mating, rats were given 0.5 mg/kg/day disulfoton in diet for 60 days, resulting in marked depression of fetal brain AChE activity (Ryan et al. 1970). In multi-generational exposure studies, third generation litters had cloudy swelling and fatty infiltration of the liver, mild nephropathy, juvenile hypoplasia of the testes in males, and depressed red blood cell AChE activity (Taylor 1965a). In a similar study, depressed brain AChE activity was seen in first generation litters in the mid- and high-dose exposure groups, 0.03 and 0.09 mg/kg/day, respectively (Hixson and Hathaway 1986).

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 disulfoton 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 disulfoton 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 disulfoton 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

Disulfoton and its metabolites have been measured in various human or animals tissues and body fluids (blood, urine, feces, liver, kidneys, and body fat) following disulfoton exposure (Brokopp et al. 1981; Hattori et al. 1982; Puhl and Fredrickson 1975; Yashiki et al. 1990). Because disulfoton is quickly metabolized, it is rarely detected in the blood or plasma of exposed individuals, but detection of the insecticide in blood provides conclusive evidence of previous exposure. About 2–3 hours after a man accidentally ingested disulfoton, 0.093 nmol/g (4.92 ng/g) of disulfoton and 4.92 nmol/g of total metabolites were detected in his blood (Yashiki et al. 1990). In another study, 1.45 nmol/g of disulfoton

was detected in the blood of a man found dead at least 24 hours after he had ingested disulfoton (Hattori et al. 1982). A total disulfoton and metabolite level of 1,095 ng/mL was measured in the blood of a woman 3.5 hours after ingestion of disulfoton (Futagami et al. 1995). In all cases, the ingested dose was unknown; therefore, a correlation between disulfoton exposure and blood concentration cannot be made.

The presence of disulfoton and/or its metabolites in the liver appears to be a sensitive indicator of disulfoton exposure, despite the limited data. Supporting evidence from animal studies indicates that disulfoton exposure could result in detectable levels in the liver (Bull 1965; Puhl and Fredrickson 1975), but monitoring of liver levels in humans would require biopsy. Dialkyl phosphate metabolites are used as biomarkers of exposure to multiple organophosphate insecticides and thus cannot be solely used to identify disulfoton exposure. Additionally, the measurement of these metabolites primarily reflects recent exposure to organophosphates. Specimens of urine collected from 31 locations across the United States, comprising the sample areas of the National Health and Nutrition Examination Survey (NHANES) from 2011–2012, reported detection (detection limit 0.1 ng/mL) of DETP at a frequency of 71% and DEDPT at a frequency of 5.4% of those tested (CDC 2019). Although no human data were located on the relationship between the concentration of urinary metabolites and the exposure dose, data from several animal studies demonstrate that 28.6–98% of the dose was accounted for in the urine 2–10 days post-exposure (Bull 1965; Lee et al. 1985; Puhl and Fredrickson 1975). An unknown amount of disulfoton sulfoxide and/or demeton S-sulfone was detected in the urine from a person exposed to an unknown amount of disulfoton (Yashiki et al. 1990). Disulfoton sulfoxide and disulfoton sulfone are specific to disulfoton but are only reported in this one study. Results from a human occupational study of pesticide formulators who had worked with disulfoton for 25 weeks showed that the metabolites DEP (0.01–4.4 ppm), DETP (0.01–1.57), DEDPT (<0.01–0.05 ppm), and DEPTh (<0.01–0.55 ppm) were detected in the urine (Brokopp et al. 1981). The mean preformulation urinary levels were 0.05 ppm DEP, 0.04 ppm DETP, 0.01 ppm DEDPT, and 0.008 ppm DEPTh. Threshold levels of these metabolites, defined as two standard deviations above the mean, were 0.13 ppm DEP, 0.12 ppm DETP, 0.06 ppm DEDPT, and 0.06 ppm DEPTh. Although the excretion of DEP varied considerably among the individuals, this metabolite was more commonly detected above the threshold level among these employees. One animal study demonstrated that a greater percentage of the disulfoton dose was eliminated as DEP (Bull 1965). This provides limited evidence that DEP is a more sensitive urinary biomarker than the other metabolites discussed.

Urine catecholamines may also serve as biomarkers of disulfoton exposure as evidenced by limited animal data, since no human data are available. Disulfoton exposure caused a 173 and 313% increase in

urinary noradrenaline and adrenaline levels in female rats, respectively, within 72 hours of exposure (Brzezinski 1969). The major metabolite of catecholamine metabolism, HMMA, was also detected in the urine from rats given acute doses of disulfoton (Wysocka-Paruszewska 1971). Organophosphates and carbamates are known to inhibit AChE, which can cause an accumulation of acetylcholine at the nerve synapses (King and Aaron 2015), and since the secretion of catecholamines is influenced by acetylcholine (Norman and Henry 2015), it is likely that other organophosphates can also cause a release of catecholamines from the adrenals and the nervous system. In addition, increased blood and urine catecholamines can be associated with overstimulation of the adrenal medulla and/or the sympathetic neurons by excitement/stress or sympathomimetic drugs, and other chemical compounds such as reserpine, carbon tetrachloride, carbon disulfide, DDT, and monoamine oxidase inhibitors (MAO) inhibitors (Brzezinski 1969). For these reasons, a change in catecholamine levels is not a specific indicator of disulfoton exposure.

Disulfoton induced the liver MFO system in animals (Stevens et al. 1973). In the same study, exposure to disulfoton orally for 3 days also increased ethylmorphine N-demethylase and NADPH oxidase activities; however, no effect on NADPH cytochrome c reductase was observed. These changes are not specific for disulfoton exposure, and these subtle liver effects require invasive techniques in humans to obtain liver tissue for performance of these enzyme assays.

3.3.2 Biomarkers of Effect

Disulfoton toxicity manifests as cholinergic toxicity symptoms such as salivation, diarrhea, pupil constriction, muscle tremors, nausea, and weight loss. These symptoms have been observed in humans accidentally exposed to disulfoton (Futagami et al. 1995; Yashiki et al. 1990) and in animals given disulfoton (Schwab et al. 1981). Ataxia, convulsions, coma, respiratory distress, and death are common signs associated with a more severe toxicosis. Nervous tissue is the most sensitive target organ.

Cholinesterase inhibition is a biomarker for other organophosphates and thus not always conclusive evidence of disulfoton toxicity (Osweiler et al. 1985). Organophosphates share a common mechanism of cholinesterase inhibition and similar adverse effects and symptoms have been observed (Robert and Reigart 2013). Depression of cholinesterase activity can indicate the possibility of more serious neurological effects, but the severity of the signs and symptoms and the degree of cholinesterase depression are not always correlated. Employees occupationally exposed to disulfoton for 9 weeks had marked depression of red blood cell AChE activity, but no clinical signs of toxicity (Wolfe et al. 1978).

Animal studies have demonstrated that brain AChE depression is a sensitive indicator of neurological effects (Carpy et al. 1975; Costa et al. 1984; Schwab and Murphy 1981; Schwab et al. 1981, 1983). Inhibition of red blood cell AChE activity or serum cholinesterase activity with or without concomitant neurological signs is a common indicator of organophosphate exposure. Red blood cell AChE activity more accurately reflects the degree of synaptic cholinesterase inhibition in nervous tissue, while serum cholinesterase activity may be associated with other sites (Goldfrank et al. 1990). In a 14-day rat study, while T-lymphocyte AChE correlated better with brain AChE activity than did red blood cell AChE, the recovery of T-lymphocyte activity recovered faster; therefore, red blood cell activity correlated better with brain AChE (Fitzgerald and Costa 1993).

AChE (also known as red blood cell cholinesterase, erythrocyte cholinesterase, or true cholinesterase) activity is typically used as a biomarker of effect for organophosphate toxicity, including disulfoton. Specifically, a reduction of an individual's activity relative to their baseline activity indicates a toxic effect; ATSDR considers a ≥20% decrease in an individual's cholinesterase activity a toxic effect. The normal range of AChE activity can be wide due to the variation in the human population; therefore, the percentage change is used instead of activity levels. In a case study, a woman had depressed red blood cell AChE activity of 3,524 IU/L at admission and 3,122 IU/L 19 days after exposure (reference range: 10,000–14,000 IU/L); observed cholinergic signs of toxicity gradually decreased over the 19-day period despite continued depression of AChE activity (Futagami et al. 1995). Cholinesterase depression was not observed in 11 employees exposed to disulfoton for ≤2.5 weeks, but the presence of urinary metabolites of disulfoton (a biomarker of exposure) indicated exposure (Brokopp et al. 1981).

Plasma cholinesterase (also known as serum cholinesterase, pseudocholinesterase, or butyryl-cholinesterase) is used to support acetylcholinesertase and clinical manifestations to diagnose organophosphate toxicity (Moon and Chun 2014; Strelitz et al. 2014; Worek et al. 2005). Experimental studies of animals exposed to disulfoton have observed that plasma cholinesterase activity decreases more rapidly than AChE but recovers to baseline more quickly (Klaus 2006a, 2006b; Sheets 2005). In one clinical case, a man who showed severe signs of cholinergic toxicity after accidentally ingesting disulfoton had depressed serum cholinesterase activity up to 8 days after exposure (Yashiki et al. 1990). A relationship between plasma cholinesterase and clinical symptoms of organophosphate poisoning has been observed (Prasad et al. 2013; Worek et al. 2005) and plasma cholinesterase appears to be most accurate for acute prognosis of organophosphate poisoning (Aygun et al. 2002). However, use of plasma cholinesterase on its own as a diagnostic tool is not agreed upon because: (1) it is most often used as a biomarker of liver function; (2) it can be altered with pregnancy, infection, and various medical illnesses;

and (3) levels can vary widely in an individual with repeat sampling (Katz and Brooks 2020; Zivkovic et al. 2014). Therefore, while assaying plasma cholinesterase activity is easier, red blood cell AChE is considered to be a more accurate biomarker for nervous system toxicity (Katz and Brooks 2020).

As previously stated, cholinesterase activity and neurological symptoms are used as biomarkers of effect for disulfoton, other organophosphates, and carbamate pesticides which may affect the toxicity of disulfoton. Furthermore, liver disease, malnutrition, infection, renal failure, and anticholinesterase pharmaceuticals, which are used to treat neurodegenerative diseases, may also lower AChE activity (Anderson et al. 2019; Khan et al. 2018; Knight et al. 2018; Moss 2020). Serum β-glucuronidase activity was increased in a dose-related manner when disulfoton was given intraperitoneally to rats (Kikuchi et al. 1981). In the same study, this effect was not observed in mice, rabbits, or guinea pigs.

Increased levels of urinary catecholamines may also be associated with accumulation of acetylcholine that resulted from AChE inhibition by disulfoton. No human data were located to support this, but limited animal data provide some evidence. Disulfoton exposure caused a 173 and 313% increase in urinary noradrenaline and adrenaline levels in rats, respectively, within 72 hours (Brzezinski 1969). The major metabolite of catecholamine metabolism, HMMA, was also detected in the urine from rats given acute doses of disulfoton (Wysocka-Paruszewska 1971).

In rats given a single oral dose of disulfoton, gamma-enolase mRNA in sciatic nerve increased by 200% two hours after the exposure and exceeded 250% 30 days after exposure (Matsuda et al. 2000). In the same study, depressed AChE mRNA levels in soleus muscle and sciatic nerve indicated disulfoton exposure 12 hours after exposure. Since gamma-enolase mRNA levels remained high for over 4 weeks following exposure, study authors suggested up-regulation of gamma-enolase mRNA as a marker of nervous system abnormality. However how an increase in gamma-enolase mRNA indicates toxicity is unclear (Matsuda et al. 2000). No additional studies were located that examined the up-regulation of gamma-enolase mRNA following organophosphate exposure. A more detailed discussion of the health effects caused by disulfoton can be found in Chapter 2.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Disulfoton can function as an inhibitor of MFO when given in one or two doses and can potentiate the toxicity of similarly related compounds. Disulfoton exhibits Type I binding, that is, binding to the oxidized form of cytochrome P-450, and when given as a single dose, competitively inhibits the

metabolism of other Type I substrates (Stevens et al. 1973). However, it was also reported that disulfoton was a noncompetitive inhibitor of rat and mouse ethylmorphine N-demethylase (Stevens and Greene 1974; Stevens et al. 1972a). When given as a single dose, disulfoton also appears to inhibit NADPH cytochrome c reductase (Stevens et al. 1973). Disulfoton was reported to inhibit hexobarbital metabolism, thereby prolonging hexobarbital sleeping time in mice (Stevens et al. 1972a). This effect was not due to inhibition of cholinesterase, nor was it due to an altered sensitivity of the brain to barbiturates, but it was associated with inhibition of hepatic MFO metabolism. These investigators also determined that disulfoton depressed microsomal metabolism of aniline as well as ethylmorphine in the mouse. A significant decrease in N-demethylase activity of aminopyrine and hydroxylase activity of acetanilide was observed in animals pretreated orally with disulfoton for 2 successive days, compared to the control group (Fawade and Pawar 1978). Disulfoton also caused decreased levels of cytochrome P450 and cytochrome b, and an increase in NADPH-linked and ascorbate-promoted lipid peroxidation.

In contrast to the inhibitory effects of acute exposure, repeated dosing with disulfoton induces the cytochrome P450 MFO system (Stevens et al. 1973). Disulfoton (1/2 LD_{50}) given orally to mice for 3 days resulted in increased activities of ethylmorphine N-demethylase and NADPH oxidase activities, but not the activity of NADPH cytochrome c reductase, the rate of reduction of cytochrome P450, or the content. Apparently, the duration of exposure determines the effect of disulfoton on the various components of the MFO system. In another study, treatment of mice orally with disulfoton (1/2 LD_{50}) for 5 days followed by administration of hexobarbital resulted in an increase in hexobarbital hydroxylase activity (Stevens et al. 1972b). Therefore, disulfoton-treated mice had shorter hexobarbital sleeping times. Microsomes from disulfoton-treated mice also had increased activity of aniline hydroxylase when aniline was added to the incubation mixture. Lower doses of disulfoton for similar time periods of exposure did not result in significant hepatic enzyme induction. The results from these studies suggest that depending on the duration of exposure, disulfoton may increase or decrease the severity of toxicity associated with chemicals that are similarly metabolized.

The toxicity of disulfoton may be altered by pretreatment with inducers or inhibitors of the hepatic microsomal drug metabolizing system. Phenobarbital causes enzyme repression of flavin-containing monooxygenase, but it also causes induction of cytochrome P450 activity (Sipes and Gandolfi 1986). Therefore, pretreatment with phenobarbital will not result in flavin monooxygenase-mediated activation of disulfoton to its active metabolite. Cytochrome P450 can activate disulfoton to its toxic metabolites as well as detoxify disulfoton by oxidative dearylation and dealkylation to less toxic metabolites (Ecobichon 1990). However, pretreatment with phenobarbital induced cytochrome P450 enzymes that functioned

more as detoxification enzymes than as activation enzymes (DuBois and Kinoshita 1968; Pawar and Fawade 1978). Although phenobarbital affects both enzyme systems differently, the net result is protection from the toxicity of disulfoton. One hundred percent protection against the toxicity of disulfoton was achieved both in mice and rats pretreated with phenobarbital and then given disulfoton orally at the LD_{50} dose level (Pawar and Fawade 1978). Pretreatment with another enzyme inducer, 3-methylcholanthrene, resulted in only 73% protection against disulfoton toxicity in both rats and mice. Rats pretreated with phenobarbital were less susceptible to the toxicity of disulfoton (DuBois and Kinoshita 1968). In this study, the LD_{50} value for the pretreated group (16.3 mg/kg) was greater than that for the control group (6.7 mg/kg), suggesting that phenobarbital pretreatment reduced the toxic effects of disulfoton by way of hepatic microsomal enzyme induction. A 3-day phenobarbital pretreatment also resulted in increased microsomal protein content and increased aminopyrine N-demethylase activity, but decreased acetanilide hydroxylase activity, in mice given disulfoton for 3 more days (Fawade and Pawar 1980).

Pretreatment with the ethylmorphine, resulted in 100% mortality in both rats and mice, and aminopyrine pretreatment resulted in 100 and 64% mortality in rats and mice, respectively, exposed to disulfoton (Pawar and Fawade 1978). The levels of electron transport chain components (cytochrome b, cytochrome c reductase, and total heme) in rats were lowered by administration of metabolic inhibitors, nickel chloride, cobalt chloride, or cycloheximide (Fawade and Pawar 1983). When given a single dose of disulfoton, the electron transport components were further decreased in rats pretreated with nickel chloride or cobalt chloride. Data from this study suggests an additive effect by disulfoton (Fawade and Pawar 1983). In a separate experiment, an additive effect between disulfoton and the tested metabolic inhibitors was suggested by the decrease in ethylmorphine N-demethylase and acetanilide hydroxylase activities when rats were given an inhibitor followed by disulfoton. In another experiment, the inhibitors decreased the activity of delta-aminolevulinic acid synthetase, but this decrease was reversed when disulfoton was administered (Fawade and Pawar 1983).

Although some steroids have been reported to reduce the toxic effects of some insecticides, the steroid ethylestrenol decreased the rate of recovery of depressed cholinesterase activity in disulfoton-pretreated rats (Robinson et al. 1978). The exact mechanism of this interaction was not determined.

Ethylestrenol alone caused a small decrease in cholinesterase activity. Rats excreted less adrenaline and more noradrenaline when given simultaneous treatments of atropine and disulfoton compared with rats given disulfoton alone (Brzezinski 1973). The mechanism of action of disulfoton on catecholamine levels

may depend on acetylcholine accumulation. In the presence of atropine, the acetylcholine effect on these receptors increases the ability of atropine to liberate catecholamines.

Cross-tolerance between disulfoton and another organophosphate, chlorpyrifos, was observed in mice (Costa and Murphy 1983b). In the same study, propoxur-tolerant mice were tolerant to disulfoton but not vice versa. Propoxur (a carbamate) is metabolized by carboxylesterases, and these enzymes are inhibited in disulfoton-tolerant animals; disulfoton-tolerant animals are more susceptible to propoxur and/or carbamate insecticides than are non-pretreated animals. In another study, disulfoton-tolerant rats were tolerant to the cholinergic effects of octamethyl pyrophosphoramide (OMPA) but not parathion (McPhillips 1969a, 1969b). The study authors were unable to explain why the insecticides OMPA and parathion caused different effects. Additionally, when two or more organophosphates are absorbed, additive toxicity is likely to occur due to similarities in their mechanism of toxicity (Robert and Reigart 2013). Among studies that examined, additive effects of exposure to organophosphates and carbamates, none examining disulfoton were located. An assay study on organophosphate and carbamate pesticides (carbaryl, carbofuran, parathion, demeton-S-methyl, and aldicarb) with similar mechanisms demonstrated an additive inhibitory effect on cholinesterase activity (Mwila et al. 2013).