2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of disulfoton. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure-inhalation, oral, and dermal; and then by health effect-death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt

at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user’s perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for disulfoton. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User’s Guide has been provided at the end of this profile (see APPENDIX A). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

2.2.1 Inhalation Exposure

2.2.1.1 Death

No studies were located regarding the lethal effects in humans after inhalation exposure to disulfoton.

In an acute inhalation study, l-hour exposure of male Sprague-Dawley rats to 202.2 mg/ m^3 disulfoton resulted in 60% mortality, while no deaths occurred in male rats exposed to ≤195.1 mg/ m^3 (Doull 1957). In Holtzman rats, a l-hour exposure resulted in death of 3 of 6 males at 180.1 mg/ m^3 and 2 of 6 females at 87.6 mg/ m^3 (DuBois 1971). No deaths occurred in males at 101.3 mg/ m^3 or in females at 75.1 mg/ m^3. LC_{50} values reported for Wistar rats were 290 mg/ m^3 in males and 63 mg/ m^3 for females exposed for 1 hour and 60 mg/ m^3 for males and 15 mg/ m^3 for females exposed for 4 hours (Thyssen 1978). When the rats were exposed to disulfoton 4 hours/day for 5 days, a concentration of 9.8 mg/ m^3 resulted in death of 9 of 10 females within l-8 days after exposure. No males died, and no deaths occurred in either sex at ≤l.8 mg/ m^3. In a 3-week study, 5 of 10 females exposed intermittently to 3.7 mg/ m^3 died after 3-12 exposures, while 3 of 20 females exposed intermittently to 3.1 mg/ m^3 intermittently died after 8-15 exposures (Thyssen 1980). No deaths occurred in the male rats in the 3-week study. Based on these data, strain differences in the lethal concentrations of disulfoton appear to exist in rats, and female rats are definitely more susceptible to the lethality of disulfoton than male rats. A l-hour exposure of female mice to 53.4 mg/ m^3 (lowest exposure concentration) resulted in 10% mortality, and 58.2 mg/ m^3 resulted in 70% mortality (Doull 1957). Male mice were not studied; therefore, data are insufficient to make comparisons of the inhalation lethality of disulfoton between male and female mice, and between rats and mice. The lethality of disulfoton appears to be related to the cholinergic effects, since such effects as tremors or convulsions were usually noted in the animals that died (see Section 2.2.1.5). The LC_{50} values in rats and the LOAEL values resulting in mortality in rats and mice are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.1.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, or body weight effects in humans after inhalation exposure to disulfoton.

TABLE 2-1. Levels of Significant Exposure to Disulfoton - Inhalation

Key[a] to figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
ACUTE EXPOSURE							
Death							
1	Rat (Sprague-Dawley)	0.5-1 hr				202.2 M (60% died)	Doull 1957
2	Rat (Holtz- man)	1 hr				87.6 F (2/6 died) 180.1 M (3/6 died)	DuBois 1971
3	Rat (Wistar)	1 hr				290 M (LC50) 63 F (LC50)	Thyssen 1978
4	Rat (Wistar)	4 hr				60 M (LC50) 15 F (LC50)	Thyssen 1978
5	Rat (Wistar)	5 d 4 hr/d				9.8 F (9/10 died)	Thyssen 1978
6	Rat (Wistar TNO/W 74)	3 d-3 wk 5 d/wk 6 hr/d				3.7 F (5/10 died)	Thyssen 1980
7	Mouse (Carworth Farms)	1 hr				53.4 F (10% died)	Doull 1957
Neurological							
8	Rat (Sprague-Dawley)	.5- 1hr				65.1 M (muscle twitching and fibrillation, ataxia, salivation, urination, defecation, lacrimation)	Doull 1957
9	Rat (Wistar)	1 hr				133 M (sluggishness, failure to 27 F groom, typical signs of cholinesterase inhibition - not otherwise described)	Thyssen 1978

TABLE 2-1. Levels of Significant Exposure to Disulfoton - Inhalation (continued)

Key [a] to figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)		LOAEL Serious (mg/m3)		Reference
10	Rat (Wistar)	4 hr					64 M 3.4 F	(sluggishness, failure to groom, typical signs of cholinesterase inhibition - not otherwise described)	Thyssen 1978
11	Rat (Wistar)	5 d 4 hr/d		0.5 [b]	1.8	(17-26% depression in erythrocyte cholinesterase activity; unspecified behavioral disorders)	9.8	(unspecified behavioral disorders and unspecified signs of cholinesterase activity depression)	Thyssen 1978
12	Mouse (Carworth Farms)	1 hr					53.4 F	(muscular twitches and fibrillations, ataxia; salivation, urination, defecation, lacrimation)	Doull 1957
INTERMEDIATE EXPOSURE									
Death									
13	Rat (Wistar TNO/W 74)	3 wk 5 d/wk 6 hr/d					3.1 F	(3/20 died)	Thyssen 1980
Systemic									
14	Rat (Fischer- 344)	3 wk 5 d/wk 6 hr/d	Bd Wt	0.7					Shiotsuka 1988

TABLE 2-1. Levels of Significant Exposure to Disulfoton - Inhalation (continued)

Key[a] to figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
15	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d	Resp	0.16 M 1.4 F	1.4 M (increased incidence of inflammation of the nasal turbinates)		Shiotsuka 1989
			Cardio	1.4			
			Gastro	1.4			
			Hemato	1.4			
			Musc/skel	1.4			
			Hepatic	1.4			
			Renal	1.4			
			Endocr	1.4			
			Derm	1.4			
			Ocular	1.4			
			Bd Wt	1.4			
16	Rat (Wistar TNO/W 74)	3 wk 5 d/wk 6 hr/d	Resp	0.1	0.5 (inflammatory changes in the respiratory tract)	3.7 F (mottled distended lungs in the rats that died)	Thyssen 1980
			Cardio	3.7			
			Gastro	0.5		3.7 F (bloated gastrointestinal tract and ulcer-like foci in the glandular mucosa in rats that died)	
			Hemato	3.7			
			Hepatic	3.7			
			Renal	3.7			
			Endocr	0.5	3.7 F (increased absolute and relative adrenal weight)		
			Ocular	3.7			
			Bd Wt	0.5	3.7 F (11-12% decreased body weight gain)		

TABLE 2-1. Levels of Significant Exposure to Disulfoton - Inhalation (continued)

Key[a] to figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
17	Rat (Wistar TNO/W 74)	3 wk 5 d/wk 6 hr/d	Resp	0.02		3.1 F (distention and discoloration of lungs in rats that died; increased inflammatory changes in the respiratory tract)	Thyssen 1980
			Cardio	3.1 F			
			Gastro	3.1 F			
			Hemato	0.02	3.1 F (decreased percentage of lymphocytes, increased percentage of polymorpho- nuclear leukocytes)		
			Hepatic	3.1 F			
			Renal	3.1 F			
			Endocr	0.02	3.1 F (increased absolute and relative adrenal weight)		
			Ocular	3.1 F			
			Bd Wt	3.1 F			
Immunological/Lymphoreticular							
18	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d		1.4			Shiotsuka 1989
19	Rat (Wistar TNO/W 74)	3 wk 5 d/wk 6 hr/d		0.1	0.5 (minimal to definite bone marrow changes accompanied by inflammatory changes in the respiratory tract)		Thyssen 1980

TABLE 2-1. Levels of Significant Exposure to Disulfoton - Inhalation (continued)

Key[a] to figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)		LOAEL Serious (mg/m3)		Reference
20	Rat (Wistar TNO/W 74)	3 wk 5 d/wk 6 hr/d		0.02	3.1 F	(decreased absolute and relative spleen weight, reactive bone marrow changes accompanied by inflammatory changes in the respiratory tract)			Thyssen 1980
	Neurological								
21	Rat (Fischer- 344)	3 wk 5 d/wk 6 hr/d		0.7					Shiotsuka 1988
22	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d		0.16	1.4	(14-31% inhibition of plasma cholinesterase, 22-34% inhibition of erythrocyte cholinesterase, 28-29% inhibition of brain cholinesterase)			Shiotsuka 1989
23	Rat (Wistar TNO/W 74)	3 wk 5 d/wk 6 hr/d			0.1	(lethargy for a brief time after exposure during the last week)	3.7	(muscle tremors, convulsion, increased salivation, difficulty breathing, 48-58% inhibition of brain cholinesterase)	Thyssen 1980

TABLE 2-1. Levels of Significant Exposure to Disulfoton - Inhalation (continued)

Key [a] to figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
24	Rat (Wistar TNO/W 74)	3 wk 5 d/wk 6 hr/d		0.02 [c]		3.1 F (muscle tremors, convulsions, increased salivation, difficulty breathing)	Thyssen 1980

[a]The number corresponds to entries in Figure 2-1.
[b]Used to derive an acute-duration inhalation minimal risk level (MRL) of 0.006 mg/m^3; concentration adjusted for intermittent exposure, converted to a human equivalent concentration, and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).
[c]Used to derive an intermediate-duration inhalation MRL of 2x10^{-4} mg/m^3; concentration adjusted for intermittent exposure, converted to a human equivalent concentration, and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Derm = dermal; Endocr = endocrine; F = female; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LC50 = lethal concentration-50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = muscular/skeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 2-1. Levels of Significant Exposure to Disulfoton – Inhalation

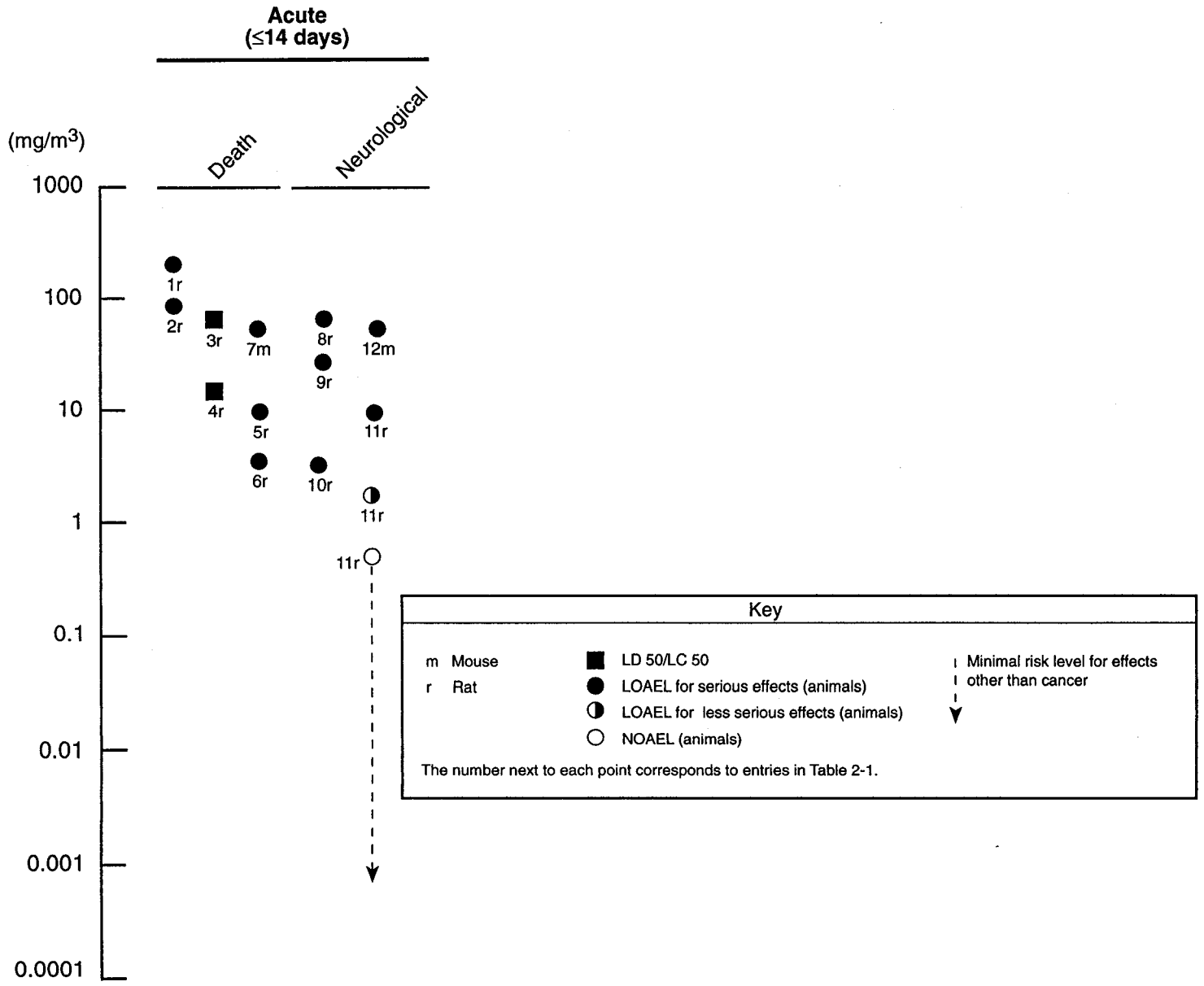
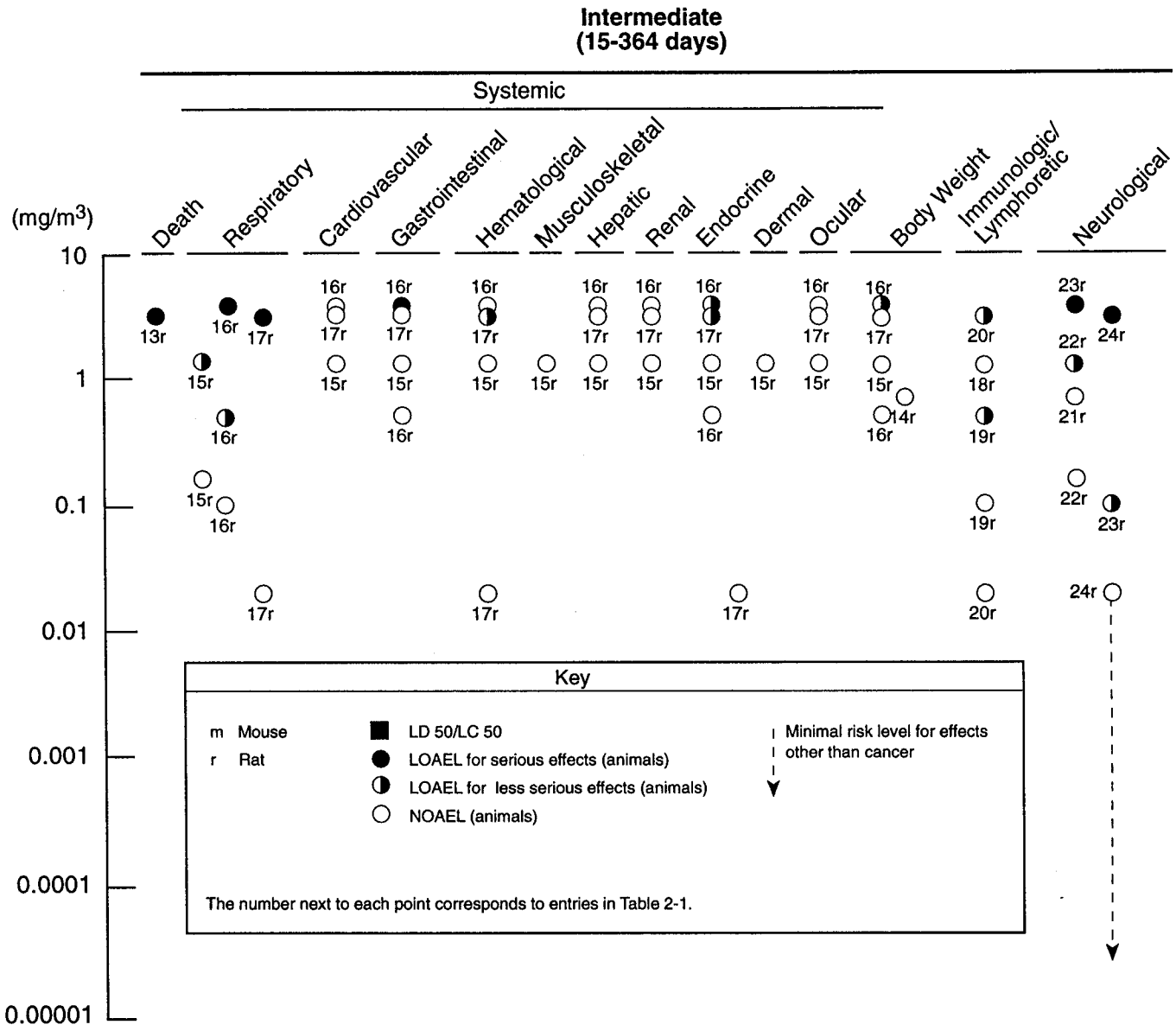


Figure 2-1. Levels of Significant Exposure to Disulfoton – Inhalation (continued)



Studies on these end points in animals exposed by inhalation to disulfoton are discussed below. The highest NOAEL values and all LOAEL values from each reliable study for these end points in animals in each duration category are recorded in Table 2-l and plotted in Figure 2-l.

Respiratory Effects. In two separate experiments in which male and female rats were exposed intermittently for 3 weeks to 0.1, 0.5, or 3.7 mg/ m^3 in the first experiment, and to 0.02 mg/ m^3 (males and females) or 3.1 mg/ m^3 (females only) in the second experiment, inflammatory changes were found throughout the respiratory tract at 0.5, 3.1, and 3.7 mg/ m^3 (Thyssen 1980). These inflammatory changes were considered to be related to reactive bone marrow changes (see Section 2.2.1.3), which were minimal in male rats and definite in females in the first experiment. Deaths occurred in the female rats exposed to 3.1 or 3.7 mg/ m^3, and mottled, distended, and discolored lungs were found upon necropsy of the rats that died. Increased incidences of inflammation of the nasal turbinates were found in male rats, but not female rats, exposed to 1.4 mg/ m^3 intermittently for 13 weeks (Shiotsuka 1989). These lesions were not found at 0.16 mg/ m^3.

Cardiovascular Effects. No treatment-related microscopic lesions were found in the hearts of rats exposed intermittently to 3.7 mg/ m^3 for 3 weeks (Thyssen 1980) or to 1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989).

Gastrointestinal Effects. In the female rats that died during intermittent exposure to 3.7 mg/ m^3 for 3 weeks, bloated gastrointestinal tracts and ulcer-like foci in the glandular mucosa were found upon necropsy (Thyssen 1980). Otherwise, no treatment-related histological effects in the gastrointestinal tract of the surviving females or in males exposed to ≤3.7 mg/ m^3 were found. Likewise, no gastrointestinal tract lesions were found in male or female rats exposed intermittently to ≤1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989).

Hematological Effects. No effects on formed elements of the blood were found upon hematological examination in rats exposed intermittently to ≤3.7 mg/ m^3 for 3 weeks (Thyssen 1980). However, in a second experiment in which female rats were similarly exposed to 3.1 mg/ m^3, a relatively low percentage of lymphocytes and high percentages of polymorphonuclear leukocytes in the differential leukocyte counts were found. These effects were regarded as a first sign of a response to the inflammation in the respiratory tract and bone marrow changes observed in these rats (see

Section 2.2.1.3). No hematological effects were observed in rats exposed intermittently to 0.02-0.5 mg/ m^3 for 3 weeks (Thyssen 1980) or to 1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989).

Musculoskeletal Effects. No gross or histological lesions were found in bones or skeletal muscle of rats exposed intermittently to ≤1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989).

Hepatic Effects. Clinical chemistry tests and histological examination of livers revealed no hepatic effects in rats exposed intermittently to ≤3.7 mg/ m^3 for 3 weeks (Thyssen 1980) or to ≤1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989).

Renal Effects. Clinical chemistry, urinalysis, and histological examination of kidneys revealed no renal effects in rats exposed intermittently to ≤3.7 mg/ m^3 for 3 weeks (Thyssen 1980) or to ≤1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989).

Endocrine Effects. No histological lesions were found in the thyroid or adrenal glands of rats exposed interrnittently to ≤3.7 mg/ m^3 for 3 weeks, but females exposed to 3.1 and 3.7 mg/ m^3 in two separate experiments had significantly increased absolute and relative adrenal weights (Thyssen 1980). Since the increase in adrenal weights was consistently observed in both experiments, it was considered to be related to disulfoton exposure. No histological effects or effects on the weight of the adrenal gland and no histological effects on the thyroids, parathyroids, pituitary, or pancreas were observed in rats exposed intermittently to ≤1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989).

Dermal Effects. No gross or histological lesions were found in the skin of rats exposed intermittently to 1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989).

Ocular Effects. No histological or ophthalmological evidence of ocular effects were found in rats exposed intermittently to ≤3.7 mg/ m^3 for 3 weeks (Thyssen 1980) or to ≤1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989).

Body Weight Effects. Female rats exposed intermittently to 3.7 mg/ m^3, but not 3.1 mg/ m^3, for 3 weeks had 12% and 11% lower body weights than controls during weeks 1 and 2, respectively, but only 5% lower body weight during week 3 (Thyssen 1980). Males similarly exposed to 3.7 mg/ m^3 had lower body weights than controls, but the difference was never >10%. No effects on body weight

were found in the rats exposed intermittently to 0.5 mg/ m^3 (Thyssen 1980) or 0.7 mg/ m^3 (Shiotsuka 1988) for 3 weeks, or 1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989).

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after inhalation exposure to disulfoton.

In two separate experiments in which male and female Wistar rats were exposed intermittently for 3 weeks to 0.1, 0.5, or 3.7 mg/ m^3 in the first experiment, and to 0.02 mg/ m^3 (males and females) or 3.1 mg/ m^3 (females only) in the second experiment, inflammatory changes were found throughout the respiratory tract at 0.5, 3.1, and 3.7 mg/ m^3 (Thyssen 1980). These inflammatory changes were considered to be related to reactive bone marrow changes. The reactive bone marrow changes were not specifically described in the study, but were regarded as minimal in male rats and definite in female rats in the first experiment. In the second experiment, but not in the first, female rats exposed to 3.1 mg/ m^3 had a relatively low percentage of lymphocytes and high percentages of polymorphonuclear leukocytes in the differential leukocyte counts. These effects were regarded as a first sign of a response to the inflammation in the respiratory tract and bone marrow changes observed in these rats. The female rats exposed to 3.1 mg/ m^3 also had decreased absolute and relative spleen weight, but histological examination of the spleen and bronchial lymph nodes revealed no treatmentrelated effects in males or females exposed to ≤3.7 mg/ m^3. In addition, histological examination of bone marrow, cervical lymph nodes, mesenteric lymph nodes, spleen, and thymus of Fischer 344 rats exposed intermittently to ≤1.4 mg/ m^3 for 13 weeks revealed no effects (Shiotsuka 1989). The highest NOAEL values and the LOAEL values for immunological and lymphoreticular effects in rats are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.4 Neurological Effects

Exposure to disulfoton can result in inhibition of cholinesterase activity in blood and at nerve synapses of muscles, secretory organs, and nervous tissue such as the brain and spinal cord (Murphy 1986). The accumulation of acetylcholine at these sites (specifically, the nerve synapses and ganglia in these organs) results in central nervous system, nicotinic, and muscarinic effects. Clinical signs and symptoms of neurotoxicity are usually observed in humans and animals that have been acutely

exposed to disulfoton or other organophosphate insecticides. Central nervous system signs and symptoms include anxiety, restlessness, depression of respiratory and circulatory centers, ataxia, convulsions, and coma. Nicotinic signs of disulfoton toxicity include muscle weakness, muscle tremors and fasciculations, and involuntary twitching. Muscle weakness that affects the respiratory muscles may contribute to dyspnea and cyanosis. Tachycardia may result from stimulation of sympathetic ganglia in cardiac tissue and may, therefore, mask the bradycardia due to the muscarinic action on the heart. Nicotinic action at the sympathetic ganglion may also result in pallor, high blood pressure, and hyperglycemia. Muscarinic signs of disulfoton toxicity include miosis, increased salivation, sweating, urination and defecation, vomiting and nausea, bronchoconstriction, increased bronchial secretions, and bradycardia that can progress to heart block.

Inhibition of the two principal human cholinesterases, acetylcholinesterase and pseudocholinesterase, may not always result in visible neurological effects (Sundlof et al. 1984). Acetylcholinesterase, also referred to as true cholinesterase, red blood cell cholinesterase, or erythrocyte cholinesterase is found in erythrocytes, lymphocytes, and at nerve synapses (Goldfrank et al. 1990). Inhibition of erythrocyte or lymphocyte acetylcholinesterase is theoretically a reflection of the degree of synaptic cholinesterase inhibition in nervous tissue, and therefore a more accurate indicator than pseudocholinesterase activity of inhibited nervous tissue acetylcholinesterase (Fitzgerald and Costa 1993; Sundlof et al. 1984). Pseudocholinesterase (also referred to as cholinesterase, butyrylcholinesterase, serum cholinesterase, or plasma cholinesterase) is found in the plasma, serum, pancreas, brain, and liver and is an indicator of exposure to a cholinesterase inhibitor.

Pseudocholinesterase and lymphocyte acetylcholinesterase activities are depressed before erythrocyte cholinesterase, suggesting that these cholinesterases are more sensitive than acetylcholinesterase (Fitzgerald and Costa 1993; Iyaniwura 1991; Sundlof et al. 1984). However, erythrocyte cholinesterase recovers more slowly (90-120 days) than pseudocholinesterase or lymphocyte acetylcholinesterase (days to weeks), and is therefore a better indicator after exposure ceases. Depression in pseudocholinesterase activity only indicates possible exposure to organophosphate(s), whereas depression in erythrocyte and lymphocyte cholinesterases also indicates a neurological effect, since they reflect inhibition of brain acetylcholinesterase activity. Recently, it was shown that the inhibition of lymphocyte acetylcholinesterase activity more closely paralleled the inhibition of brain acetylcholinesterase than did erythrocyte acetylcholinesterase activity (Fitzgerald and Costa 1993). In this profile, inhibition in lymphocyte or erythrocyte acetylcholinesterase activity and not

pseudocholinesterase activity is considered as an adverse neurological effect. Based on an evaluation by Kaloyanova and El Batawi (1991), ATSDR considers a 60% or greater acetylcholinesterase inhibition as a serious effect and less than 60%, but greater than 20%, acetylcholinesterase inhibition as a less serious effect.

Nervous system effects may occur in humans after occupational exposure to disulfoton (Wolfe et al. 1978). In this study, mean disulfoton concentrations of 0.460-0.633 mg/ m^3 caused a 22.8% depression in erythrocyte cholinesterase activity in workers at a pesticide-fertilizer mixing operation. The workers were exposed to disulfoton for 9 weeks, and there were no reports of adverse clinical signs due to disulfoton exposure. The study was limited in that baseline blood cholinesterase activities were obtained 2 weeks after the initial exposure and were compared with cholinesterase activities at 9 weeks. Therefore, the actual depression in cholinesterase activity over a 9-week period was probably >22.8%. In addition, these workers were also dermally exposed to disulfoton (see Section 2.2.3.4); therefore, the 22.8% depression in cholinesterase activity was probably due to both inhalation and dermal exposure. Despite these limitations, the study concluded that because this depression in cholinesterase activity was only associated with dry mixing operations, the wet mixing operations are less hazardous to workers.

Neurological effects, such as muscle twitching, ataxia, and increased salivation, urination, defecation, and lacrimation were observed in male Sprague-Dawley rats exposed to ≥65.1 mg/ m^3 and in female Carworth Farms mice exposed to ≥53.4 mg/ m^3 for 1 hour (Doull 1957). Female rats and male mice were not included in this study. However, the greater susceptibility of female rats to the cholinergic effects of disulfoton was demonstrated in several experiments in an acute inhalation study using Wistar rats (Thyssen 1978). In the LC_{50} determinations in this study, sluggishness, failure to groom, and typical signs of cholinesterase inhibition (not otherwise described) were observed in male rats exposed to ≥133 mg/ m^3 and in females exposed to ≥27 mg/ m^3 for 1 hour. These signs of toxicity were observed at lower exposure levels when rats were exposed for 4 hours (in males exposed to ≥64 mg/ m^3 and in females exposed to ≥3.4 mg/ m^3). These effects were transient, lasting for about 24 hours after exposure. In an experiment designed to examine cholinesterase activity in rats exposed to 0.5, 1.8, or 9.8 mg/ m^3 for 4 hours/day for 5 days, erythrocyte cholinesterase activity was depressed by 30-32% of controls in males exposed to 9.8 mg/ m^3 and by 17-26% in females at both 1.8 and 9.8 mg/ m^3. In addition, all rats were reported to display unspecified behavioral disorders at ≥1.8 mg/ m^3 and unspecified signs of cholinergic toxicity at 9.8 mg/ m^3. No inhibition of erythrocyte

cholinesterase activity and no signs of cholinergic toxicity were observed at 0.5 mg/ m^3. Based on this NOAEL of 0.5 mg/ m^3, an acute-duration inhalation MRL of 0.006 mg/ m^3 was calculated as described in footnote "b" in Table 2-l. No significant decrease in the activity of brain, serum, or submaxillary gland cholinesterase was found in female rats exposed to 0.14-0.7 mg/ m^3 disulfoton for 1 hour/day for 5-10 days (DuBois and Kinoshita 1971). No clinical signs of disulfoton toxicity or other details were reported.

Signs of cholinergic toxicity and depressions in cholinesterase activities were also observed in rats exposed to disulfoton for intermediate durations. In Wistar rats exposed intermittently to 0.1, 0.5, or 3.7 mg/ m^3 for 3 weeks, exposure to 0.1 mg/ m^3 resulted in brief periods of lethargy after exposure ended during the last week; exposure to 0.5 mg/ m^3 resulted in lethargy and failure to groom in males during the last week and in females during the second and last week; and exposure to 3.7 mg/ m^3 resulted in muscle tremors, convulsion, increased salivation, and dyspnea in males starting at the end of the first week and in females during the first week (Thyssen 1980). Erythrocyte cholinesterase activity was inhibited in males by 24-28% and in females by 27-32% at 3.7 mg/ m^3. Brain cholinesterase activity was inhibited in males by 48% at 3.7 mg/ m^3 and in females by 30% at 0.5 mg/ m^3 and 58% at 3.7 mg/ m^3. In a second 3-week experiment to determine a no-effect level for cholinesterase inhibition in Wistar male and female rats, no clinical signs of neurological effects and no effects on plasma, erythrocyte, or brain cholinesterase were observed at 0.02 mg/ m^3 (Thyssen 1980). Female rats exposed to 3.1 mg/ m^3 had muscle tremors, convulsion, increased salivation, and dyspnea, confirming the results in the first experiment. Male rats were not exposed to 3.1 mg/ m^3 in the second experiment. Based on the NOAEL of 0.02 mg/ m^3, an intermediate-duration inhalation MRL of $2x10^{-4}$ mg/ m^3 was calculated as described in footnote "c" in Table 2-l. In Fischer rats exposed intermittently to 1.4 mg/ m^3 for 13 weeks, erythrocyte cholinesterase activity was inhibited by 22-28% in males and 26-34% in females, and brain cholinesterase activity was inhibited by 29% in males and 28% in females (Shiotsuka 1989). Cholinesterase activities were not affected at $\leq$0.16 mg/ m^3, and no effects on brain weight or histological evidence of lesions in the brain, optic nerve, sciatic nerve, or spinal cord were found at any exposure level. In a similar study in Fischer 344 rats exposed to lower concentrations for 3 weeks, no significant differences in brain cholinesterase activities were found at 0.006-0.7 mg/ m^3 (Shiotsuka 1988). Erythrocyte cholinesterase activity was statistically consistently decreased at 0.7 mg/ m^3, but the decreases were never greater than 17% of control levels. The highest NOAEL values and all reliable LOAEL values for neurological effects in rats and mice for each duration category are recorded in Table 2- 1 and plotted in Figure 2- 1.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to disulfoton.

In rats exposed intermittently to 0.02-3.7 mg/ m^3 for 3 weeks, measurements of the testes and ovary weights and histological examination of the testes and ovaries revealed no compound-related effects (Thyssen 1980). Similarly, no effects on testis or ovary weight and no compound-related histological lesions in epididymides, prostate, seminal vesicles, testicles, cervix, mammary glands, ovaries, or uterus were found in rats exposed intermittently to ≤1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989). Reproductive studies in animals given disulfoton by the oral route have demonstrated that disulfoton produces effects on reproductive parameters (see Section 2.2.2.5); therefore, the above inhalation concentration levels cannot be considered NOAEL values for reproductive effects because these inhalation studies did not examine reproductive performance or outcomes.

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to disulfoton.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after inhalation exposure to disulfoton.

Genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

No studies were located regarding cancer in humans after inhalation exposure to disulfoton.

In a 13-week study in rats exposed intermittently to ≤1.4 mg/ m^3, the author reported that comprehensive histological examination of organs and tissues revealed no treatment-related neoplastic

lesions (Shiotsuka 1989). However, chronic-duration inhalation studies, which would be more appropriate to assess possible carcinogenicity, were not located for disulfoton.

2.2.2 Oral Exposure

2.2.2.1 Death

Only one study was located involving death in humans after ingestion of disulfoton (Hattori et al. 1982). In this case report, a 30-year-old man was found dead after consuming an unknown amount of disulfoton. He was believed to have been dead for at least 24 hours. Autopsy and histopathological examination revealed miosis, bubbling saliva from the mouth, pulmonary edema and hemorrhage, swelling of the glomemlus, and congestion of most organs, which suggested that the man had ingested an organophosphate. Analysis of urine and blood samples confirmed that disulfoton was the toxicant responsible for the death.

The dose of disulfoton associated with death following acute oral exposure in animals depends on the sex, species, and the duration of the exposure. As seen from Table 2-2 and Figure 2-2, female rats and mice were generally more sensitive than male rats and mice, and rats generally appeared to be more sensitive than mice, to disulfoton given orally. LD_{50} values ranged from 1.9 to 3.2 mg/kg in female rats, 6.2-12.5 mg/kg in male rats (Bombinski and DuBois 1958; Crawford and Anderson 1974; Gaines 1969; Mihail 1978; Pawar and Fawade 1978), 2.7-8.2 mg/kg in female mice, and 5.8-19.3 mg/kg in male mice (Mihail 1978; Pawar and Fawade 1978; Stevens et al. 1972a). In one LD_{50} determination in rats, deaths occurred within 6 minutes to 2 days in males and 4 minutes to 3 days in females (Gaines 1969). Oral LD_{50} values of 10 mg/kg in rats of unspecified sex (Schafer 1972), of 8.9-10.8 mg/kg in male guinea pigs (Bombinski and DuBois 1958; Crawford and Anderson 1973), and of 12.7 mg/kg in female guinea pigs (Crawford and Anderson 1973) have also been reported. A dose of 18 mg/kg was determined to be the minimum dose at which mortality occurred in wild deer mice of unspecified sex given disulfoton by gavage (Schafer and Bowles 1985).

Deaths occurred on the day of treatment in 4 of 6 female rats at a dose of 2.5 mg/kg and in 1 of 9 female rats at a dose of 1.5 mg/kg, but no deaths occurred in male rats given ≤5.2 mg/kg (Sheets 1993a). However, in another study using the same strain of rats (Sprague-Dawley), 1 of 5 male rats died after receiving one dose of 3.5 mg/kg disulfoton, while two more rats died after receiving the

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Rat (Sprague-Dawley)	once (G)				12.5M (LD50) 2.6 F (LD50)	Bombinski and DuBois 1958
2	Rat (NS)	once (GW)				2.0 F (2/4 died)	Crawford and Anderson 1974
3	Rat (Sherman)	once (GO)				6.8M (LD50) 2.5 F (LD50)	Gaines 1969
4	Rat (Wistar)	once (GO)				6.2M (LD50) 1.9 F (LD50)	Mihail 1978
5	Rat (Hindustan antibiotics)	once (G)				7.2M (LD50) 3.2 F (LD50)	Pawar and Fawade 1978
6	Rat (NS)	once (GO)				10 (LD50)	Schafer 1972
7	Rat (Sprague-Dawley)	1-23 d 1x/d for 3 days at a time (GO)				3.5M (3/5 died)	Schwab et al. 1981
8	Rat (Sprague-Dawley)	once (GO)				1.5 F (1/9 died on the day of treatment)	Sheets 1993a
9	Mouse (MMRI)	once (GO)				7.0M (LD50) 8.2 F (LD50)	Mihail 1978

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
10	Mouse (Hindustan antibiotics)	once (G)				5.8M (LD50) 2.7F (LD50)	Pawar and Fawade 1978
11	Mouse (wild deer mouse)	once (G)				18 (mortality of an unspecified number of mice)	Schafer and Bowles 1985
12	Mouse (Swiss-Webster)	once (GO)				19.3M (LD50)	Stevens et al. 1972a
13	Mouse (Swiss)	1-10 d 1x/d (GO)				9.6M (2/8 died)	Stevens et al. 1972b
14	Gn pig (NS)	once (G)				10.8M (LD50)	Bombinski and DuBois 1958
15	Gn pig (NS)	once (GW)				8.9M (LD50) 12.7F (LD50)	Crawford and Anderson 1973
	Systemic						
16	Rat (Wistar)	once (GO)	Endocr		6.25 F (173 and 313% increase in urinary noradrenaline and adrenaline levels, respectively)		Brzezinski 1969
17	Rat (Wistar)	once (GO)	Endocr		5.0M (decrease in adrenal gland catecholamine levels, increase in plasma catacholamines)		Brzezinski 1972

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
18	Rat (Wistar)	once (GO)	Endocr		5.0M (increase in urinary adrenaline and noradrenaline)		Brzezinski 1973
19	Rat (Wistar)	once (GO)	Endocr		5.0M (increase in urinary catecholamines by 41-250%)		Brzezinski and Ludwicki 1973
20	Rat (Wistar)	once (GO)	Endocr		1.25 (increase in urinary levels of adrenalin and noradrenalin)		Brzezinski and Rusiecki 1970
21	Rat (Sprague-Dawley)	10 d 1x/d (GO)	Bd Wt		2.0M (32% reduction in weight gain)		Costa et al. 1984
22	Rat (Sprague-Dawley)	10 d 1x/d (GO)	Bd Wt			2.0M (50% reduced body weight gain)	Costa et al. 1986
23	Rat (Hindustan antibiotics)	once (GO)	Hepatic		2.0M (increased lipid peroxidation)		Fawade and Pawar 1983
24	Rat (Long-Evans)	1-2 wk 1x/d (GO)	Bd Wt		2.0M (temporary but significant ($p<0.025$) decreased body weight gain at day 3 with recovery by day 5-6)		Fitzgerald and Costa 1992
25	Rat (Long-Evans)	1-2 wk 1x/d (GO)	Bd Wt		2.0M (significantly ($p<0.025$) reduced body weight gain beginning at day 3 with recovery by day 5-6)		Fitzgerald and Costa 1993

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
26	Rat (Wistar)	once (GO)	Resp	0.5 F	1.0 (dyspnea)		Mihail 1978
27	Rat (Holtzman)	9 d (F)	Bd Wt	0.38 F	1.0 F (10-12% reduced body weight gain at all weighing times)		Schwab and Murphy 1981
28	Rat (Sprague-Dawley)	1-23 d 1x/d for 3 days at a time (GO)	Bd Wt		2.0 M (approximately 20% reduced body weight gain)		Schwab et al. 1981
29	Rat (Sprague-Dawley)	1-10 d 1x/d (GO)	Bd Wt		2.0 M (weight loss not otherwise specified)		Schwab et al. 1983
30	Rat (Sprague-Dawley)	once (GO)	Musc/skel	5.2			Sheets 1993a
			Ocular	5.2			
31	Rat (Wistar)	once (GO)	Endocr	0.26 F	0.52 F (increased excretion of 1.25 M 4-hydroxy-3-methoxy-mandelic acid in urine)		Wysocka-Parusz ewska 1971
32	Mouse (Hindustan antibiotics)	2-4 d 1x/d (GO)	Hepatic		0.5 M (increased lipid peroxidation)		Fawade and Pawar 1978
33	Mouse (Hindustan antibiotics)	3 d 1x/d (GO)	Hepatic		1.0 M (increased lipid peroxidation)		Fawade and Pawar 1980

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
34	Mouse (MMRI)	once (GO)	Resp	2.5	5.0 (dyspnea)		Mihail 1978
	Neurological						
35	Rat (Wistar)	once (GO)			5.0M (decrease in brain catecholamine levels)		Brzezinski 1972
36	Rat (Wistar)	once (GO)				5.0M (33.8 -96.7% inhibition of blood acetylcholinesterase activity)	Brzezinski and Ludwicki 1973
37	Rat (Sprague-Dawley)	10 d (GO)				2M (89% inhibition of brain acetylcholinesterase activity)	Costa and Murphy 1983
38	Rat (Sprague-Dawley)	10 d 1x/d (GO)				2M (50% reduction in pancreatic acetylcholinesterase activity, salivation, lacrimation, diarrhea)	Costa et al. 1984
39	Rat (Sprague-Dawley)	10 d 1x/d (GO)				2M (decreased number of muscarinic receptors in cerebral cortex 84% inhibition of brain acetyl cholinesterase)	Costa et al. 1986
40	Rat (NS)	once (GW)				0.5 F (tremors)	Crawford and Anderson 1974
41	Rat (Long-Evans)	1-2 wk 1x/d (GO)				2M (typical signs of cholinergic toxicity, not specified, including diarrhea, decrease in brain receptor density)	Fitzgerald and Costa 1992

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
42	Rat (Long-Evans)	1-2 wk 1x/d (GO)				2M (60-84% decrease in brain cholinesterase activity, diarrhea, flaccidity, malaise)	Fitzgerald and Costa 1993
43	Rat (CD)	10 d Gd 6-15 1x/d (G)		0.1 [b] F	0.3 F (41% inhibition of plasma and erythrocyte cholinesterase activity in dams)	1.0 F (82-90% inhibition of plasma and erythrocyte cholinesterase activity in dams)	Lamb and Hixson 1983
44	Rat (Wistar)	once (GO)		0.5 F		1.0 F (muscle twitching cramps, salivation)	Mihail 1978
45	Rat (Holtzman)	9 d (F)			0.38 F (30-35% inhibition of brain and diaphragm acetyl cholinesterase)	1.0 F (tremors, diarrhea, excessive urination, fasciculations, exophthalmia)	Schwab and Murphy 1981
46	Rat (Sprague-Dawley)	1-23 d 1x/d for 3 days at a time (GO)				2.0M (exophthalmia, salivation, excessive urination and defecation, tremors)	Schwab et al. 1981
47	Rat (Sprague-Dawley)	1-10 d 1x/d (GO)				2.0M (salivation, lacrimation, excessive urination and diarrhea, fasciculations, tremors, 15-51% inhibition of ileal acetylcholinesterase activity)	Schwab et al. 1983

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
48	Rat (Sprague-Dawley)	once (GO)		0.24		0.76 F (muscle fasciculations, decrease vocalization, minimal head or body movement, 53% decrease in erythrocyte cholinesterase activity) 1.5M (muscle fasciculation, tremors, minimal head or body movement)	Sheets 1993a
49	Rat (Holtzman)	1 wk (F)			0.26 F (50% inhibition of brain acetylcholinesterase activity)		Su et al. 1971
50	Mouse (MMRI)	once (GO)		2.5		5.0 (muscle twitches, clonic cramps, salivation)	Mihail 1978
	Reproductive						
51	Mouse (NMRI/ ORIG)	once (GO)		5M			Herbold 1980
	Developmental						
52	Rat (CD)	10 d Gd 6-15 1x/d (G)		0.3	1.0 (delayed ossification of parietal bones and sternebrae)		Lamb and Hixson 1983
53	Rabbit (New Zealand)	13 d Gd 6-18 1x/d (G)		1.5			Tesh et al. 1982

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	INTERMEDIATE EXPOSURE						
	Death						
54	Rat (Albino)	30 d (F)				2.5M (death of 4/71)	Robinson et al. 1978
55	Rat (Fischer 344)	48 d ad lib (F)				1.31 F (1/12 died)	Sheets 1993b
56	Mouse (Charles River)	4 wk (F)				26F (5/25 died)	Clark et al. 1971
	Systemic						
57	Rat (Wistar)	76 d 1x/d every 2d (GO)	Endocr		0.625 M (increase in urinary levels of adrenalin and noradrenalin)		Brzezinski and Rusiecki 1970
58	Rat (Fischer- 344)	6 mo ad lib (F)	Bd Wt	0.07			Christenson and Wahle 1993

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
59	Rat (Sprague-Dawley)	F0: 15 wk premating; F1b: 13 wk premating & through production and weaning of F2a & F2b (F)	Bd Wt	0.03	0.09 (6-10% and 9-11% decrease in body weight gain in F1 parental females and males, respectively, during premating period)		Hixson and Hathaway 1986
60	Rat (Wistar)	90 d (F)	Resp	0.55			Klotzsche 1972
			Cardio	0.55			
			Gastro	0.55			
			Hemato	0.55			
			Musc/skel	0.55			
			Hepatic	0.55			
			Renal	0.55			
			Endocr	0.55			
			Derm	0.55			
			Ocular	0.55			
			Bd Wt	0.55			
61	Rat (Albino)	30 d (F)	Bd Wt		2.5 M (29% reduced body weight gain)		Robinson et al. 1978
62	Rat (Holtzman)	30-62 d (F)	Bd Wt	0.38 F	1.0 F (10-12% reduced body weight gain at all weighing times)		Schwab and Murphy 1981

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
63	Rat (Fischer- 344)	13 wk ad lib (F)	Musc/skel	1.31			Sheets 1993b
			Ocular	1.31			
			Bd Wt	1.31			
64	Rat (Holtzman or Charles River)	141-178 d (F)	Bd Wt	0.5 F		1.25 F (40% reduced body weight gain)	Stavinoha et al. 1969
65	Mouse (CF-LP)	13 wk (F)	Resp	0.71			Rivett et al. 1972
			Cardio	0.71			
			Gastro	0.71			
			Hemato	0.71			
			Hepatic	0.71			
			Renal	0.71			
			Endocr	0.71			
			Ocular	0.71			
			Bd Wt	0.71			
	Immunological/Lymphoreticular						
66	Rat (Wistar)	90 d (F)		0.55			Klotzsche 1972
67	Mouse (CF-LP)	13 wks (F)		0.71			Rivett et al. 1972

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Neurological						
68	Rat (Fischer- 344)	6 mo ad lib (F)		0.03 F 0.06 M	0.07 F (22-29% inhibition in erythrocyte cholinesterase activity)		Christenson and Wahle 1993
69	Rat (Charles River)	3 mo (F)			0.5 M (59% inhibition of brain acetylcholinesterase activity)		Clark and Pearson 1973
70	Rat (NS)	2 mo (F)			2.5 (increased permeabilitiy of brain tissue to copper ferricyanide)		Clark and Stavinoha 1971
71	Rat (NS)	8-16 wk (F)		0.05	0.1 (inhibition of brain and red blood cell cholinesterase activity)		Doull and Vaughn 1958
72	Rat (Fischer 344)	3-6 mo (F)		0.05 M	0.18 M (46-50% inhibition of erythrocyte cholinesterase)	0.75M (71-82% inhibition of erythrocyte cholinesterase)	Hayes 1985
					0.06 F (14-22% inhibition of erythrocyte cholinesterase)	0.21 F (68-69% inhibition of erythrocyte cholinesterase)	

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
73	Rat (Sprague-Dawley)	F0: 15 wk premating; F1b: 13 wk premating & through production and weaning of F2a & F2b (F)		0.03		0.09 F (tremor in the F0 females during the production of the F1 generation)	Hixson and Hathaway 1986
74	Rat (Wistar)	90 d (F)		0.07 M 0.11 F	0.34 M (30-40% inhibition of 0.55 F plasma and erythrocyte cholinesterase)		Klotzsche 1972
75	Rat (Albino)	30 d (F)				2.5M (unspecified typical signs of anticholinesterase, poisoning, inhibition of cholinesterase activity of 77.2% in brain, 81.9% in the stomach, 70.3% in the diaphragm)	Robinson et al. 1978
76	Rat (Albino)	60-95 d (F)				0.5 (51.6% and 81.3% inhibition of male and female brain cholinesterase activity, respectively)	Ryan et al. 1970
77	Rat (Holtzman)	30-62 d (F)				0.38 F (75% inhibition of brain and 50% inhibition of diaphragm acetylcholinesterase)	Schwab and Murphy 1981

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
78	Rat (Fischer- 344)	13 wk ad lib (F)		0.071 F		0.351 F (muscle fasciculation, urine stain, 79-80% inhibition of erythrocyte cholinesterase activity, 64% inhibition of brain cholinesterase activity)	Sheets 1993b
				0.063 M		0.270M (61-67% inhibition of erythrocyte cholinesterase activity; 35% inhibition of brain cholinesterase activity)	
79	Rat (Holtzman or Charles River)	141-178 d (F)				0.5 F (72% inhibition of brain acetylcholinesterase activity)	Stavinoha et al. 1969
80	Mouse (NS)	2 mo (F)			19.5 (increased permeability of brain tissue to copper ferricyanide)		Clark and Stavinoha 1971
81	Mouse (Charles River)	4-12 wk (F)				21.7 (increased exploratory behavior)	Clark et al. 1971
82	Mouse (CF-LP)	13 wk (F)		0.14 F 0.63 M	0.71 F (27-37% inhibition of red blood cell and plasma cholinesterase activity)		Rivett et al. 1972
83	Dog (Beagle)	5 mo 5d/wk 1x/d (C)				0.5 (80% inhibition of erythrocyte cholinesterase)	Hikita et al. 1973

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference
84	Dog (Beagle)	40 wk (F)			0.06	(22-50% inhibition of erythrocyte cholinesterase; 33-36% inhibition of plasma cholinesterase)			Hoffman et al. 1975
	Reproductive								
85	Rat (Sprague-Dawley)	F0: 15 wk premating; F1b: 13 wk premating & through production and weaning of F2a & F2b (F)		0.009	0.03	(decreased F2b litter counts and litter weights)	0.09	(decreased % sperm-positive F0 & F1 females; decreased maternal F0 & F1 weight during gestation & lactation; decreased litter counts, viability & lactation indices, increased dead births & % dead births)	Hixson and Hathaway 1986
86	Rat (Albino)	60-95 d (F)					0.5	(2/5 females failed to become pregnant)	Ryan et al. 1970
	Developmental								
87	Rat (Sprague-Dawley)	F0: 15 wk premating; F1b: 13 wk premating & through production and weaning of F2a & F2b (F)		0.009 [c]	0.03	(24-32% inhibition of brain cholinesterase activity in F1a pups)			Hixson and Hathaway 1986

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
88	Rat (Albino)	60-95 d (F)			0.5 (32.1% inhibition of fetal brain cholinesterase activity)		Ryan et al. 1970
89	Rat (Holtzman)	3 generations (F)			0.1 (30-40% inhibition of erythrocyte cholinesterase in F3b weanlings) 0.5 (cloudy swelling and fatty livers, mild nephropathy, juvenile hypoplasia of testes in F3b weanlings)		Taylor 1965a
	CHRONIC EXPOSURE						
	Death						
90	Rat (Fischer 344)	104-106 wk (F)				1.02 F (increased mortality)	Hayes 1985

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Systemic						
91	Rat (Sprague-Dawley)	1.5-2yr (F)	Resp	0.1			Carpy et al. 1975
			Cardio	0.1			
			Gastro	0.1			
			Hemato	0.1			
			Musc/skel	0.1			
			Hepatic	0.1			
			Renal	0.1			
			Endocr	0.1			
			Derm	0.1			
			Ocular	0.1			
			Bd Wt	0.1			

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
92	Rat (Fischer 344)	104-106 wk (F)	Resp	0.21 F	1.02 F (granulomatous and suppurative inflammation of the lungs) 0.75 M		Hayes 1985
			Cardio	1.02 F			
			Gastro	0.21 F 0.75 M	1.02 F (mucosal hyperplasia and chronic inflammation of the forestomach)		
			Hemato	1.02 F			
			Musc/skel	0.21 F 0.75 M		1.02 F (skeletal muscle atrophy due to debilitation)	
			Hepatic	1.02 F			
			Renal	1.02 F			
			Endocr	0.18 M 1.02 F	0.75 M (pancreatic atrophy)		
			Derm	0.21 F 0.18 M	1.02 F (acanthosis, 0.75 M hyperkeratosis, ulcer of the skin)		
			Ocular	0.06 F 0.18 M	0.21 F (cystic degeneration of Harderian gland)	1.02 F (corneal neovascularization) 0.75M	
			Bd Wt	0.21 F 0.18 M	1.02 F (11-19% decrease in 0.75 M body weight gain)		

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference
93	Mouse (CD-1)	23 mo (F)	Resp	2.53				Hayes 1983
			Cardio	2.53				
			Gastro	2.53				
			Hemato	2.53				
			Musc/Skel	2.53				
			Hepatic	2.53				
			Renal	2.53				
			Endocr	2.53				
			Derm	2.53				
			Ocular	2.53				
			Bd Wt	2.53				
94	Dog (Beagle)	2 yr (F)	Resp	0.14				Hoffman et al. 1975
			Cardio	0.14				
			Gastro	0.14				
			Hemato	0.14				
			Musc/skel	0.14				
			Hepatic	0.14				
			Renal	0.14				
			Endocr	0.14				
			Ocular	0.14				
			Bd Wt	0.14				
95	Dog (Beagle)	2 yr 5 d/wk 1x/d (C)	Ocular			0.63	(myopia, astigmatism, degenerationof ciliary muscle cells)	Ishikawa and Miyata 1980

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Immunological/Lymphoreticular						
96	Rat (Sprague-Dawley)	1.5-2 yr (F)		0.1			Carpy et al. 1975
97	Rat (Fischer 344)	104-106 wk (F)		0.21 F 0.18 M	1.02 F (splenic lymphoid follicle depletion) 0.75 M (plasma cell hyperplasia in the mandibular lymph nodes)		Hayes 1985
98	Mouse (CD-1)	23 mo (F)		2.53			Hayes 1983
99	Dog (Beagle)	2 yr (F)		0.14			Hoffman et al. 1975
	Neurological						
100	Rat (Sprague-Dawley)	1.5-2 yr (F)		0.05 M	0.06 M (26-37%inhibition of brain cholinesterase) 0.09 F		Carpy et al. 1975
101	Rat (Fischer 344)	104-106 wk (F)		0.05 M		0.18M (46-67% inhibition of erythrocyte cholinesterase, 53% inhibtion of brain cholinesterase, optic nerve degeneration)	Hayes 1985
					0.06 F (14-24% inhibition of erythrocyte cholinesterase, 21% inhibition of brain cholinesterase)	0.21 F (57-77% inhibition of erythrocyte cholinesterase, 53% inhibition of brain cholinesterase, optic nerve degeneration, rough coat)	

TABLE 2-2 Levels of Significant Exposure to Disulfoton - Oral (continued)

Key [a] to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference
102	Mouse (CD-1)	23 mo (F)		0.5 M			2.13M 2.53 F	(significant inhibition of erythrocyte, plasma, and brain cholinesterase by 56-82%, 50-79%, and 44-46%, respectively)	Hayes 1983
103	Dog (Beagle)	2 yr (F)		0.03	0.14	(46-53% inhibition of erythrocyte cholinesterase, 54-70% inhibition of plasma cholinesterase, 34.4% inhibition of brain cholinesterase in males)			Hoffman et al. 1975
104	Dog (Beagle)	2 yr 5 d/wk 1x/d (C)					0.5	(necrosis and atrophy of optic nerve and retina)	Uga et al. 1977
	Reproductive								
105	Rat (Fischer 344)	104-106 wk (F)		0.21 F 0.75 M	1.02 F	(uterine cystic hyperplasia)			Hayes 1985

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.001 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).
[c]Used to derive an intermediate-duration oral MRL of $9x10^{-5}$ mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).
[d]Used to derive a chronic-duration oral MRL of $6x10^{-5}$ mg/kg/day; dose divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

ad lib = ad libitum; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; d = day(s); Derm = dermal; Endocr = endocrine; F = female; (F) = feed; F0 = parental generation; F1 = first filial generation; F1a = first set of litters in the first filial generation; F1b = second set of litters in the first filial generation; F2a = first set of litters in the second filial generation; F2b = second set of litters in the second filial generation; F3B = second set of litters in the third filial generation; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage, oil vehicle; (GW) = gavage, water vehicle; Hemato = hematological; LD50 = lethal dose - 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); Musc/skel = muscular/skeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); x = time(s); yr = years(s)

Figure 2-2. Levels of Significant Exposure to Disulfoton – Oral

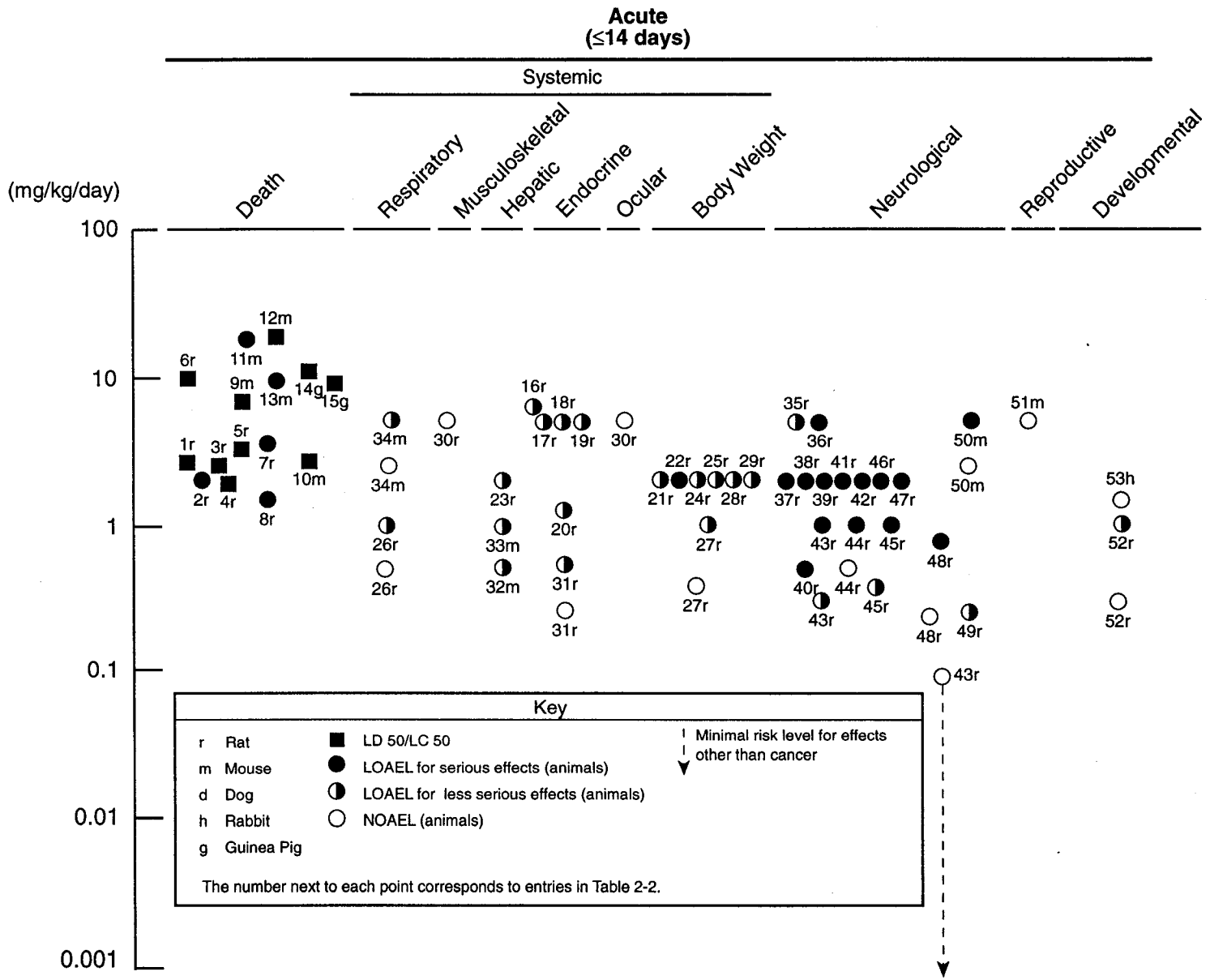


Figure 2-2. Levels of Significant Exposure to Disulfoton – Oral (continued)

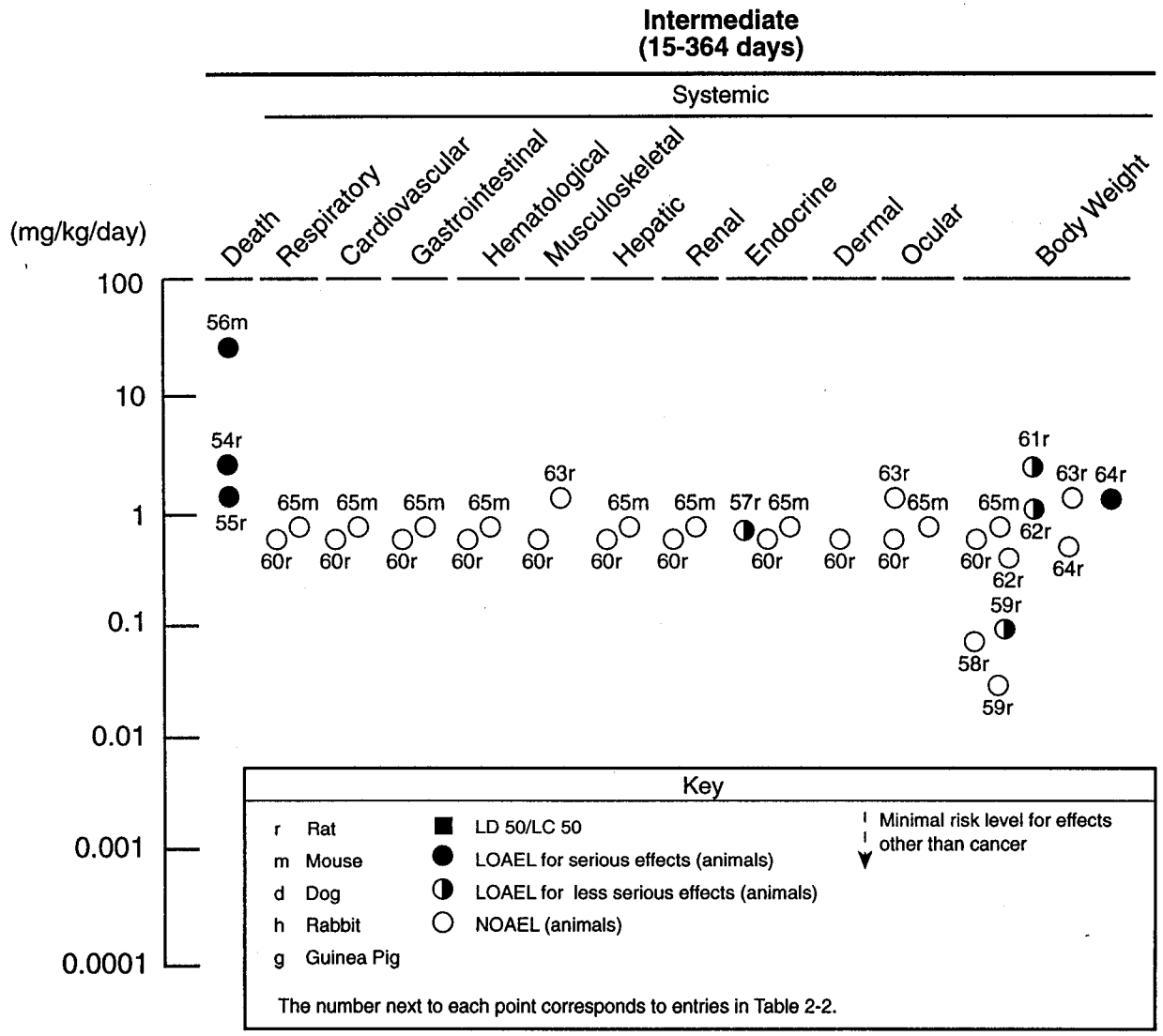


Figure 2-2. Levels of Significant Exposure to Disulfoton – Oral (continued)

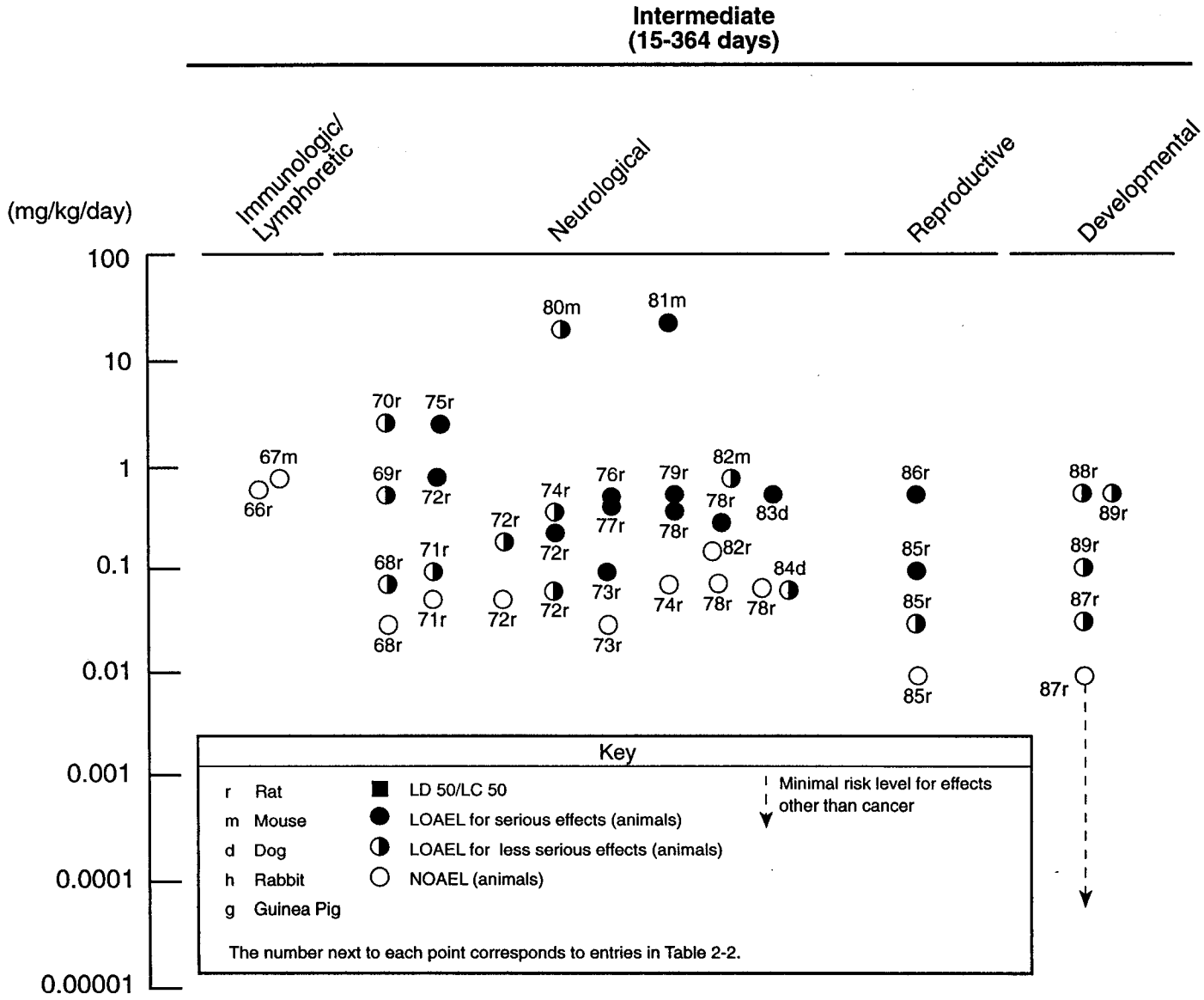
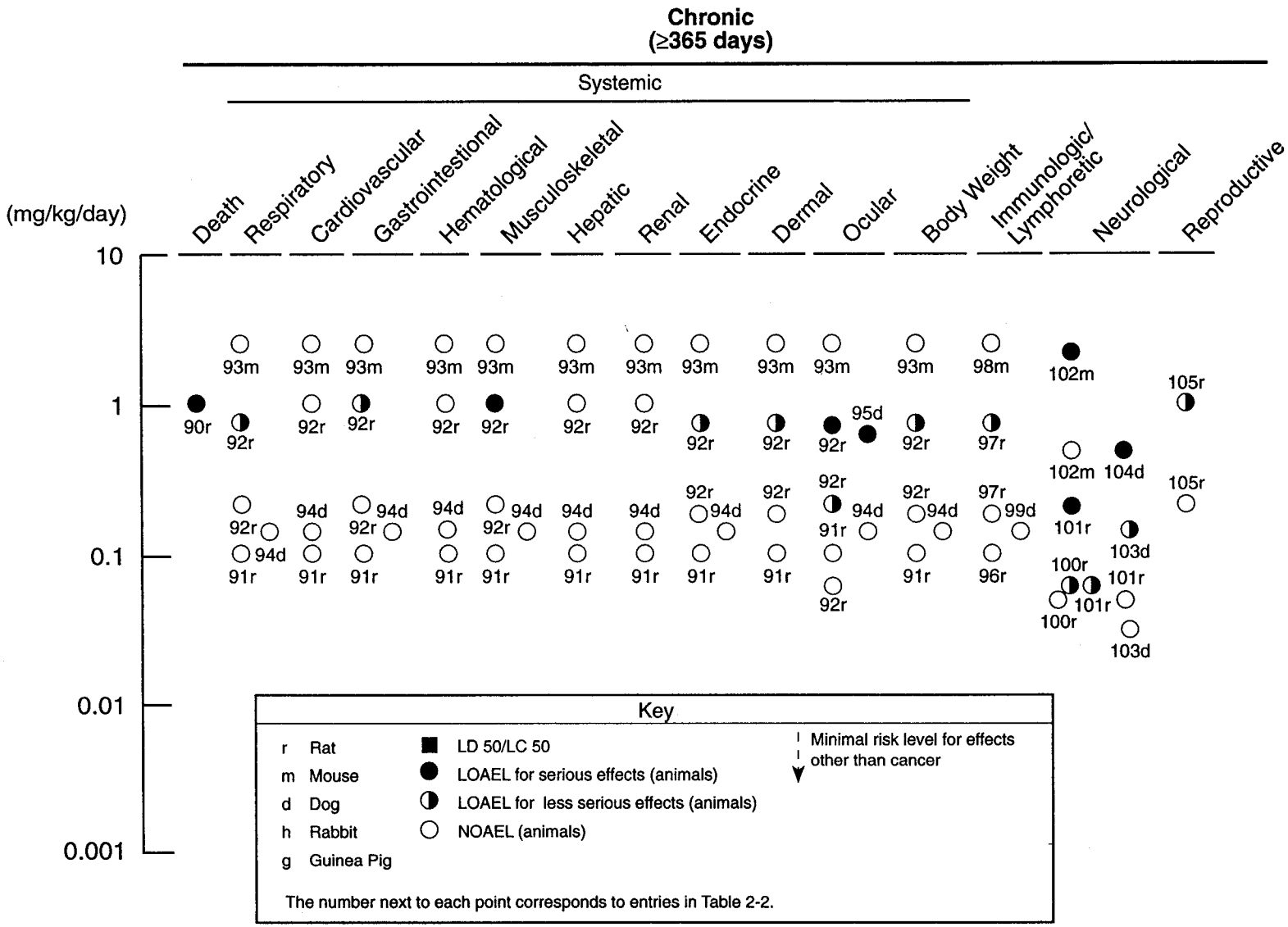


Figure 2-2. Levels of Significant Exposure to Disulfoton – Oral



same dose for 3 consecutive days (Schwab et al. 1981). In the same study, 1 of 8 rats died after receiving either 2.5 or 3.5 mgfkgfday for 6 days. When groups of mice were given 50% of the derived LD_{50} (9.6 mg/kg) for 3, 5, and 10 days, mortality was 2 of 8, 2 of 8, and 9 of 20, respectively (Stevens et al. 1972b). The results suggest that even at half the acute LD_{50} dose, almost half of the mice given disulfoton for 10 days died.

In intermediate-duration studies, 1 of 12 female rats given 1.3 1 mg/kg/day disulfoton in the diet was found dead on day 48 due to cholinergic effects (tremor, muscle fasciculation) (Sheets 1993b). In addition, 4 of 71 male rats died when given a diet providing 2.5 mg/kg/day for 30 days (Robinson et al. 1978), and 5 of 25 female mice died when given a diet providing 26 mg/kg/day disulfoton for 4 weeks (Clark et al. 1971). The reason that mice could tolerate a higher dose in the diet for 4 weeks compared with single dose LD_{50} values is probably related to the method of administration (i.e., continuous intermittent exposure during feeding versus a single bolus dose by gavage).

In a 2-year dietary study, female rats in the high dose group (1.02 mg/kg/day) had a 40% mortality rate during the last week of the study compared with 12% in controls (Hayes 1985). While the mortality rate in the control group was unusually low, the 40% mortality rate in the high dose female rats was also increased when compared with historical controls, in which the mortality rate ranged from 18 to 34%. No increase in the mortality rate of male rats was observed. Furthermore, no increase in the mortality was reported for mice exposed to 2.13 mg/kg/day (males) or 2.53 mg/kg/day (females) disulfoton in the diet for 23 months (Hayes 1983). These results support the conclusion that rats are more sensitive than mice and that female rats are more sensitive than male rats to the lethal effects of disulfoton.

The causes of death in these studies were not specifically mentioned, but disulfoton is a cholinesterase inhibitor, and animals exposed to disulfoton typically exhibit cholinergic signs of toxicity (see Section 2.2.2.4).

The LD_{50} values and the doses associated with death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

Studies regarding the systemic effects that have been observed in humans and animals after oral exposure to disulfoton are discussed below. The highest NOAEL values and all LOAEL values from each reliable study for each systemic effect in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. Intra-alveolar bleeding, edema of the lungs, and blood in the bronchus were observed at autopsy in a man who had been dead for at least 24 hours after ingesting an unknown quantity of disulfoton (Hattori et al. 1982). This was the only information found regarding respiratory effects in humans after oral exposure to disulfoton.

Breathing difficulties were observed in rats given a single gavage dose of 1.0 mg/kg and in mice given 5.0 mg/kg disulfoton (Mihail 1978). Rats given 0.5 mg/kg and mice given 2.5 mg/kg did not display breathing disorders.

No histopathological lesions were found in the lungs of rats exposed to 0.34 mg/kg/day (males) or 0.55 mg/kg/day (females) (Klotzsche 1972) or mice exposed to 0.63 mg/kg/day (males) or 0.71 mg/kg/day (females) (Rivett et al. 1972) in the diet for 90 days, or in rats exposed to ≤0.21 mg/kg/day (Carpy et al. 1975; Hayes 1985), in mice exposed to 2.13 mg/kg/day (males) or 2.53 mg/kg/day (females) (Hayes 1983), or in dogs exposed to 0.14 mg/kg/day (Hoffman et al. 1975) in the diet for up to 2 years. In rats exposed to disulfoton in the diet for 2 years, granulomatous and suppurative inflammation of the lungs was found in the high dose groups (0.75 mg/kg/day in males and 1.02 mg/kg/day in females) (Hayes 1985). The lung inflammation was considered to be due to aspiration of the food particles, which in turn may have been associated with the debilitation observed in the high dose groups.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to disulfoton.

No histopathological lesions were found in the hearts of rats exposed to ≤0.55 mg/kg/day (Klotzsche 1972) or mice exposed to ≤0.71 mg/kg/day (Rivett et al. 1972) in the diet for 90 days, or in rats exposed to 0.1 mg/kg/day (Carpy et al. 1975) or ≤1.02 mg/kg/day (Hayes 1985), in mice exposed to

≤2.53 mg/kg/day (Hayes 1983), or in dogs exposed to 0.14 mg/kg/day (Hoffman et al. 1975) in the diet for up to 2 years.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after oral exposure to disulfoton.

No histopathological lesions were found in the gastrointestinal tracts of rats exposed to 0.34 mg/kg/day (males) or 0.55 mg/kg/day (females) (Klotzsche 1972) or mice exposed to 0.63 mg/kg/day (males) or 0.71 mg/kg/day (females) (Rivett et al. 1972) in the diet for 90 days, or in rats exposed to 0.1 mg/kg/day (Carpy et al. 1975), in mice exposed to 2.13 mg/kg/day (males) or 2.53 mg/kg/day (females) (Hayes 1983), or in dogs exposed to 0.14 mg/kg/day (Hoffman et al. 1975) in the diet for up to 2 years. However, increased incidences of mucosal hyperplasia and chronic inflammation of the forestomach were observed in female rats given 1.02 mg/kg/day disulfoton in the diet for 2 years (Hayes 1985). The mucosal hyperplasia was usually diffuse but was sometimes more locally severe and accompanied by inflammation, fibrosis, and ulceration. The forestomach lesions were not observed in male rats at ≤0.75 mg/kg/day or in females at ≤0.21 mg/kg/day.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to disulfoton.

Limited information from animal studies suggests that intermediate- or chronic-duration exposure to disulfoton is not associated with hematological effects. No hematological effects were observed in rats fed ≤0.55 mg/kg/day of disulfoton (Klotzsche 1972) or in mice fed ≤0.71 mg/kg/day (Rivett et al. 1972) for 90 days. In 2-year feeding studies, disulfoton did not cause any hematological effects in rats (Carpy et al. 1975; Hayes 1985), mice (Hayes 1983), or dogs (Hoffman et al. 1975).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to disulfoton.

Degeneration of ciliary muscle cells was found in the eyes of dogs given disulfoton at doses ≥0.63 mg/kg/day for 2 years (Ishikawa and Miyata 1980; Suzuki and Ishikawa 1974). The degenerative changes consisted of the presence of unique membranous structures, displacement of myofilaments, and lack of clearly defined organelles. The authors suggested that the microsomal

oxidation of disulfoton to an active metabolite that can destroy microsomes may account for the destructive changes in the ciliary muscle cells (Suzuki and Ishikawa 1974) or that cholinergic innervation of the iris-sphincter and ciliary muscle by disulfoton resulted in edema of the ciliary muscles (Ishikawa and Miyata 1980). The degeneration of these cells was believed to be the cause of myopia (see Ocular Effects below) in these dogs.

Histological examination of the gastrocnemius muscle of rats given a single gavage dose of ≤5.2 mg/kg (Sheets 1993a) or ≤1.31 mg/kg/day disulfoton in the diet for 13 weeks (Sheets 1993b) revealed no treatment-related lesions. No histopathological muscular or skeletal lesions were found in rats exposed to 0.34 mg/kg/day (males) or 0.55 mg/kg/day (females) (Klotzsche 1972) or mice exposed to 0.63 mg/kg/day (males) or 0.71 mg/kg/day (females) (Rivett et al. 1972) in the diet for 90 days, or in rats exposed to ≤0.21 mg/kg/day (Carpy et al. 1975; Hayes 1985), in mice exposed to 2.13 mg/kg/day (males) or 2.53 mg/kg/day (females) (Hayes 1983), or in dogs exposed to 0.14 mg/kg/day (Hoffman et al. 1975) in the diet for up to 2 years. However, reduced skeletal muscle size and skeletal muscle atrophy were observed in female rats given 1.02 mg/kg/day disulfoton in the diet for 2 years (Hayes 1985). The skeletal muscle atrophy corresponded to the generalized debilitation in the high dose females.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to disulfoton.

In animals, the hepatic effects associated with oral exposure to disulfoton included alterations in liver microsomal enzyme activities, lipid peroxidation, and changes in liver weight. The ability of disulfoton to affect microsomal enzyme activities appears to depend upon the dose, the duration of dosing, and the time between dosing and enzyme assays. Microsomal enzyme induction is considered to be nonadverse unless the induction of enzymes can be linked to more serious liver effects. A single oral dose (9.6 mg/kg) of disulfoton caused a significant ($p<0.05$) decrease in *in vitro* mouse liver ethylmorphine N-demethylase and reduced nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome c reductase activities, but no significant effect on NADPH oxidase, when assayed 1 hour after dosing (Stevens et al. 1973). However, a significant increase in liver ethylmorphine N-demethylase and NADPH oxidase activities, but no significant effect on NADPH cytochrome c reductase activity or cytochrome P-450 content, was observed in mice given 9.6 mg/kg/day disulfoton for 3 days and sacrificed 24 hours later for *in vitro* enzyme assays. When mice were

treated with 8 mg/kg/day for 5 days, the content of cytochrome P-450 was also significantly increased. Treatment of mice with 9.6 mg/kg/day disulfoton for 3, 5, or 10 days resulted in significant shortening of the hexobarbital sleeping time, compared with controls, and stimulated the *in vitro* side chain oxidation of hexobarbital and the hydroxylation of aniline (Stevens et al. 1972b). Significant increases in microsomal protein content and delta-aminolevulinic acid synthetase activity, and significant decreases in ethylmorphine N-demethylase, aminopyrine N-demethylase, and acetanilide hydroxylase activities were found in the livers from rats given 2 mg/kg or mice given 0.5 or 1.0 mg/kg/day disulfoton for l-4 days (Fawade and Pawar 1978, 1980, 1983). Disulfoton also caused an increase in NADPH-dependent and ascorbate-promoted lipid peroxidation and a decrease in electron transport elements. The reduction in electron transport elements was thought to be due to loss of integrity of the membranes and structural alterations in the membrane phospholipids, leading to increased lipid peroxidation. Disulfoton or its oxygenated metabolite may also have changed the conformation of heme protein, leading to enhanced lipid peroxidation (Fawade and Pawar 1978).

In intermediate-duration studies, no effects on clinical chemistry indices of liver toxicity and no histopathological hepatic lesions were found in rats given 0.34 mg/kg/day (males) or 0.55 mg/kg/day (females) (Klotzsche 1972) or in mice given 0.63 mg/kg/day (males) or 0.71 mg/kg/day (females) (Rivett et al. 1972) in the diet for 90 days. However, a slight increase in liver weight was observed in female mice at 0.71 mg/kg/day (Rivett at al. 1972).

Similarly, in chronic feeding studies, no clinical chemistry or histological evidence of liver toxicity was found in rats (Carpy et al. 1975; Hayes 1985), mice (Hayes 1983), or dogs (Hoffman et al. 1975). However, trends towards increased liver weights in male rats and decreased liver weights in female rats fed disulfoton for 1.5-2.0 years were observed (Carpy et al. 1975). The reason for these opposite trends in male and female rats is not clear.

Renal Effects. The only information found regarding renal effects in humans after oral exposure to disulfoton was swelling of the glomerulus at autopsy in a man who had been dead for at least 24 hours after ingesting an unknown quantity of disulfoton (Hattori et al. 1982).

Few data were located regarding renal effects in animals after oral exposure to disulfoton, and the evidence for renal effects due to disulfoton ingestion is inconclusive. Urinalysis and histological examination revealed no renal effects in rats given ≤0.55 mg/kg/day disulfoton (Klotzsche 1972) or in

mice given ≤0.71 mg/kg/day disulfoton (Rivett et al. 1972) in the diet for 90 days, in rats given ≤1.02 mg/kg/day in the diet for 1.5-2 years (Carpy et al. 1975; Hayes 1985), in mice given ≤2.53 mg/kg/day in the diet for 23 months (Hayes 1983), or in dogs given 0.14 mg/kg/day in the diet for 2 years (Hoffman et al. 1975). Trends towards increased kidney weights in male rats and decreased kidney weights in female rats fed disulfoton for 1.5-2 years were observed (Carpy et al. 1975). The reason for these opposite trends in male and female rats is not clear. In another study, absolute and relative kidney weights were significantly increased in females mice fed 2.53 mg/kg/day, but not in male mice fed 2.13 mg/kg/day, disulfoton for 23 months (Hayes 1983). The increased kidney weight was thought to be associated with an insignificant increase in the incidence of malignant lymphoma in the kidney. Since the kidney tumors were not believed to be the result of disulfoton treatment, the toxicological significance of the increased kidney weight is not clear.

Endocrine Effects. No studies were located regarding endocrine effects in humans after oral exposure to disulfoton.

Disulfoton exposure altered catecholamine levels in animals, and this hormonal imbalance may be associated with elevated acetylcholine levels (Brzezinski 1969, 1972, 1973; Brzezinski and Ludwicki 1973; Brzezinski and Rusiecki 1970; Wysocka-Paruszewska 1970, 1971). In these studies, acute dosing with disulfoton caused increases in urinary and plasma noradrenaline and adrenaline levels, accompanied by decreases of adrenaline in the adrenal glands, in rats. In addition, the major urinary metabolite of catecholamine metabolism, 4-hydroxy-3-methoxymandelic acid (HMMA), was recovered in the urine from rats given acute doses of disulfoton (Wysocka-Paruszewska 1970, 1971). The maximum level of HMMA in the urine occurred 72 hours after exposure, which coincides with the time period for maximum urine catecholamine levels. There was a direct relationship between blood cholinesterase inhibition and catecholamine (adrenaline and noradrenaline) levels in the urine and blood (Brzezinski and Ludwicki 1973). Maximum inhibition of cholinesterase activity and maximum plasma catecholamine occurred during the first l-2 hours after exposure. However, catecholamine levels returned to normal more rapidly than cholinesterase activity. It was proposed that high levels of acetylcholine, which are normally associated with cholinesterase activity inhibition, caused a release of catecholamines from the stores in the adrenals.

Elevated catecholamine concentrations in the urine were also observed in rats dosed with 0.625 mg/kg/day of disulfoton every other day for 76 days (Brzezinski and Rusiecki 1970). Urinary

catecholamine levels plateaued between 16 and 36 days, followed by a gradual decline for the next 40 days. However, these levels were still elevated at day 76.

In 13-week dietary studies, measurement of organ weight and histological examination of adrenals, pancreas, pituitary, and thyroid revealed no effects in rats at doses ≤0.55 mg/kg/day (Klotzsche 1972) or mice at dose ≤0.1 mg/kg/day (Rivett et al. 1972)

There was a trend towards increased pituitary weights in male rats and decreased pituitary weights in female rats fed disulfoton for 1.5-2.0 years (Carpy et al. 1975). The reason for the opposite trends in organ weights in males and females and the toxicological significance for these effects is not clear. Male rats given a high dose (0.75 mg/kg/day) of disulfoton in the diet for 2 years had a significantly increased incidence of pancreatic atrophy, seen as small focal areas of shrunken acinar cells (Hayes 1985). No histopathological lesion in the pancreas were observed in females at doses ≤1.02 mg/kg/day, and no histopathological lesions in the adrenal, pituitary, thyroid, or parathyroid were found in the male or female rats at any dose. In other chronic dietary studies, no organ weight changes or histopathological lesions in the adrenals, pancreas, thyroid, parathyroid, or pituitary were found in mice at doses ≤2.53 mg/kg/day (Hayes 1983) or dogs at doses ≤0.14 mg/kg/day (Hoffman et al. 1975). The Hoffman study also found no changes or histopathological lesions in parotid in dogs.

Dermal Effects. No studies were located regarding dermal effects in humans after oral exposure to disulfoton.

In animals, histological examination of skin revealed no lesions in rats exposed to 0.34 mg/kg/day (males) or 0.55 mg/kg/day (females) (Klotzsche 1972) in the diet for 90 days, or in rats exposed to 0.1 mg/kg/day (Carpy et al. 1975) or in mice exposed to 2.13 mg/kg/day (males) or 2.53 mg/kg/day (females) (Hayes 1983) in the diet for up to 2 years. However, acanthosis, hyperkeratosis, ulceration of the skin, exudate formation, and epithelial inclusion cysts were increased in male rats exposed to 0.75 mg/kg/day and female rats exposed to 1.02 mg/kg/day disulfoton in the diet for 2 years (Hayes 1985). No increase in skin lesions was found in the male rats at 0.18 mg/kg/day or in female rats at 0.21 mg/kg/day.

Ocular Effects. The only information regarding ocular effects in humans comes from an epidemiological study in which a marked increase of myopia in young children was observed

(Ishikawa and Miyata 1980). The increase coincided with an increased use of disulfoton in combination with other organophosphates to treat food crops. As discussed below, disulfoton caused myopia in Beagle dogs, providing supportive evidence that disulfoton probably contributed to the development of myopia in the young children.

Ocular effects such as myopia and astigmatism have been observed in dogs. Myopia and astigmatism occurred after 12 months in Beagle dogs given ≥0.63 mg/kg/day disulfoton for 2 years (Ishikawa and Miyata 1980; Suzuki and Ishikawa 1974). The myopia became progressively worse until cessation of dosing. As discussed above for musculoskeletal effects, histological examination of the ciliary muscle cells revealed degenerative changes that were considered to be the cause of the myopia. Cystic degeneration of the Harderian gland was observed in male rats exposed to 0.75 mg/kg/day and in female rats exposed to ≥0.21 mg/kg/day disulfoton in the diet for 2 years (Hayes 1985). In the same study, the incidence of cornea1 neovascularization was significantly increased in the high dose rats (0.75 mg/kg/day in males and 1.02 mg/kg/day in females), while no ocular lesions were found in the male rats at 0.18 mg/kg/day or in the female rats at 0.06 mg/kg/day. In other studies, ophthalmological and histological examination of eyes revealed no lesions in rats given a single gavage dose of 5.2 mg/kg (males) or 1.5 mg/kg (females) (Sheets 1993a); or in rats exposed to ≤1.08 mg/kg/day (males) or ≤1.31 mg/kg/day (females) (Klotzsche 1972; Sheets 1993b) or mice exposed to 0.63 mg/kg/day (males) or 0.71 mg/kg/day (females) (Rivett et al. 1972) in the diet for 90 days; or in rats exposed to 0.1 mg/kg/day (Carpy et al. 1975), in mice exposed to 2.13 mg/kg/day (males) or 2.53 mg/kg/day (females) (Hayes 1983), or in dogs exposed to 0.14 mg/kg/day (Hoffman et al. 1975) in the diet for up to 2 years.

Body Weight Effects. Weight loss or decreased body weight gain is commonly observed in animals after acute exposure to disulfoton and is one of the typical signs of cholinergic toxicity of cholinesterase inhibitors (see Section 2.2.1.4). The weight loss or reduced weight gain usually occurs early in the dosing regimen, but the rate of weight gain recovers with repeated dosing as the animals become tolerant (Costa et al. 1984, 1986; Fitzgerald and Costa 1992, 1993; Schwab and Murphy 1981; Schwab et al. 1981). Many of the acute oral studies conducted in animals showing initial body weight loss were designed to study the phenomenon and mechanism of tolerance development (see Section 2.3.5). Rats treated with 2.0 or 2.5 mg/kg/day disulfoton by gavage for l-10 days initially exhibited a 20-50% reduction in weight gain (Costa et al. 1984, 1986; Schwab et al. 1981, 1983). In another study, rats exhibited an unspecified, but significant ($p<0.01$), decrease in body weight gain

within 3 days of a 9-day disulfoton feeding regimen that provided 1 mg/kg/day (Schwab and Murphy 1981). In all of these studies, the effect on weight gain diminished with repeated dosing, suggesting that the rats became tolerant to disulfoton. In addition, a more severe weight loss (≈20%) was observed in rats given 3.5 mg/kg/day of disulfoton for 3 days than in rats that had previously received a 2.5 mg/kg/day dose for 6 days and then a 3.5 mg/kg/day dose for an additional 6 days (Schwab et al. 1981). Although changes in neurological effects are more commonly employed, these studies suggested that body weight changes can be used to monitor the development of tolerance in rats.

Although the acute studies suggest that with repeated dosing body weight gain recovers after the initial decrease, the body weight remains lower than the control body weight, as demonstrated in intermediate-duration studies. Rats given 2.5 mg/kg/day disulfoton for 30 days gained 29% less in body weight gain than controls (Robinson et al. 1978). In a 62-day feeding study, significantly ($p<0.01$) lower body weights were seen in rats within 3 days at 1 mg/kg/day disulfoton (Schwab and Murphy 1981). Although the rats recovered some of the body weight, the body weights were still significantly depressed at all weighing times during the 62-day exposure. A 40% decrease in body weight gain was observed in rats given 1.25 mg/kg/day, but not 0.5 mg/kg/day, disulfoton in the diet for 141-178 days (Stavinoha et al. 1969). Weight changes were used as the major criterion for tolerance development rather than the less objective neurological signs of cholinergic poisoning. The time for tolerance development increased as the dose of disulfoton increased. In an extensive reproductive study, body weight gain was marginally depressed by 6-10% in F_1 parental females and 9-l 1% in F_1 parental males receiving 0.09 mg/kg/day disulfoton in the diet during the premating period of 13 weeks (Hixson and Hathaway 1986). In other intermediate-duration dietary studies, no effects on body weight gain were observed in rats given ≤l.3 1 mg/kg/day (Christenson and Wahle 1993; Klotzsche 1972; Sheets 1993b) or in mice given ≤0.71 mg/kg/day (Rivett et al. 1972).

In rats given disulfoton in the diet for 2 years, body weight gain was decreased by ll-19% in females at 1.02 mg/kg/day, but not at 0.21 mg/kg/day, and in males at 0.75 mg/kg/day, but not at 0.18 mg/kg/day (Hayes 1985). In other chronic-duration studies, no effects on body weight were observed in rats given 0.1 mg/kg/day in the diet (Carpy et al. 1975), in mice given ≤2.53 mg/kg/day in the diet (Hayes 1983), or in dogs given 0.14 mg/kg/day in the diet (Hoffman et al. 1975).

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding the immunological or lymphoreticular effects in humans after oral exposure to disulfoton.

In 13-week feeding studies, histological examination of lymph nodes, spleen, and bone marrow of rats at doses ≤0.55 mg/kg/day (Klotzsche 1972) and of lymph nodes, spleen, and thymus of mice at ≤0.71 mg/kg/day (Rivett et al. 1972) revealed no treatment-related lesions. In rats given the high concentration of disulfoton in the diet for 2 years, males (0.75 mg/kg/day) had a significantly increased incidence of plasma cell hyperplasia in the mandibular lymph nodes, and females (1.02 mg/kg/day) had a significantly increased incidence of splenic lymphoid follicle depletion (Hayes 1985). The author suggested that plasma cell hyperplasia in the mandibular lymph nodes was probably a response to upper respiratory tract inflammation, which may have been due to aspiration of ingested food particles. Histological examination of the mesenteric lymph node or thymus revealed no treatment-related lesions in either sex at any dose. In other chronic dietary studies, no treatmentrelated lesions were found in the lymph nodes, spleen, thymus, or bone marrow of rats at ≤0.1 mg/kg/day (Carpy et al. 1975), mice at ≤2.53 mg/kg/day (Hayes 1983), or dogs at ≤0.14 mg/kg/day (Hoffman et al. 1975).

Down-regulation of cholinergic muscarinic receptors in T-lymphocytes and significantly inhibited acetylcholinesterase activity in T-lymphocytes were found in rats given 2 mg/kg/day disulfoton by gavage for l-2 weeks (Fitzgerald and Costa 1993). The inhibition of T-lymphocyte acetylcholinesterase activity paralleled that in the brain. The immunological significance of these neurological effects (see Section 2.2.2.4) is not known.

2.2.2.4 Neurological Effects

Exposure to disulfoton can result in inhibition of acetylcholinesterase activity, with consequent accumulation of acetylcholine at nerve synapses and ganglia leading to central nervous system, nicotinic, and muscarinic effects (see Section 2.2.1.4 for more extensive discussion).

In a human case-report study, a 30-year-old man was found dead after consuming an unknown amount of disulfoton (Hattori et al. 1982). Bubbling saliva in the oral cavity and constricted pupils were

evidence of muscarinic effects. Pulmonary edema and blood in the lungs and bronchus suggested that death was primarily due to respiratory failure brought on by disulfoton intoxication. Severe signs and symptoms of disulfoton toxicosis (miosis, salivation, masseteric spasms, and monoplegia) were observed in a man within 2-3 hours of consuming 34 heaping tablespoons of disulfoton (Yashiki et al. 1990). The coincidental depression in serum cholinesterase activity and the occurrence of severe clinical signs suggests that a very severe toxic dose of disulfoton was consumed, and death may have occurred were it not for the medical treatment.

Disulfoton can interfere with neurotransmitter levels (e.g., acetylcholine, catecholamines) in the brain of affected animals (Brzezinski 1972; Costa et al. 1986). A single dose of disulfoton caused a lower noradrenaline level in the brain of rats compared with the controls, with the greatest decreases (about 0.175 µg /mL and 0.1 µg /mL compared with 0.3 µg /rnL in control) at 15 and 120 minutes after dosing (Brzezinski 1972). Adrenaline levels in the brain, however, showed little difference from control levels, being slightly increased at 60 minutes and slightly decreased at 120 minutes. The rats exhibited typical cholinergic signs of disulfoton toxicosis (not otherwise specified). In other acute exposure studies, brain cholinesterase activity was significantly depressed when rats were given disulfoton for 7-10 days, suggesting elevated brain acetylcholine levels (Costa and Murphy 1983a; Costa et al. 1986; Schwab and Murphy 1981; Su et al. 1971).

Muscle twitching, clonic cramps, and increased salivation were observed in rats given a single gavage dose of 1.0 mg/kg and in mice given a single oral gavage dose of 5.0 mg/kg (Mihail 1978). Rats given 0.5 mg/kg and mice given 2.5 mg/kg did not develop these signs. However, in another study, rats given a single gavage dose of 0.5 mg/kg had tremors (Crawford and Anderson 1974). In an extensive neurotoxicity screening study, rats were given single gavage doses of disulfoton (0.24, 1.5, and 5.2 mg/kg in males; 0.24, 0.76, and 1.5 mg/kg for females) (Sheets 1993a). Clinical signs of cholinergic intoxication consisted of muscle fasciculation, tremors, ataxia, oral stain, urine stain, diarrhea, or decreased activity in the high dose males (5.2 mg/kg) and high dose females (1.5 mg/kg) and muscle fasciculation in the mid dose females (0.76 mg/kg). A battery of functional observational tests revealed effects in both males and female at the mid and high doses. At the mid dose and high dose, these effects included muscle fasciculation, ataxia, and minimal head or body movement during open field observation in both sexes and a lower incidence of vocalizations upon removal from the home cage in females. High dose males also had uncoordinated righting reflex. Results of motor and locomotor activity tests revealed a 55% and 51% reduced motor activity in high dose males and

females, respectively, and 64% and 62% reduced locomotor activity in high dose males and females, respectively. Erythrocyte cholinesterase activity was inhibited by 21% in high dose males, 75% in high dose females, and 53% in mid dose females. No treatment-related effects were observed for brain weight and extensive histopathological examination of the brain, spinal cord, peripheral nerves (sciatic, tibial, sural), optic nerves, or gasserian ganglion.

Animals exposed to disulfoton develop typical signs of cholinergic toxicity associated with inhibition of brain acetylcholinesterase activity after a few oral doses (Costa et al. 1984; Schwab and Murphy 1981; Schwab et al. 1981, 1983). However, with subsequent dosing, the severity of the overt cholinergic effects diminish, while cholinesterase remains inhibited. This phenomenon is known as tolerance (see Section 2.3.5). Male rats given 2.0 or 2.5 mg/kg/day of disulfoton for 1-14 days initially exhibited exophthalmia, excessive salivation, urination and defecation, diarrhea, fasciculations, generalized tremors, flaccidity, and malaise (Costa et al. 1984; Fitzgerald and Costa 1992, 1993; Schwab et al. 1981, 1983). Similar effects were observed in female rats after 3 days on a diet that provided 1 mg/kg/day disulfoton (Schwab and Murphy 1981). A diet that provided 0.38 mg/kg/day did not cause overt signs of toxicity, but brain acetylcholinesterase was inhibited by 30-35%. The severity of these signs diminished after an unspecified time with repeated dosing, but the signs did not completely disappear (Costa et al. 1984; Schwab and Murphy 1981; Schwab et al. 1981, 1983). When rats were given 3.5 mg/kg/day for 3-4 days, these clinical signs were more severe than those exhibited by rats pretreated with 2.5 mg/kg/day of disulfoton for 6 days and then given 3.5 mg/kg/day for 6 more days (Schwab et al. 1981). Thus, the rats pretreated with 2.5 mg/kg/day for 6 days became tolerant to even higher doses of disulfoton. In the same study, heart, ileum, forebrain, and hindbrain cholinesterase activity was moderately but significantly depressed in rats given seven daily doses of 2 mg/kg/day of disulfoton, followed by four daily doses of 3 mg/kg/day. Furthermore, a 50% reduction in pancreatic acetylcholinesterase activity was observed in rats given 2 mg/kg/day for 10 days despite the disappearance of clinical cholinergic signs after a few doses (Costa et al. 1984). This depression in cholinesterase activity suggests that the mechanism associated with disulfoton toxicity was not impaired, despite the disappearance of overt neurological signs of toxicity following repeated doses of disulfoton.

Disulfoton caused muscular tremors, unsteadiness, and ataxia in pregnant rabbits after exposure to 1.5-3.0 mg/kg/day on days 6-18 of gestation (Tesh et al. 1982). Doses of 0.3 or 1.0 mg/kg/day disulfoton did not affect the pregnant rabbits. In a reproductive study, tremors were observed in high

dose (0.09 mg/kg/day) F_0 female rats, but not the mid dose (0.03 mg/kg/day) F_0 females, during the production of the F_1 generation (Hixson and Hathaway 1986). Pregnant rats given disulfoton during gestation had significantly inhibited plasma and erythrocyte cholinesterase activity by 82-90% at 1 mg/kg/day and by 41% at 0.3 mg/kg/day, but not at 0.1 mg/kg/day (Lamb and Hixson 1983). Basec on this NOAEL (0.1 mg/kg/day), an acute oral MRL of 0.001 mg/kg/day was calculated as described in footnote "b" in Table 2-2.

An intermediate-duration extensive neurotoxicity screening study similar to the acute study described above was conducted in rats fed disulfoton in the diet that provided doses of 0.063, 0.270, or 1.08 mg/kg/day for males and 0.71, 0.315 or 1.31 mg/kg/day for females (Sheets 1993b). Clinical signs of cholinergic intoxication consisted of muscle fasciculation, perianal stains, and increased reactivity in high dose males and females and increased incidence of urine stains in mid and high dose females. A battery of functional observational tests revealed effects in high dose males and mid and high dose females. The effects in high dose rats included muscle fasciculations, tremors, increased defecation, decreased forelimb grip strength, decreased movement, and increased urine stain. Muscle fasciculations and increased urine stain were also seen in mid dose females. Automated measures of motor and locomotor activity were reduced on each test occasion (weeks 4, 8, and 13) in the high dose males and females. Erythrocyte cholinesterase activity was inhibited by 95100% in high dose rats and 61-80% in mid dose rats. Brain cholinesterase activity was inhibited by 35% in mid dose males and 64% in mid dose females and by 75% in high dose males and 87% in high dose females. No treatment-related effects were observed for brain weight and extensive histopathological examination of the brain, spinal cord, peripheral nerves (sciatic, tibial, sural), optic nerves, or gasserian ganglion.

In intermediate-duration studies, typical signs of cholinergic poisoning are generally seen only during the first few days, after which they diminish. However, cholinesterase activity usually remains inhibited during exposure. Characteristic signs (not otherwise specified) of anticholinesterase poisoning were observed in rats fed disulfoton for 30 days, and some of the rats recovered (Robinson et al. 1978). Brain, stomach, and diaphragm cholinesterase activity were severely depressed. In a 62-day feeding study, rats developed severe cholinergic signs of disulfoton toxicity after 3 days on a diet providing 1 mg/kg/day disulfoton (Schwab and Murphy 1981). The severity of these signs decreased but never completely disappeared after 62 days. Brain and diaphragm cholinesterase activity was depressed at day 6 and remained depressed throughout the study. Sex and strain differences in rats may influence the ability of disulfoton to inhibit cholinesterase or to elevate acetylcholine levels.

Brain cholinesterase activity was significantly depressed to about the same extent in Holtzman rats and Charles River rats fed disulfoton for 141-178 days, but only the Charles River rats had elevated brain acetylcholine levels (Stavinoha et al. 1969). Cholinesterase activity of erythrocytes and the brain was inhibited to a greater extent or at lower doses in female rats than in male rats given disulfoton in the diet for intermediate durations (Christenson and Wahle 1993; Doull and Vaughn 1958; Hayes 1985; Klotzsche 1972; Ryan et al. 1970). In mice fed diets providing 0.63 mg/kg/day (males) or 0.71 mg/kg/day (females), cholinesterase was inhibited in all tissues, especially in females, but the tissues were not specified (Rivett et al. 1972). In a chronic study in which dogs were given capsules containing disulfoton for 2 years, erythrocyte cholinesterase activity was inhibited by 80% after 5 months of exposure to ≥0.5 mg/kg/day and remained depressed throughout the 2-year duration (Hikita et al. 1973). A 22-50% inhibition of erythrocyte cholinesterase activity and a 33-36% inhibition of plasma cholinesterase activity were found in dogs given diets containing disulfoton at a dose of 0.06 mg/kg/day for 40 weeks (Hoffman et al. 1975).

Disulfoton has also been studied for behavioral effects. Rats fed ≥0.5 mg/kg/day disulfoton for 90 days had significantly depressed brain acetylcholinesterase levels (59-74% below control), but the treated rats had shorter maze running times and made fewer mistakes than the controls (Clark and Pearson 1973). This unexpected result (improved learning) at reduced brain cholinesterase levels led the authors to question the “critical level of 60% reduction” for neurobehavioral effects. In another behavioral experiment, there was an unexplained increase in exploratory behavior in mice fed disulfoton for 12 weeks (Clark et al. 1971). Dietary exposure of rats and mice to 2.5 mg/kg/day disulfoton for 2 months resulted in an increase in the permeability of spinal cord and brain stem tissues in both species (Clark and Stavinoha 1971). The nature of this change in permeability was not further investigated.

In a chronic-duration study, a decrease in relative and absolute brain weights was observed in male rats, but an increase in brain weights was observed in female rats fed disulfoton for 1.5-2.0 years (Carpy et al. 1975). The reason for and the toxicological significance of these opposite trends in males and females is not clear. In the same study, plasma, erythrocyte, and brain cholinesterase activity was significantly inhibited in both male and female rats. A dose of 0.1 mg/kg/day resulted in a 21% inhibition of brain cholinesterase activity in female rats. At 0.05 mg/kg/day, brain cholinesterase was inhibited by 11% in male rats. In another chronic dietary study in rats which provided doses of 0.05, 0.18, and 0.75 mg/kg/day in males and 0.06, 0.21, and 1.02 mg/kg/day in

females, erythrocyte cholinesterase activity was inhibited by ≤19% and 14-24% in low dose males and females respectively, 46-67% and 57-77% in mid dose males and females, respectively, and 71-82% and 75-86% in high dose males and females, respectively (Hayes 1985). Brain cholinesterase activity was inhibited by 15% and 21% in low dose males and females, respectively; 53% in both mid dose males and females; and 79% and 82% in high dose males and females, respectively. Relative brain weight was significantly increased in both high dose males and females. Histological examination revealed a dose-related increased incidence of optic nerve degeneration that was statistically significant in mid dose males and mid and high dose females. No treatment-related lesions were found in the brain, sciatic nerve, or spinal cord. Based on the LOAEL of 0.06 mg/kg/day for erythrocyte and brain cholinesterase inhibition in female rats, a chronic oral MRL of $6x10^{-5}$ mg/kg/day was calculated as described in footnote "d" in Table 2-2. Significant depression of erythrocyte, plasma, and brain cholinesterase activity was also found in mice fed disulfoton for 23 months at doses of 2.13 mg/kg/day (males) and 2.53 mg/kg/day (females) (Hayes 1983). Beagle dogs did not exhibit profound changes in general appearance or behavior when fed disulfoton (0.03 or 0.14 mg/kg/day) for 2 years (Hoffman et al. 1975). However, significant depression of plasma and erythrocyte and brain cholinesterase activity occurred at 0.14, but not at 0.03 mg/kg/day. No histological lesions were found in the brain. Necrosis and atrophy of the optic nerve and retina was observed in dogs given disulfoton (0.5-1.5 mg/kg/day) for 2 years (Uga et al. 1977). The authors regarded the pathological changes in the retina as mild; however, the nerve fibers in the optic nerve were reduced in number.

The highest NOAEL values and all LOAEL values for neurological effects in all reliable studies in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to disulfoton.

In a dominant lethal test, treatment of male mice with a single oral dose of 5 mg/kg disulfoton had no effect on male fertility (Herbold 1980). In a three-generation reproductive study, exposure of male and female rats to disulfoton in the diet at 0.5 mg/kg/day resulted a "slight" reduction of litter sizes in the third generation (Taylor 1965a). This study was limited by data reporting deficiencies such as lack of statistical analysis, incomplete necropsy report, and insufficient histopathological data. A more extensive multigeneration study was conducted in male and female rats exposed to disulfoton in the

diet that provided doses of 0.009, 0.03, and 0.09 mg/kg/day (Hixson and Hathaway 1986). At 0.09 mg/kg/day, decreased reproductive performance occurred, evidenced by a decreased percentage of females placed for mating and decreased percentage of sperm-positive F_0 and F_1 parental females. In addition, decreased maternal weight of F_0 and F_1 dams during gestation and lactation, decreased litter counts, viability index, and lactation index, and increased dead births and percentage of dead births occurred in both generations at 0.09 mg/kg/day. A decrease in F_{2b} litter counts and litter weights occurred at 0.03 mg/kg/day. Gross and histological examination of the ovary, vagina, uterus, testes, epididymis, seminal vesicles, and prostate of the F_0 and F_1 parents revealed no treatment-related lesions. In an intermediate-duration study, exposure of male and female rats to diets providing 0.5 mg/kg/day disulfoton for 60 days prior to mating and/or during mating resulted in the failure of two of five females to become pregnant (Ryan et al. 1970). Histological examination of reproductive organs of males (testes, epididymides, seminal vesicles, prostate glands) and females (ovaries, uteri, mammary glands) did not reveal any treatment-related lesions in rats fed 0.34 mg/kg/day (males) or 0.55 mg/kg/day (females) (Klotzsche 1972) or in mice fed 0.63 mg/kg/day (males) or 0.71 mg/kg/day (females) (Rivett et al. 1972) for 90 days, or in rats fed 0.1 mg/kg/day (Carpy et al. 1975), in mice fed 2.13 mg/kg/day (males) or 2.53 mg/kg/day (females) (Hayes 1983), or in dogs fed 0.14 mg/kg/day (Hoffman et al. 1975) for up to 2 years. However, uterine cystic hyperplasia was observed in female rats given disulfoton in the diet at 1.02 mg/kg/day, but not at 0.21 mg/kg/day, for 2 years (Hayes 1985). Histological examination of the cervix, mammary glands, ovaries, prostate gland, seminal vesicles, and testes revealed no effects in the rats at any dose level. In the studies that did not assess reproductive function, the dose levels that were not associated with histopathological lesions in reproductive organs cannot be considered as NOAEL values.

The reliable NOAEL value for reproductive performance and the LOAEL values for reproductive effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Developmental Effects

No studies were located regarding development effects in humans after oral exposure to disulfoton.

Pregnant rats given disulfoton on days 6-15 of gestation had decreased plasma and erythrocyte cholinesterase activity at ≥0.3 mg/kg/day (Lamb and Hixson 1983). Fetotoxic effects included increased incidences of incomplete ossified parietal bones and stemebrae at 1.0 mg/kg/day, but not at

0.3 mg/kg/day. This was considered as evidence of growth retardation due to maternal toxicity rather than specific fetotoxic effects. There was no evidence of soft tissue, external, or skeletal malformations. Pregnant rabbits given high doses of disulfoton (1.5-3.0 mg/kg/day) died or exhibited cholinergic signs of disulfoton toxicity (Tesh et al. 1982). Generally, there were no adverse effects on fetal survival, growth, or development. Because of the high mortality of the dams, the initial high dose (3.0 mg/kg/day) was reduced to 2.0 mg/kg/day and finally to 1.5 mg/kg/day. In animals that received doses in the range of 1.5-3.0 mg/kg or a combination of doses, no fetotoxic effects were observed in the offspring. Thus, 1.5 mg/kg/day is considered the NOAEL for developmental effects in this study. In an intermediate-duration study, exposure of male and female rats to diets providing 0.5 mg/kg/day disulfoton for 60 days prior to mating and/or during mating resulted in a 32.1% depression in fetal brain cholinesterase activity (Ryan et al. 1970). In a three-generation study in rats, cloudy swelling and fatty infiltration of the liver, mild nephropathy (females), and juvenile hypoplasia of the testes were observed in F_{3b} litters (Taylor 1965a). These litters also had significantly depressed erythrocyte cholinesterase activities. In another multigeneration study in rats, brain cholinesterase activity was inhibited by 24% and 32% in male and female F_{1a} pups, respectively, at 0.03 mg/kg/day and by 50% and 59% in male and female F_{1a} pups, respectively, at 0.09 mg/kg/day (Hixson and Hathaway 1986). No inhibition of brain cholinesterase was found in the F_{1a} pups at 0.009 mg/kg/day, and no grossly observable developmental abnormalities were found in any pups in the F_1 or F_2 generation. Based on this NOAEL of 0.009 mg/kg/day for brain cholinesterase inhibition in pups, an intermediate oral MRL of $9x10^{-5}$ mg/kg/day was calculated as described in footnote “c” in Table 2-2. The highest NOAEL values and all LOAEL values for developmental effects in each reliable study in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after oral exposure to disulfoton.

Disulfoton was negative in a dominant lethal test in male mice treated orally with a single dose of 5 mg/kg (Herbold 1980) and in an erythrocyte micronucleus test in mice dosed with 6 or 12 mg/kg/day for 2 days (Herbold 1981). Disulfoton also did not induce micronuclei in erythrocytes of mice dosed with 2, 4, or 8 mg/kg disulfoton (EPA 1984a; Sandhu et al. 1985), but whether disulfoton was administered by the oral or intraperitoneal route was not clear. Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to disulfoton.

No histological evidence of a carcinogenic effect was observed in rats fed ≤0.1 mg/kg/day disulfoton for 1.5-2.0 years (Carpy et al. 1975), in rats fed ≤1.02 mg/kg/day disulfoton for 2 years (Hayes 1985), or in mice fed ≤2.53 mg/kg/day disulfoton for 23 months (Hayes 1983). The study by Carpy et al. (1975) was limited by insufficient necropsy and histological data and by dosing manipulations. In addition, there was no evidence of carcinogenicity in Beagle dogs fed disulfoton (0.02-0.14 mg/kg/day) for 2 years (Hoffman et al. 1975).

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to disulfoton.

Dermal LD_{50} values suggest that, irrespective of strain, female rats are more sensitive than male rats when disulfoton is administered dermally. The dermal LD_{50} for disulfoton was determined to be 15.9 and 3.6 mg/kg in male and female Wistar rats, respectively (Mihail 1978). In Sherman rats, the dermal LD_{50} was determined to be 15 and 6 mg/kg in males and females, respectively (Gaines 1969). In male Sprague-Dawley rats, the dermal LD_{50} was determined to be 20 mg/kg (DuBois 1957). A dermal LD_{50} value of 0.285 mL/kg (187 mg/kg) was reported for rats given a liquid formulation containing 65.7% disulfoton (Weil et al. 1971). When a granular formulation containing 10% disulfoton was applied at a dose of 1,280 mg/kg, one of four rats died. The difference in dermal LD_{50} values is probably related to the different formulations of disulfoton. In a range-finding study, 2 of 2 rabbits died after 1 or 2 applications of 10 mg/kg/day disulfoton was applied to the shorn, unabraded skin and left for 6 hours (Flucke 1986). None of the rabbits similarly treated with 0.4 or 2.0 mg/kg/day for 5 days died. In a 3-week experiment, similar treatment of rabbits 5 days/week resulted in death of 5 of 5 females after l-6 treatments and of 5 of 5 males after 3-10 treatments with 6.5 mg/kg/day. None of the rabbits treated with ≤1.6 mg/kg/day for 3 weeks died. The rabbits that died in these experiments exhibited persistent cholinergic signs of intoxication (muscle spasms, dyspnea, salivation) before death.

The LD_{50} values in rats and the LOAEL values for death of rabbits after dermal exposure to disulfoton are recorded in Table 2-3.

2.2.3.2 Systemic Effects

No studies were located regarding musculoskeletal or ocular effects in humans or animals after dermal exposure to disulfoton. The highest NOAEL values and all LOAEL values from each reliable study for systemic end points in animals are recorded in Table 2-3.

Respiratory Effects. No studies were located regarding respiratory effects in humans after dermal exposure to disulfoton.

In rats exposed to disulfoton applied to clipped dorsal skin at doses of 2.5-20 mg/kg, breathing difficulties were noted (Mihail 1978), but it was not clear at which doses this effect was seen. In a 3-week study, in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week, necropsy of the rabbits that died within 2 weeks during treatment (100%) with the high dose of 6.5 mg/kg/day revealed distended, pale, mottled, and fluid-containing lungs (Flucke 1986). The organs and tissues of the high dose rabbits were not examined histologically, but gross and histological examination of the lungs of rabbits similarly treated with ≤1.6 mg/kg/day for 3 weeks revealed no treatment-related lesions.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after dermal exposure to disulfoton.

Acute dermal exposure to unspecified doses of organophosphorus insecticides, including disulfoton, was reported to cause protein degeneration and significant circulatory disruptions in heart muscles of rats, cats, and rabbits (Kundiev and Rappoport 1967). This study is limited by reporting deficiencies regarding incidences, and by uncertainty about whether the effects were observed in all species and whether disulfoton was among the insecticides causing these effects. In a 3-week study in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week, gross and histological examination of the heart revealed no treatment-related lesions at ≤1.6 mg/kg/day (Flucke 1986).

TABLE 2-3. Levels of Significant Exposure to Disulfoton - Dermal

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
ACUTE EXPOSURE						
Death						
Rat (Sprague-Dawley)	once				20 M (LD50)	DuBois 1957
Rat (Sherman)	once				15 M (LD50) 6.0 F (LD50)	Gaines 1969
Rat (Wistar)	once				15.9 M (LD50) 3.6 F (LD50)	Mihail 1978
Rabbit (New Zealand)	1-2 d 6 hr/d				10 (2/2 died)	Flucke 1986
Rabbit (New Zealand)	1-2 wk 5 d/wk 6 hr/d				6.5 (10/10 died)	Flucke 1986

TABLE 2-3. Levels of Significant Exposure to Disulfoton - Dermal (continued)

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference
Systemic							
Rabbit (New Zealand)	1-2 wk 5 d/wk 6 hr/d	Resp			6.5	(distended, pale, mottled, fluid containing lungs in rabbits that died)	Flucke 1986
		Gastro			6.5	(marked intussusception of the ileum in one female that died)	
		Hepatic			6.5	(lobular pattern in the liver of rabbits that died)	
		Renal			6.5	(pale kidneys, with reddened renal pelvis and indistinct structure in rabbits that died)	
		Derm	6.5				
		Bd Wt			6.5	(little or no feed intake and distinct weight loss up to time of death)	
Immunological/Lymphoreticular							
Rabbit (New Zealand)	1-2 wk 5 d/wk 6 hr/d				6.5	(small pale spleen in rabbits that died)	Flucke 1986
Neurological							
Rabbit (New Zealand)	1-5 d 6 hr/d		2		10	(2/2 rabbits exhibited unspecified cholinergic signs and died after 1 or 2 doses)	Flucke 1986
Rabbit (New Zealand)	1-2 d 6 hr/d				6.5	(muscle spasms, dyspnea, salivation)	Flucke 1986

TABLE 2-3. Levels of Significant Exposure to Disulfoton - Dermal (continued)

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
INTERMEDIATE EXPOSURE						
Systemic						
Rabbit (New Zealand)	3 wk 5 d/wk 6 hr/d	Resp	1.6			Flucke 1986
		Cardio	1.6			
		Hemato	1.6			
		Hepatic	1.6			
		Renal	1.6			
		Endocr	1.6			
		Derm	1.6			
		Bd Wt	1.6			
Neurological						
Rabbit (New Zealand)	3 wk 5 d/wk 6 hr/d		0.4	1.6 F (21-33% inhibition of erythrocyte cholinesterase activity)		Flucke 1986

Bd Wt = body weight; d = day(s); Derm = dermal; Endocr = endocrine; F = female; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LD50 = lethal dose 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after dermal exposure to disulfoton.

In a 3-week study in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week, necropsy of the rabbits that died within 2 weeks during treatment (100%) with the high dose of 6.5 mg/kg/day revealed marked intussusception of the ileum of one female (Flucke 1986). The gastrointestinal tract of the high dose rabbits or of rabbits similarly treated with ≤1.6 mg/kg/day for 3 weeks were not examined histologically.

Hematological Effects. No studies were located regarding hematological effects in humans after dermal exposure to disulfoton. No hematological effects in rabbits were found at ≤1.6 mg/kg/day in a 3-week study in which disulfoton was applied to the shorn, unabraded skin and left for 6 hours, 5 days/week (Flucke 1986).

Hepatic Effects. No studies were located regarding hepatic effects in humans after dermal exposure to disulfoton.

Acute dermal exposure to unspecified doses of organophosphorus insecticides, including disulfoton, was reported to cause protein degeneration and significant circulatory disruptions in the livers of rats, cats, and rabbits (Kundiev and Rappoport 1967). This study is limited by reporting deficiencies regarding incidences, and by uncertainties about whether the effects were observed in all species and whether disulfoton was among the insecticides causing these effects. In a 3-week study in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week, 100% of the rabbits died within 2 weeks during treatment. Necropsy of the rabbits treated with the high dose of 6.5 mg/kg/day revealed a lobular pattern in the liver (Flucke 1986). The organs and tissues of the high dose rabbits were not examined histologically, but clinical chemistry results and gross and histological examination of the liver of rabbits similarly treated with ≤1.6 mg/kg/day for 3 weeks revealed no treatment-related hepatic effects. Slight increases in the absolute and relative liver weights were found in male rabbits at 1.6 mg/kg/day, but the absence of clinical chemistry and histological effects indicates that the liver weight change was not toxicologically significant.

Renal Effects. No studies were located regarding renal effects in humans after dermal exposure to disulfoton.

Acute dermal exposure to unspecified doses of organophosphorus insecticides, including disulfoton, was reported to cause protein degeneration and significant circulatory disruptions in the kidneys of rats, cats, and rabbits (Kundiev and Rappoport 1967). This study is limited by reporting deficiencies regarding incidences, and uncertainties about whether the effects were observed in all species and whether disulfoton was among the insecticides causing these effects. In a 3-week study in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week, 100% of the rabbits died within 2 weeks during treatment. Necropsy of the rabbits treated with the high dose of 6.5 mg/kg/day revealed pale kidneys, with reddened renal pelvis and indistinct structure (Flucke 1986). The organs and tissues of the high dose rabbits were not examined histologically, but clinical chemistry and urinalysis results and gross and histological examination of the kidney of rabbits similarly treated with ≤1.6 mg/kg/day for 3 weeks revealed no treatment-related renal effects.

Endocrine Effects. No studies were located regarding endocrine effects in humans after dermal exposure to disulfoton.

In a 3-week study in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week, gross and histological examination of the adrenal and thyroid glands revealed no treatment-related lesions at ≤1.6 mg/kg/day (Flucke 1986).

Dermal Effects. No studies were located regarding dermal effects in humans after dermal exposure to disulfoton.

Acute dermal exposure to unspecified doses of organophosphorus insecticides, including disulfoton, was reported to cause microscopic changes in the blood vessels and fibrous structures in the skin of rats, cats, and rabbits (Kundiev and Rappoport 1967). Collagen and elastin fibers in the skin and the blood vessels in the skin appeared to have been affected. These effects were thought to have further increased the absorption of the insecticides by the skin. This study is limited by reporting deficiencies regarding incidences, and questions about whether the effects were observed in all species and whether disulfoton was among the insecticides causing these effects. In a 3-week study in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week, the treated areas of the skin were observed daily for signs of inflammation (redness and swelling) (Flucke 1986). In the rabbits that died within 2 weeks during treatment with the high dose of 6.5 mg/kg/day (100%) and in the rabbits treated with ≤1.6 mg/kg/day for 3 weeks, no indication of local irritation was found.

The skin of the high dose rabbits was not examined histologically, but histological examination of the skin of rabbits treated with ≤1.6 mg/kg/day for 3 weeks revealed no treatment-related lesions.

Body Weight Effects. No studies were located regarding body weight effects in humans after dermal exposure to disulfoton.

In a 3-week study in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week, little or no feed intake and distinct weight loss occurred up to the time of death in the rabbits that died within 2 weeks during treatment. Necropsy of the rabbits treated with the high dose of 6.5 mg/kg/day (100%) (Flucke 1986). No effects on body weight were found in rabbits similarly treated with ≤1.6 mg/kg/day for 3 weeks.

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after dermal exposure to disulfoton.

In a 3-week study in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week, 100% of the rabbits died within 2 weeks during treatment. Necropsy of the rabbits treated with the high dose of 6.5 mg/kg/day revealed small and pale spleens in some cases (Flucke 1986). The organs and tissues of the high dose rabbits were not examined histologically, but gross and histological examination of the spleens of rabbits similarly treated with ≤1.6 mg/kg/day for 3 weeks revealed no treatment-related lesions. The NOAEL value and the LOAEL value for effects on the spleen in rabbits are recorded in Table 2-3.

2.2.3.4 Neurological Effects

Exposure to disulfoton can result in inhibition of acetylcholinesterase activity, with consequent accumulation of acetylcholine at nerve synapses and ganglia leading to central nervous system, nicotinic, and muscarinic effects (see Section 2.2.1.4 for more extensive discussion).

A farmer who had worn disulfoton~ontaminated gloves for several days developed signs of disulfoton toxicity (weakness, fatigue, and cyanosis) and had to be hospitalized (Savage et al. 1971). Because a

considerable amount (not otherwise specified) of disulfoton was detected in the serum and because blood cholinesterase activity was severely depressed, it can be assumed that the patient had absorbed a considerable amount of disulfoton through the skin. The patient recovered following treatment for the toxicosis. Severe neurological signs and symptoms were not reported by workers exposed to disulfoton during wet or dry mix operations at mean doses of 0.013-0.23 mg/kg/day for 9 weeks at a pesticide-fertilizer mixing plant (Wolfe et al. 1978). However, erythrocyte cholinesterase activity was depressed by 22.8% from week 2-9 of the study in workers involved in dry mix operations (0.23 mg/kg/day). No depression in blood cholinesterase activity was observed in workers involved with wet mix operations (0.013 mg/kg/day). These workers were also exposed to disulfoton by the inhalation route (see Section 2.2.1.4). Therefore, the depression in erythrocyte cholinesterase activity may be due to both absorption of disulfoton through the respiratory tract and through the skin. No significant reductions in plasma or erythrocyte cholinesterase activities occurred in three employees at a pesticide formulating plant exposed to disulfoton (unspecified doses) for 25 weeks (Brokopp et al. 1981). Similarly, no reductions in cholinesterase activity were found for eight employees exposed for shorter periods.

Disulfoton caused muscle twitching and clonic cramps in male and female rats after acute dermal exposure to doses 2.5-20 mg/kg (Mihail 1978), but it was not clear at which doses these signs were observed. These neurological effects persisted for an unspecified time after disulfoton was removed from the skin. Disulfoton also caused depression in skin cholinesterase activity in rats, cats, and rabbits given acute unspecified dermal doses (Kundiev and Rappoport 1967). This study is limited by reporting deficiencies regarding incidences and uncertainty about whether the effects were observed in all species. In a range-finding study, 2 of 2 rabbits died after 1 or 2 applications of 10 mg/kg/day disulfoton were applied to the shorn, unabraded skin and left for 6 hours (Flucke 1986). The rabbits exhibited cholinergic signs of intoxication (not otherwise specified) before death. None of the rabbits similarly treated with 0.4 or 2.0 mg/kg/day for 5 days showed cholinergic signs or died. In a 3-week experiment, similar treatment of rabbits 5 days/week resulted in death of 5 of 5 females after l-6 treatments and of 5 of 5 males after 3-10 treatments with 6.5 mg/kg/day. Persistent cholinergic signs (muscle spasm, dyspnea, salivation) were observed in the high dose females after 1 or 2 treatments and in high dose males after 2 treatments. No clinical signs of cholinergic intoxication were seen in the rabbits treated with 0.4 or 1.6 mg/kg/day, but erythrocyte cholinesterase activity was inhibited by 21-33% in the female rabbits treated with 1.6 mg/kg/day. The highest NOAEL values

and the LOAEL values for neurological effects in rabbits for the acute and intermediate duration are recorded in Table 2-3.

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after dermal exposure to disulfoton. In a 3-week study, in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week, gross and histological examination of the testes, epididymides, ovaries, and uterus revealed no treatment-related lesions at ≤1.6 mg/kg/day (Flucke 1986). Slight increases in the absolute and relative testes weights were found in male rabbits at 1.6 mg/kg/day, but the absence of histological effects indicates that the testes weight change was not toxicologically significant. Reproductive function was not evaluated.

No studies were located regarding the following effects in humans or animals after dermal exposure to disulfoton:

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

2.3 TOXICOKINETICS

The toxicokinetics of disulfoton in humans and animals depends on its physicochemical characteristics and its metabolism. The lipophilicity of disulfoton indicates that the insecticide should be easily absorbed by oral, inhalation, and dermal routes. No bioavailability data were located for inhalation and dermal exposure. However, disulfoton is almost completely absorbed from the gastrointestinal tract within 2 days after oral exposure. Animal studies suggest that disulfoton is widely distributed primarily to the liver and in smaller quantities to the kidney, fat, skin, muscle, brain, and other organs.

Disulfoton and/or its metabolites are excreted mainly in the urine of humans and animals, with minor amounts excreted in the feces and expired air.

Disulfoton causes neurological effects in humans and animals. The mechanism of action on the nervous system depends on the metabolism of disulfoton to active metabolites. The liver is the major site of metabolic oxidation of disulfoton to disulfoton sulfoxide, disulfoton sulfone, demeton S-sulfoxide and demeton S-sulfone, which inhibit acetylcholinesterase in nervous tissue. These four active metabolites are more potent inhibitors of acetylcholinesterase than disulfoton. Cytochrome P-450 monooxygenase and flavin adenine dinucleotide monooxygenase are involved in this metabolic activation. The active metabolites ultimately undergo nonenzymatic and/or enzymatic hydrolysis to more polar metabolites that are not toxic and are excreted in the urine.

The inhibition of acetylcholinesterase increases the amount of acetylcholine at nerve synapses, which causes an overstimulation of cholinergic nerves and effector organs. Depending on the dose and its duration, the resulting cholinergic effects are usually reversible within several days to 2 weeks after disulfoton exposure has been discontinued. Prolonged exposure to disulfoton results in diminished cholinergic signs, and affected animals develop tolerance. The down-regulation of cholinergic receptors due to accumulation of acetylcholine may be associated with disulfoton-induced tolerance.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No studies were located regarding absorption in humans or animals after inhalation exposure to disulfoton.

2.3.1.2 Oral Exposure

Disulfoton and/or its metabolites have been detected in the blood and urine of humans who consumed unknown amounts of disulfoton solution (Hattori et al. 1982; Yashiki et al. 1990). 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, at ≈2 hours after ingestion (Yashiki et al. 1990). The 4.92 nmol/g blood concentration corresponded to 1.35 µg disulfoton/g. Gastrointestinal absorption was not yet

complete, since 3.3 mg of disulfoton were recovered from the stomach contents, which was also collected at ≈2 hours after the ingestion. The concentration of metabolites in the urine was not quantitated. While these data indicate 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 lo-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 in males. As seen in Section 2.2, female rats are more sensitive to the toxic effects of disulfoton than male rats; therefore, the females were given a lower dose. 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 mg/kg 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, with 61-72% excreted in urine of females and 31-48% excreted in the urine of males in the first 4 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). However, the intraperitoneal LD_{50} values were slightly lower than the oral LD_{50} values, suggesting that gastrointestinal absorption is <100%.

2.3.1.3 Dermal Exposure

No studies were located regarding absorption in humans or animals after dermal exposure to disulfoton. However, data on lethality, other signs of toxicity, and acetylcholinesterase inhibition in animals after dermal exposure (see Section 2.2.3) suggest that disulfoton can be absorbed from the skin.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution in humans or animals after inhalation exposure to disulfoton.

2.3.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to disulfoton.

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 (kidney, plasma, fat, whole blood, skin, muscle, 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-l.5 mg/kg/day disulfoton in

capsules for 2 years, disulfoton was detected in the kidney (0.06 ppm), urine (0.06 ppm), liver (0.02 ppm), serum (0.04 ppm), 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 tissue, thymus, spleen, erythrocytes, extraocular muscle, and muscles of the extremities and torso.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals after dermal exposure to disulfoton.

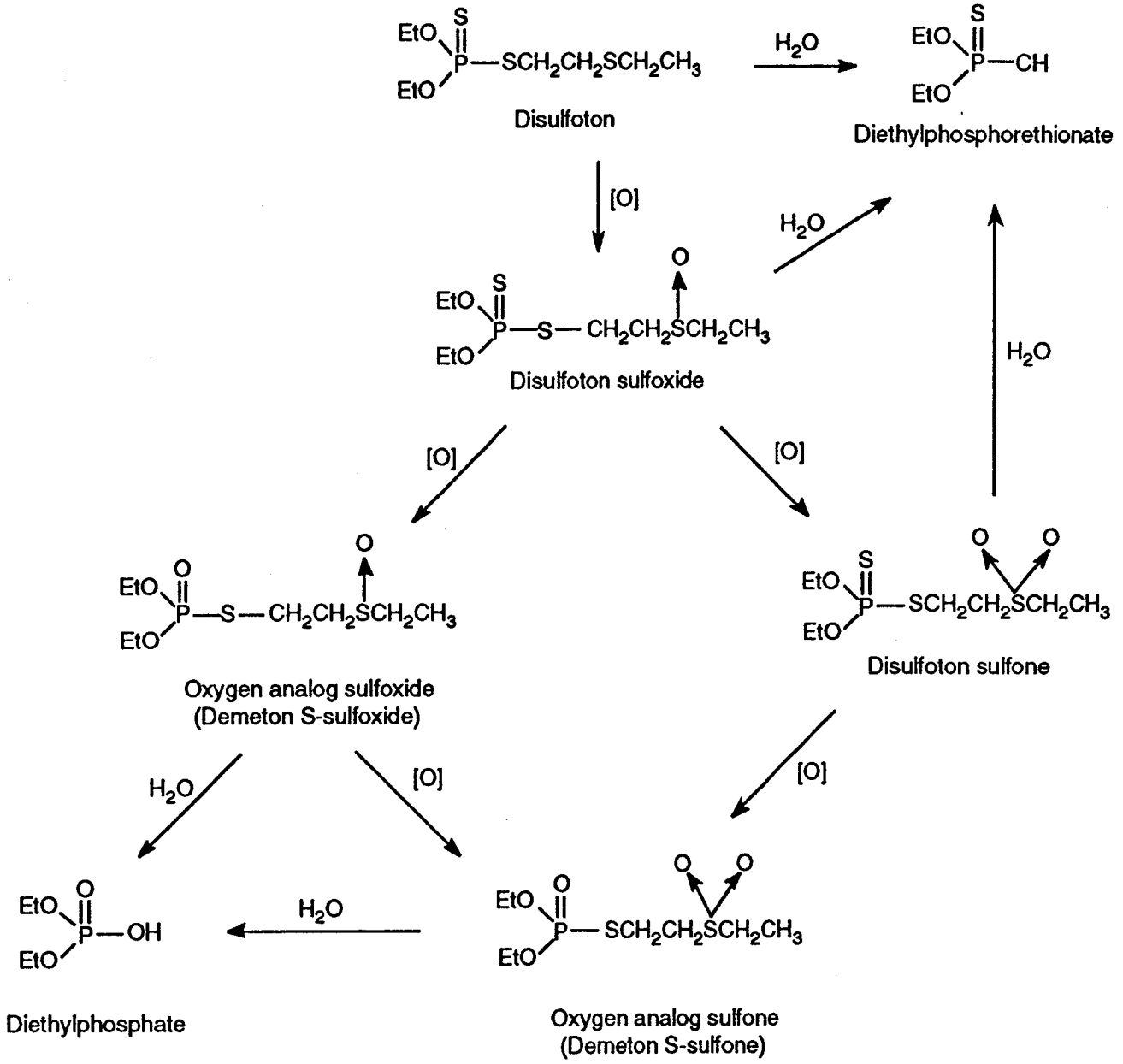
2.3.3 Metabolism

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 2-3). 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.

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, diethyl phosphate (DEP), diethyl thiophosphate (DETP), and diethyl dithiophosphate (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. Workers exposed mainly to disulfoton at a pesticide formulating plant had excreted the metabolites DEP, DETP, DEDPT, and diethyl phosphorothiolate (DEPTh) in urine after dermal and possibly inhalation exposure to disulfoton (Brokopp et al. 1981).

Studies in rats and mice indicate that the same pathways operate in humans and rodents. Unidentified urinary metabolites in mice injected intraperitoneally with 32ρ-disulfoton were described as hydrolysis

FIGURE 2-3. Metabolic Pathways for Disulfoton*



* Adapted from WHO 1976

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 also 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. Furthermore, 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 2-3, 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 of the pathways, however, cannot be deduced from relative percentages of metabolites formed because the final urinary metabolites are common products of several of the intermediate metabolites (see Figure 2-3). 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 males, while only demeton S-sulfone was apparent in the urine of females (Lee et al. 1985). However, after dosing with 0.2 mg/kg/day for 14 days, the pattern in males and females 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 the demeton S-sulfone.

The studies described above support the accepted theory (Eto 1974) 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. These active metabolites bind to ubiquitous cholinesterase 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 P-450 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 P-450 mixed-function oxidase (MFO) system. The components of the MFO system include cytochrome P-450, the terminal oxidase, and NADPH, and NADPH-dependent cytochrome c reductase (Stevens and Green 1974). Generally, anticholinesterase insecticides such as disulfoton bind to oxidized cytochrome P-450 to form a disulfoton:cytochrome P-450 complex, which usually produces some form of a Type I spectra (Stevens et al. 1973). An electron is then transferred from cytochrome c reductase to cytochrome P-450 (Gillette et al. 1972), thereby reducing the disulfoton:cytochrome P-450 complex. Molecular oxygen then binds to this complex to form a disulfoton:reduced cytochrome P-450:0_2 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 P-450 (Hildebrandt and Estabrook 1971).

Flavin monooxygenase specifically oxidizes sulfides to (R)-(+)-sulfoxide enantiomers, while cytochrome P-450-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 P-450) may be required to form sulfones (Tynes and Hodgson 1985). Disulfoton interacted with cytochrome P-450 to markedly inhibit the metabolism of p-nitroanisole and parathion, both of which have rather high affinities for cytochrome P-450. These findings underscore the fact that cytochrome P-450 and flavin monooxygenase both have the potential to participate in the oxidation of the disulfoton.

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 similar species. 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). In addition, pig liver flavin monooxygenase was more saturable by disulfoton than the mouse liver enzyme (Tynes and Hodgson 1985). Flavin monooxygenase in pig liver also had a higher affinity (lower K_m) 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.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

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

2.3.4.2 Oral Exposure

No studies were located regarding the rate or extent of excretion in humans after oral 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 (see Section 2.2). 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 mg/kg 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, with 61-72% excreted in urine of females and 3148% excreted in the urine of males in the first 4 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.

2.3.4.3 Dermal Exposure

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

2.3.4.4 Other Routes of Exposure

No studies were located regarding excretion in humans after other routes of exposure to disulfoton.

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 metabolites lo-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).

2.3.5 Mechanisms of Action

Disulfoton is readily absorbed from the gastrointestinal tract (Hattori et al. 1982; Lee et al. 1985; Puhl and Fredrickson 1975; Yashiki et al. 1990). Although information regarding the rate and extent of absorption of disulfoton after inhalation and dermal exposure was not located, the lipophilic nature of disulfoton suggests that it is probably absorbed by passive diffusion by these routes as well.

Although there are no known intermediary proteins or other mechanisms associated with distribution of disulfoton to effector organs or to organs where the compound is likely to be metabolized, binding studies with other organophosphates suggest that serum albumin may be an important protein involved with distribution to effector organs (Braeckman et al. 1983). Available data suggest that more disulfoton is distributed initially to the liver than to any other organ (Puhl and Fredrickson 1975). The detection of disulfoton metabolites in the liver of rats is consistent with this observation (Bull 1965). The active metabolites of disulfoton are known to depress the activity of serum, erythrocyte, and brain cholinesterase (see Section 2.2.2.4).

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 an oral study in rats, the metabolites, disulfoton sulfoxide, disulfoton sulfone, demeton S-sulfoxide, and demeton S-sulfone resulted in mortality and signs of toxicity at lower doses than did disulfoton (Crawford and Anderson 1974).

No information was located regarding the mechanism of excretion of disulfoton and/or its metabolites. Because disulfoton and its active metabolites are relatively lipophilic, excretion by passive diffusion is the probable mechanism.

The acute toxic effects of organophosphate insecticides are due primarily to accumulation of acetylcholine at muscarinic and nicotinic cholinergic receptors (Costa et al. 1982b). The accumulation of this substrate is due to the inhibition of cholinesterase activity by the active metabolites of the organophosphate (Murphy et al. 1984). In many instances, humans and animals exhibit signs of toxicity resembling excessive stimulation of cholinergic nerves. Atropine, a cholinergic blocking agent, has been used to demonstrate that excessive acetylcholine accumulation is related to the mechanism of toxicity (Bombinski and DuBois 1958). Cholinesterase enzyme kinetics provide further evidence supporting the proposed mechanism. Studies indicate that the enzyme-acetylcholine complex and the enzyme-organophosphate complex usually are formed at the same rate (Murphy et al. 1984). However, the enzyme-acetylcholine complex is hydrolyzed at a much faster rate than the enzyme-organophosphate complex; the enzyme is therefore regenerated faster in the forrner case. Dephosphorylation of the enzyme is a slow reaction, and, therefore rate-limiting.

Signs of disulfoton toxicity, such as muscle tremors, fasciculations, lacrimation, and salivation, in animals are generally observed after a few daily doses, but begin to diminish in severity as exposure to disulfoton continues (Bombinski and DuBois 1958). This phenomenon is known as tolerance. Tolerance appears to be a reproducible phenomenon that does not depend on the organophosphate insecticide used, the route of administration, or the animal species (Costa et al. 1982b). Several possible mechanisms have been proposed to explain this phenomenon.

Evidence suggests that tolerance involves the cholinergic system and not the opiate system (Costa and Murphy 1986). Tolerance to chronic treatment with disulfoton was once thought to be due to refractoriness of the cholinergic receptor to acetylcholine (Brodeur and DuBois 1964). More recently, tolerance has been associated more specifically with alterations of cholinergic receptor density, rather than with changes in binding affinity (Costa et al. 1981, 1982a, 1990; McDonald et al. 1988). Radiolabeled ligands, such as [^{3}H] quinuclidinyl benzilate ([^{3}H] QNB), which are muscarinic antagonists that can bind to recognition sites on muscarinic receptors, were used in these studies to demonstrate that disulfoton caused a reduced number of receptor binding sites in central and peripheral nerve tissue. However, the binding affinity was not changed in disulfoton-tolerant animals. Furthermore, the alteration in cholinergic muscarinic receptors was not due to direct binding of disulfoton and/or its metabolites to the receptor, but due to an adaptive mechanism to overstimulation by endogenous acetylcholine (Costa et al. 1981). Disulfoton also caused modulation of Ml and M2 muscarinic receptor subtypes in the brain of disulfoton-tolerant rats (Fitzgerald and Costa 1992). However, recovery of M2 muscarinic receptor binding after termination of exposure was slower compared to the Ml subtype, particularly in the hippocampus. Alteration of muscarinic binding sites in lymphocytes was also demonstrated (Costa et al. 1990; Fitzgerald and Costa 1993). The rate of recovery of cholinesterase is much slower than the rate of recovery of [^{3}H] QNB binding in disulfoton- tolerant animals (Costa et al. 1981). McDonald et al. (1988) hypothesized that the decrease in muscarinic receptor densities in the hippocampus, cortex, and striatum of the brain was the reason for memory loss in animals that became tolerant to disulfoton after repeated intraperitoneal dosing (see Section 2.4). The data from earlier intraperitoneal behavioral studies are not consistent with this finding (Costa and Murphy 1982). However, these two studies measured different neurobehavioral end points in different species. In addition, this memory loss has not been reported in humans. While cholinergic muscarinic signs diminish in severity in disulfoton-tolerant animals, nicotinic cholinergic signs may persist (McPhillips 1969a). It was proposed that nicotinic receptors were stable, while muscarinic receptors were labile to acetylcholine accumulation in disulfoton-tolerant animals (Costa et al. 1982a). However, a decrease in nicotinic acetylcholine receptors in the brain was subsequently found after repeated administration of disulfoton in rats (Costa and Murphy 1983a).

As supporting evidence for this mechanism (i.e., reduced density of muscarinic receptor binding sites), subsensitivity to other cholinergic agonists was demonstrated in disulfoton-tolerant animals. The mechanism for the subsensitivity to cholinergic agonists is probably related to changes in agonist binding as well as loss of surface receptor labeling by hydrophilic ligands such as

N-methylscopolamine (Costa et al. 1982b; Schwab et al. 1983). Animals made tolerant to disulfoton were resistant to the lethal or adverse effects of cholinergic agonists, such as carbachol (Brodeur and DuBois 1964; Costa et al. 1981; Schwab and Murphy 1981) and oxotremorine (Costa et al. 1982b; McPhillips 1969a), which are not hydrolyzed by acetylcholinesterase. Tissues from animals tolerant to disulfoton such as the ilea (Foley and McPhillips 1973; McPhillips 1969b; McPhillips and Dar 1967) and the atria (Perrine and McPhillips 1970; Schwab et al. 1983), were resistant to the effects of carbachol and/or oxotremorine. Because the uterus and vas deferens have a relatively sparse parasympathetic innervation compared to the ileum and do not receive a steady flow of impulses via this system, these tissues were not as subsensitive to carbachol as the ileum (Foley and McPhillips 1973). Thus, acetylcholine accumulation may be a prerequisite for tolerance development.

Other hypotheses have been tested in attempts to explain the mechanism of tolerance. Tolerance may be associated with interference of acetylcholine synthesis at the presynaptic junction (Costa et al. 1982b). However, no difference in the uptake of choline occurred in the hippocampus from disulfoton-tolerant mice compared with controls, suggesting that the availability of choline was not limiting. Noncholinergic mechanisms may also be involved in the development of tolerance. In one study, the atria from disulfoton-tolerant rats were subsensitive to carbachol and oxotremorine, but there was no alteration in binding of [^{3}H] QNB when compared with the controls (Schwab et al. 1983). This finding is inconsistent with the "altered receptor" hypothesis that suggests tolerance is associated with a decrease in radioligand binding and a concomitant subsensitivity to cholinergic agonists. The authors proposed that in addition to receptor loss, other mechanisms distal to [^{3}H] QNB binding sites or removed from the receptor complex may contribute to the subsensitivity of the atria and other tissues to cholinergic agonist. These and other related mechanisms remain to be studied.

2.4 RELEVANCE TO PUBLIC HEALTH

The lipophilic properties of disulfoton suggest that the compound is likely to be absorbed readily by the lungs, gastrointestinal tract, and the skin. Toxicokinetic data in humans and animals show that disulfoton is readily and extensively absorbed by the gastrointestinal tract. Disulfoton and/or its metabolites are distributed rapidly to tissues such as the liver, kidney, brain, and adipose tissue. Although the compound is relatively lipophilic, its accumulation in these organs does not appear to be appreciable. Fifty to ninety percent of the dose administered to rats was eliminated in the urine within

4-32 hours. Disulfoton is rapidly metabolized to its oxygen analogs, which are in turn rapidly detoxified to water soluble nontoxic metabolites that are eliminated via the urine.

The most significant and sensitive effects resulting from acute, intermediate, or chronic exposure to disulfoton by the inhalation, oral, or dermal route are neurological. The neurological effects are the result of inhibition of acetylcholinesterase by active metabolites of disulfoton at nerve synapses; subsequent accumulation of acetylcholine results in overstimulation of the distal neuron, and more specifically, in cholinergic effects. Examples of cholinergic effects include salivation, miosis, muscle tremors, and urinary and fecal incontinence. Convulsions, coma, and death have been associated with severe disulfoton intoxication. Studies in chickens indicated that disulfoton does not cause delayed neurotoxicity. Body weight loss is also a common finding in animals and is associated with cholinesterase inhibition. In addition, disulfoton may alter catecholamine levels in body tissues. The effects on catecholamine levels appear to be related to the accumulation of acetylcholine. Optic nerve atrophy and necrosis have also been associated with chronic oral exposure to disulfoton in dogs.

The inhibition of erythrocyte acetylcholinesterase is a very sensitive biomarker of this acetylcholinesterase inhibition at the nerve synapses, and, therefore, can be regarded as a sensitive effect as well. Some of these effects have been observed in occupationally exposed individuals and in a man who accidentally ingested disulfoton. Results from several animal studies support these findings. The cholinergic effects are usually associated with acute exposure to disulfoton. Prolonged exposure to disulfoton (5-10 days) generally results in a decrease in the severity of the cholinergic toxicity, while cholinesterase remains inhibited. This phenomenon, known as tolerance, has been observed in animals exposed to disulfoton for prolonged periods. A number of studies have investigated the mechanism of tolerance development.

Hepatic effects have not been observed in humans, but mild hepatic effects were observed in animals after oral exposure to disulfoton. These hepatic effects include alterations in liver microsomal enzyme activities and lipid peroxidation. No concurrent histopathological changes in the liver were observed. Although hepatic effects should be of little concern to exposed humans, caution is advised with concomitant use of prescription or nonprescription drugs that share a common metabolic pathway with disulfoton. Results from oral studies suggest that disulfoton may cause myopia in animals and possibly humans. In one chronic dietary study in rats, effects on eyes (degeneration of the Harderian gland and cornea1 neovascularization), lungs (inflammation), stomach (mucosal hyperplasia and

inflammation of the forestomach), skeletal muscle (atrophy due to debilitation), pancreas (atrophy), ant spleen and lymph nodes were seen in some rats, mostly at the highest doses, but the most sensitive effects were associated with cholinesterase inhibition. Other than the cholinergic effects, none of the effects were seen in mice or dogs given chronic oral doses, and their relevance to humans are unknown. Other effects observed in rats exposed to disulfoton by the inhalation route include inflammation of the nasal turbinates and bone marrow changes, which were related to decreased lymphocytes and inflammatory changes in the lungs.

No data were located regarding reproductive or developmental effects in humans. However, disulfoton has caused lower pregnancy rates and reduced litter sizes in animals after oral exposure for intermediate durations. Fetotoxic effects, such as increased incidences of incomplete ossification of parietal bones and stemebrae, have been reported in the fetuses of rat dams exposed orally during gestation. In addition, depression of fetal brain and erythrocyte cholinesterase, fatty infiltration of the liver, mild nephropathy, and juvenile hypoplasia of the testes occurred in litters of rats exposed orally in multiple-generation reproductive studies. Although placental transfer of disulfoton and/or its active metabolites appears to occur, no data were located regarding transfer via the dam’s milk.

Disulfoton has been tested for genotoxicity in a variety of assays with mostly negative results; however, the few positive results indicate genotoxic potential. Carcinogenicity was not observed in Beagle dogs, rats, or mice fed disulfoton for 2 years.

Employees at hazardous waste sites, employees at pesticide mixing and formulating plants, and farm workers are more likely to be exposed to disulfoton than individuals in other occupations. Neurotoxic effects have been observed in occupationally exposed persons. However, no human data were located to identify susceptible subpopulations. Animal data suggest that female animals and young animals are more susceptible to disulfoton toxicosis. Based on the results from animal studies, women and children could also be more susceptible than men to toxic effects of disulfoton.

Minimal Risk Levels for Disulfoton

Inhalation MRLs

- An MRL of 0.006 mg/ m^3 has been derived for acute-duration inhalation exposure (14 days or less) to disulfoton.

The MRL is based on a NOAEL of 0.5 mg/ m^3 for decreased acetylcholinesterase activity in rats exposed to disulfoton 4 hours/day for 5 days in a study by Thyssen (1978). The NOAEL was adjusted for intermittent exposure, converted to a human equivalent concentration, and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability). Inhibition of erythrocyte cholinesterase activity and unspecified behavioral disorders were observed at 1.8 mg/ m^3, and unspecified signs of cholinergic toxicity were observed at 9.8 mg/ m^3. Similar effects were observed in rats or mice exposed to higher concentrations for shorter durations (Doull 1957; Thyssen 1978). The NOAEL value of 0.5 mg/ m^3 is supported by another study, in which no significant decrease in the activity of brain, serum, or submaxillary gland cholinesterase was found in rats exposed to 0.14-0.7 mg/ m^3 for 1 hour/day for 5-10 days (DuBois and Kinoshita 1971). Mild depression of erythrocyte cholinesterase activity was reported in workers exposed by the inhalation and dermal routes (Wolfe et al. 1978).

- An MRL of $2x10^{-4}$ mg/ m^3 has been derived for intermediate-duration inhalation exposure (15-364 days) to disulfoton.

The intermediate MRL is based on a NOAEL of 0.02 mg/ m^3 for decreased acetylcholinesterase activity in rats exposed to disulfoton 6 hours/day, 5 days/week for 3 weeks in a study by Thyssen (1980). The NOAEL was adjusted for intermittent exposure, converted to a human equivalent concentration, and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability). In the Thyssen (1980) study, 2 separate 3-week experiments were conducted. In the first experiment, rats exposed to 0.1, 0.5, or 3.7 mg/ m^3 showed concentrationrelated increased severity of cholinesterase inhibition and cholinergic signs of toxicity, with the lowest exposure level of 0.1 mg/ m^3 associated with lethargy during the last week of exposure. At 0.5 mg/ m^3, lethargy and failure to groom were observed during the second and third weeks; and at 3.7 mg/ m^3, muscle tremors, convulsions, inhibition of brain cholinesterase, and death were observed. Because a

NOAEL could not be established in the first experiment, the second experiment used an exposure level of 0.02 mg/m^3, which resulted in no inhibition of cholinesterase activity or signs of cholinergic toxicity, and an exposure level of 3.1 mg/ m^3 to confirm the effects seen at 3.7 mg/ m^3 in the first experiment. In a 13-week inhalation study, inhibition of erythrocyte and brain cholinesterase activity was observed in rats exposed to 1.4 mg/ m^3, but not 0.16 mg/ m^3, disulfoton 6 hours/day, 5 days/week (Shiotsuka 1989). Other effects of intermediate-inhalation exposure of rats to disulfoton include inflammatory changes in the respiratory tract associated with bone marrow changes at 20.5 mg/ m^3 and decreased percentages of lymphocytes with increased polymorphonuclear leukocytes at 3.1 mg/ m^3, increased absolute and relative adrenal weight at 3.1 and 3.7 mg/ m^3 (Thyssen 1980), and increased incidence of inflammation of the nasal turbinates at 1.4 mg/ m^3 (Shiotsuka 1989).

An MRL has not been derived for chronic-duration inhalation exposure to disulfoton because no chronic-duration inhalation studies were located.

Oral MRLs

- An MRL of 0.001 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to disulfoton.

The MRL was based on a NOAEL of 0.1 mg/kg/day for decreased cholinesterase activity in rats treated with disulfoton by gavage on gestation days 6-15 (Lamb and Hixson 1983). Erythrocyte and plasma cholinesterase activities were significantly inhibited at 0.3 mg/kg/day. Numerous acute oral studies in rats and mice have found significantly depressed brain or other tissue cholinesterase activities (Costa and Murphy 1983a; Costa et al. 1984, 1986; Schwab and Murphy 1981; Schwab et al. 1981, 1983; Su et al. 1971). Most of these studies used higher doses than those in the Lamb and Hixson (1983) study. However, a 50% inhibition of brain cholinesterase activity was found in rats exposed to disulfoton in the diet at 0.26 mg/kg/day for 1 week (Su et al. 1971), which supports the LOAEL of 0.3 mg/kg/day in the Lamb and Hixson (1983) study. In an extensive acute oral neurotoxicity study in rats, a NOAEL of 0.24 mg/kg for erythrocyte cholinesterase activity was found (Sheets 1993a); however this NOAEL value is essentially the same as the LOAEL values of 0.26 mg/kg/day in the Su et al. (1971) study and 0.3 mg/kg/day in the Lamb and Hixson (1983) study. Other effects of disulfoton in acute oral studies include depression of body weight gain (Schwab and Murphy 1981; Schwab et al. 1981, 1983), interference with catecholamine levels in body tissues

(Brzezinski 1969; Brzezinski and Ludwicki 1973; Brzezinski and Rusiecki 1970; Wysocka-Paruszewska 1970, 1971, 1972), and lipid peroxidation in the liver (Fawade and Pawar 1978, 1980, 1983). None of these effects occurred at doses lower than the acute oral NOAEL of 0.1 mg/kg/day for neurological effects. Furthermore, the NOAEL is an order of magnitude lower than the dose (1 mg/kg/day) associated with delayed ossification in fetal rats (Lamb and Hixson 1983).

- An MRL of $9x10^{-5}$ mg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to disulfoton.

The MRL was based on a NOAEL of 0.009 mg/kg/day for decreased brain cholinesterase activity in F_{1a} pups in a multigeneration feeding study in rats by Hixson and Hathaway (1986). At the LOAEL of 0.03 mg/kg/day, the brain cholinesterase activity was inhibited 24-32% in the F_{1a} pups and litter counts and litter weights were decreased in F_{2b} litters. At 0.09 mg/kg/day, effects included tremors in the F_0 females during the production of the F_1 generation, decreased reproductive performance, decreased maternal F_0 and F_1 weight during gestation and lactation, decreased litter counts and viability and lactation indices, and increased dead births and percentage dead births. Numerous intermediate-duration oral studies in rats, mice, and dogs have found significantly depressed brain or other tissue cholinesterase activities (Clark and Pearson 1973; Doull and Vaughn 1958; Hayes 1985; Hoffman et al. 1975; Klotzsche 1972; Rivett et al. 1972; Robinson et al. 1978; Ryan et al. 1970; Schwab and Murphy 1981; Sheets 1993b; Stavinoha et al. 1969; Vaughn et al. 1958). All of these studies reported cholinesterase inhibition at higher doses than those in the Hixson and Hathaway (1986) study. In addition, other intermediate-duration oral developmental and reproductive studies in animals reported depression of brain or erythrocyte cholinesterase activity in the offspring of rats and reduced litter sizes or failure to produce litters at doses ≥0.1 mg/kg/day (Ryan et al. 1970; Taylor 1965a). In addition, cloudy swelling or fatty livers, mild nephropathy, and juvenile hypoplasia of the testes occurred in F_3 litters (Taylor 1965a).

- An MRL of $6x10^{-5}$mg/kg/day has been derived for chronic-duration oral exposure (≥365 days) to disulfoton.

The MRL is based on a LOAEL of 0.06 mg/kg/day for acetylcholinesterase inhibition in rats exposed to disulfoton in the diet for 2 years in a study by Hayes (1985) using an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to human, and 10 for human

variability). The chronic-duration oral studies have found inhibition of brain, erythrocyte, and plasma cholinesterase in rats given ≥0.06 mg/kg/day, but not ≤0.05 mg/kg/day, disulfoton in the diet for 1.5-2 years (Carpy et al. 1975; Hayes 1985) and in mice given 2.13 mg/kg/day (males) and 2.53 mg/kg/day (females), but not ≤0.5 mg/kg/day, disulfoton for 23 months (Hayes 1983). Dogs treated with 0.5 mg/kg/day disulfoton in capsules (Uga et al. 1977) and rats given ≥0.18 mg/kg/day in the diet (Hayes 1985) for 2 years had optic nerve degeneration. Systemic effects of disulfoton in chronic oral studies were ocular effects (degeneration of ciliary muscles cells, myopia, and astigmatism) in dogs at 0.63 mg/kg/day (Ishikawa and Miyata 1980) and cystic degeneration of the Harderian gland at 0.21 mg/kg/day and cornea1 neovascularization at 0.75 mg/kg/day in rats (Hayes 1985). In addition, rats given disulfoton in the diet for 2 years had granulomatous and suppurative inflammation of the lungs, pancreatic atrophy, dermal lesions, decreased body weight gain, and plasma cell hyperplasia in the mandibular lymph nodes at ≥0.75 mg/kg/day, and mucosal hyperplasia and chronic inflammation of the forestomach and splenic lymphoid follicle depletion at 1.02 mg/kg/day. In a a-year feeding study in dogs, erythrocyte and plasma cholinesterase activities were significantly inhibited at a time-weighted-average dose of 0.14 mg/kg/day, but not at 0.03 mg/kg/day (Hoffman et al. 1975). Although NOABL values of 0.03 mg/kg/day in dogs (Hoffman et al. 1975) and of 0.05 mg/kg/day in rats (Carpy et al. 1975; Hayes 1985) were found, the NOAEL values would result in MRLs higher than the intermediate-duration oral MRL. Thus, the chronic-duration oral MRL of $6x10^{-5}$ mg/kg/day was based on the LOAEL value of 0.06 mg/kg/day in female rats in the study by Hayes (1985), using an uncertainty factor of 1,000.

The chronic-duration oral MRL for disulfoton is $6x10^{-5}$ mg/kg/day, and the EPA chronic oral RfD is $4x10^{-5}$ mg/kg/day (IRIS 1994). Both of these values are based on the same study (Hayes 1985) and the identical end point. Even though the MRL and the RfD are essentially the same, they have minor differences due to the manner in which the exposure doses were calculated. The LOAEL of 0.04 mg/kg/day used by EPA was calculated by multiplying the analytical dietary concentration of 0.8 ppm (nominal concentration of 1 ppm) by the reference rat food consumption factor of 0.05. However, Hayes (1985) provided an equivalent dose of 0.08 mg/kg/day for the nominal concentration of 1 ppm, based on actual food consumption and body weight data. The LOAEL of 0.06 mg/kg/day used in deriving the chronic oral MRL was obtained by multiplying the 0.08 mg/kg/day dose, corresponding to the nominal concentration of 1 ppm, by the analytical concentration of 0.8 ppm.

Death. No studies were located regarding death in humans after inhalation or dermal exposure to disulfoton. One case report of human death after acute oral exposure to disulfoton was found (Hattori et al. 1982). Because an unknown amount was ingested, the lethal dose was not determined. Autopsy results suggested that death may have been due to asphyxia resulting from respiratory failure. Pulmonary edema is associated with disulfoton-induced overstimulation of secretory glands and bronchial secretions in the respiratory tract.

High mortality was found at 202.2 mg/ m^3 in male Sprague-Dawley rats (Doull 1957), at 180.1 mg/ m^3 in male Holtzman rats and 87.6 mg/ m^3 in female Holtzman rats (DuBois 1971), and at 53.4 mg/ m^3 in female mice (Doull 1957) after l-hour exposures; and in female Wistar rats, but not males, at intermittent exposures ≥3.1 mg/ m^3 for 3-15 exposures (Thyssen 1980). LC_{50} values reported for Wistar rats were 290 mg/ m^3 in males and 63 mg/ m^3 for females exposed for 1 hour, and 60 mg/ m^3 for males and 15 mg/ m^3 for females exposed for 4 hours (Thyssen 1978). Thus, strain-, sex-, and species-related differences in the inhalation lethality of disulfoton exist. Acute oral LD_{50} values for rats and mice also suggest that female rats and mice are more sensitive to disulfoton (1.9-8.2 mg/kg) than male rats and mice (5.8-19.3 mg/kg) (Bombinski and DuBois 1958; Crawford and Anderson 1974; Gaines 1969; Mihail 1978; Pawar and Fawade 1978; Stevens et al. 1972a) and that rats are generally more sensitive than mice to disulfoton. However, female guinea pigs appear to be less sensitive than male guinea pigs (LD_{50} of 12.7 mg/kg for females and 8.9 mg/kg for males) (Crawford and Anderson 1973). Acute dermal LD_{50} studies (Gaines 1969; Mihail 1978), and acute intraperitoneal LD_{50} studies (Bombinski and DuBois 1958) also indicate that female rats are more sensitive than male rats and that young animals are more sensitive than adult animals. The acute dermal LD_{50} in rats ranges from 3.6 to 187 mg/kg, depending on the formulation (Weil et al. 1971). Acute dermal exposure resulted in deaths in rabbits at doses of 6.5-10 mg/kg/day (Flucke 1986). In most of these studies, the cause of death was not reported, but may have been due to the cholinergic effects and hypoxia resulting from bronchoconstriction, excessive respiratory secretions, and erratic, slowed heart rate.

Although the dose levels of disulfoton that would cause death in humans are not known, disulfoton levels in the workplace, in the ambient environment, in drinking water, or at hazardous waste sites are probably not high enough to cause death.

Systemic Effects.

Respiratory Effects. Information on respiratory effects due to exposure to disulfoton is very limited. Exposure to disulfoton causes overstimulation of the muscarinic cholinergic receptors in the respiratory tract (Murphy 1986). This usually results in excessive bronchial secretions, bronchoconstriction, and eventually respiratory failure. Pulmonary edema and hemoptysis were recognized as probable causes of death in a man who ingested an unknown amount of disulfoton (Hattori et al. 1982). Studies regarding inhalation exposure were concerned primarily with lethality or cholinesterase inhibition. However, in intermediate-duration inhalation studies in rats, inflammation of the nasal turbinates at 1.4 mg/ m^3 (Shiotsuka 1989) and inflammatory changes throughout the respiratory tracts, possibly related to bone marrow changes, at 0.5 mg/ m^3 (Thyssen 1980) were observed. Breathing difficulties were observed in rats given a single gavage dose of 1.0 mg/kg, mice given a single gavage dose of 5.0 mg/kg, and rats given single dermal applications of disulfoton (Mihail 1978). In a chronic dietary study in rats, granulotamous and suppurative inflammation of the lungs occurred at the highest dietary concentration, but may have been due to aspiration of food particles, since the high dose rats showed considerable debilitation (Hayes 1885). However, no histopathological lesions were found in the lungs of rats (Klotzsche 1972) or mice (Rivett et al. 1972) exposed to disulfoton in the diet for intermediate durations, or in mice (Hayes 1983) or dogs (Hoffman et al. 1975) exposed in the diet for up to 2 years. The likelihood that respiratory effects would occur in humans exposed to disulfoton in the ambient environment or at hazardous waste sites is negligible.

Cardiovascular Effects. Exposure to disulfoton may cause acetylcholine-mediated overstimulation of muscarinic receptors in the heart, which can result in bradycardia progressing to fatal heart block (Murphy 1986). However, no studies were located regarding cardiovascular effects in humans after inhalation, oral, or dermal exposure to disulfoton. No histopathological lesions were found in the hearts of rats exposed by the inhalation route for intermediate durations (Shiotsuka 1989; Thyssen 1980), in rats (Klotzsche 1972) or mice (Rivett et al. 1972) exposed in the diet for intermediate durations, in rats (Carpy et al. 1975; Hayes 1985), mice (Hayes 1983), or dogs (Hoffman et al. 1975) exposed in the diet for chronic durations, or in rabbits exposed dermally for intermediate duration (Flucke 1986). However, acute dermal exposure to unspecified doses of organophosphorus insecticides, including disulfoton, reportedly caused protein degeneration and significant circulatory disruptions in heart muscles of rats, cats, and rabbits (Kundiev and Rappoport 1967). As discussed in Section 2.2.3, this study is limited by reporting deficiencies. No changes in blood pressure or heart

rate were found in rats injected intraperitoneally with disulfoton for ≤30 days (McPhillips and Dar 1967; Perrine and McPhillips 1970). This information is too limited, however, to allow any analysis of the likelihood of cardiovascular effects occurring in humans exposed to disulfoton in any scenario.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after exposure to disulfoton. Necropsy of rats that died after intermittent inhalation exposure to 3.7 mg/ m^3 for 3 weeks revealed bloated gastrointestinal tracts and ulcer-like foci in the glandular mucosa of the forestomach (Thyssen 1980). Mucosal hyperplasia and chronic inflammation of the forestomach was found in rats given 1.04 mg/kg/day disulfoton in the diet for 2 years (Hayes 1985). However, no histopathological lesions were found in the gastrointestinal tracts of rats exposed intermittently by inhalation to 3.1 mg/ m^3 for 3 weeks (Thyssen 1980) or 1.4 mg/ m^3 for 13 weeks (Shiotsuka 1989), in rats exposed to 0.55 mg/kg/day (Klotzsche 1972) or mice exposed to 0.71 mg/kg/day (Rivett et al. 1972) in the diet for 90 days, in rats exposed to ≤0.75 mg/kg/day (Carpy et al. 1975; Hayes 1985), mice exposed to 2.53 mg/kg/day (Hayes 1983), or dogs exposed to 0.14 mg/kg/day (Hoffman et al. 1975) in the diet for 2 years, or in rabbits treated dermally with 1.6 mg/kg/day for 3 weeks (Flucke 1986). The likelihood that gastrointestinal effects would occur in humans exposed to disulfoton in the ambient environment or at hazardous waste sites is negligible.

Hematological Effects. No studies were located regarding hematological effects in humans after exposure to disulfoton. Rats exposed intermittently for 3 weeks to 3.1 mg/ m^3, but not 3.7 mg/ m^3, had decreased percentages of lymphocytes and increased percentages of polymorphonuclear leukocytes, which represented an early response to the bone marrow changes (see Immunological and Lymphoreticular Effects below) (Thyssen 1980). No hematological effects were found in rats exposed by inhalation to lower concentrations for 13 weeks (Shiotsuka 1989); in 90-day dietary studies in rats (Klotzsche 1972) and mice (Rivett et al. 1972); in the 2-year dietary studies in rats (Carpy et al. 1975; Hayes 1985), mice (Hayes (1983), or dogs (Hoffman et al. 1975); or in the 3-week dermal study in rabbits (Flucke 1986). The results of the animal studies indicate that the potential for hematological effects is not a concern for humans exposed to disulfoton.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after exposure to disulfoton. Disulfoton injected intraperitoneally in rats for 10 days had no effect on the contractile mechanism of smooth muscle of the ileum (McPhillips 1969b). No histopathological muscular or skeletal lesions were found in rats dosed once by gavage with disulfoton (Sheets 1993a),

rats (Klotzsche 1972) or mice (Rivett et al. 1972) exposed to disulfoton in the diet for 90 days, or in rats (Carpy et al. 1975), mice (Hayes 1983), or dogs (Hoffman et al. 1975) exposed in the diet for up to 2 years. However, female rats exposed to the highest dietary concentrations of disulfoton for 2 years had skeletal muscle atrophy, which was probably related to the general debilitation of these rats (Hayes 1985). Degenerative changes in the ciliary muscle cells of the eye were observed in dogs exposed orally to disulfoton for 2 years (Ishikawa and Miyata 1980; Suzuki and Ishikawa 1974). These histopathological changes in the muscle were thought to be associated with myopia, also observed in the dogs (see Ocular Effects below). Since high incidences of myopia found in children corresponded with an increased use of disulfoton in combination with other organophosphates to treat food crops (Ishikawa and Miyata 1980), the possibility that degeneration of ciliary muscle cells occurs in humans exposed to disulfoton cannot be ruled out.

Hepatic Effects. No studies were located regarding hepatic effects in humans after exposure to disulfoton. No hepatic effects were found in rats exposed by inhalation (Shiotsuka 1989; Thyssen 1980) or dermally (Flucke 1986) to disulfoton for intermediate durations. However, acute dermal exposure to unspecified doses of organophosphorus insecticides, including disulfoton, reportedly caused protein degeneration and significant circulatory disruptions in the liver of rats, cats, and rabbits (Kundiev and Rappoport 1967). As discussed in Section 2.2.3, this study is limited by reporting deficiencies. Acute oral exposure of animals to disulfoton has resulted in alterations in hepatic microsomal enzymes (Fawade and Pawar 1978, 1980, 1983; Stevens et al. 1972b, 1973). Although inhibition or induction of hepatic microsomal enzymes by disulfoton in the absence of liver pathology is not an adverse effect, animal studies have demonstrated that disulfoton-pretreated animals are more susceptible to the toxic effects of several drugs and anesthetics (see Section 2.6). In addition, lipid peroxidation was also observed in animals after oral exposure to disulfoton (Fawade and Pawar 1978). Fatty infiltration of the liver was observed in F_{3b} offspring in a three-generation oral study (Taylor 1965a). Although no histopathological changes were observed, increased liver weights were observed in female mice exposed orally for intermediate durations (Rivett et al. 1972) and in male rats exposed orally for chronic durations (Carpy et al. 1975). The increased liver weight may be associated with liver enzyme induction. However, an unexplained decrease in liver weights was observed in female rats (Carpy et al. 1975). Clinical chemistry and histological examination revealed no hepatic effects in rats (Carpy et al. 1975; Hayes 1985), dogs (Hoffman et al. 1975), or mice (Hayes 1983) exposed to disulfoton in the diet for up to 2 years. In an intraperitoneal study, injection of rats, but not mice, rabbits, or guinea pigs, resulted in a dose-related increase in serum β-glucuronidase activity at

≥2 mg/kg, but no histopathological lesions in the liver (Kikuchi et al. 1981). In another intraperitoneal study, injection of rats with 1 mg/kg/day for 10 days resulted in cytoplasmic RNA in much smaller clumps in the periportal and centrilobular areas of the liver than in control rats (Clark and Stavinoha 1969). The results from these animal studies indicate that hepatic effects in humans exposed to disulfoton would be mild, if they occurred.

Renal Effects. Glomerular swelling was among the postmortem changes observed in a man who died after he drank an unknown amount of disulfoton (Hattori et al. 1982). However, most organs were congested at autopsy, suggesting that the renal pathology was one of the sequelae of death. Inconclusive evidence that disulfoton causes renal effects was found in animals. In one study, kidney weights of male rats increased while kidney weights of female rats decreased during a 2-year feeding study (Carpy et al. 1975). The toxicological significance of these opposite trends is not clear. Urinalysis and histological examination of kidneys from animals exposed to disulfoton in the diet for intermediate (Klotzsche 1972; Rivett et al. 1972) and chronic durations (Carpy et al. 1975; Hayes 1983, 1985; Hoffman et al. 1975), by inhalation for intermediate durations (Shiotsuka 1989; Thyssen 1980), or dermally for intermediate duration (Flucke 1986) revealed no renal effects. However, acute dermal exposure to unspecified doses of organophosphorus insecticides, including disulfoton, reportedly caused protein degeneration and significant circulatory disruptions in the kidneys of rats, cats, and rabbits (Kundiev and Rappoport 1967). As discussed in Section 2.2.3, this study is limited by reporting deficiencies. These animal studies suggest that the potential for renal effects is not a concern for humans exposed to disulfoton.

Endocrine Effects. No studies were located regarding endocrine effects in humans after exposure to disulfoton. No histopathological evidence of lesions in endocrine organs, such as adrenals, thyroids, pituitary, and pancreas, were found in animals exposed dermally (Flucke 1986) or by inhalation (Shiotsuka 1989; Thyssen 1980); however, female rats exposed to 3.1 and 3.7 mg/ m^3 in two separate intermediate-duration inhalation experiments had significantly increased absolute and relative adrenal weights (Thyssen 1980). Since the increase in adrenal weights was consistently observed in both experiments, it was considered to be related to disulfoton exposure. Oral and intraperitoneal exposure of animals to disulfoton has also resulted in increased urinary or plasma levels of adrenaline or noradrenaline or their metabolites, accompanied by decreases in adrenaline in the adrenal glands (Brzezinski 1969, 1972, 1973; Brzezinski and Ludwicki 1973; Brzezinski and Rusiecki 1970; Wysocka-Pamszewska 1970, 1971), indicating interference with catecholamine levels in body tissues.

These effects may be related to elevated acetylcholine levels, which may cause a release of catecholamines from the stores in the adrenals. Various scenarios involving human exposure to disulfoton might result in an increase in the amount of adrenaline released from the adrenals.

Histological examination of endocrine tissues in animals exposed orally to disulfoton for intermediate (Klotzsche 1972; Rivett et al. 1972) or chronic durations (Carpy et al. 1975; Hayes 1983, 1985; Hoffman et al. 1975) has generally shown no treatment-related lesions. However, male rats exposed chronically to the high dietary concentration had pancreatic atrophy (Hayes 1985). Increased pituitary weight in male rats and decreased pituitary weight in female rats were reported in another chronic feeding study (Carpy et al. 1975). The toxicological significance of these findings is unknown.

Dermal Effects. No studies were located regarding dermal effects in humans after exposure to disulfoton. Dermal lesions consisting of acanthosis, hyperkeratosis, ulceration of the skin, exudate formation, and epithelial inclusion cysts were found in male and female rats exposed chronically to the high dietary concentration of disulfoton (Hayes 1985). However, in intermediate-duration studies, histological examination of skin revealed no lesions in rats exposed by inhalation (Shiotsuka 1989), in rabbits exposed dermally (Flucke 1986), or in rats (Klotzsche 1972) or mice (Rivett et al. 1972) exposed in the diet for 90 days. In a chronic-duration study in rats (Carpy et al. 1975), mice (Hayes 1983), or dogs (Hoffman et al. 1975) exposed in the diet for up to 2 years, histological examination of the skin also revealed no lesions. Acute dermal exposure to unspecified doses of disulfoton appeared to have caused microscopic changes in the blood vessels and fibrous structures in the skin of rats, cats, and rabbits (Kundiev and Rappoport 1967). As discussed in Section 2.2.3, this study is limited by reporting deficiencies. Therefore, exposure to disulfoton in the workplace, in the environment, in drinking water or food, or at hazardous sites might cause dermal lesions in humans.

Ocular Effects. An epidemiological study suggests that the increased use of disulfoton in combination with other organophosphates to treat food crops may have increased the incidence of myopia in children (Ishikawa and Miyata 1980). To determine whether the myopia was associated with disulfoton exposure specifically, dogs were given disulfoton orally for 2 years. Myopia, astigmatism, and associated degenerative changes in the ciliary muscle (see Musculoskeletal Effects above) were observed (Ishikawa and Miyata 1980; Suzuki and Ishikawa 1974). The dog studies support the evidence for disulfoton-induced myopia in humans. Although other factors may have contributed to the occurrence of myopia, histopathological changes in the ciliary muscle of the dogs suggest a strong

cause and effect relationship. Cystic degeneration of the Harderian gland and comeal neovascularization were observed in rats exposed chronically to disulfoton in the diet (Hayes 1985). However, in other studies, histological or ophthalmological examination of the eyes revealed no lesions in rats (Klotzsche 1972) or mice (Rivett et al. 1972) exposed in the diet for 90 days, or in rats (Carpy et al. 1975), mice (Hayes 1983), or dogs (Hoffman et al. 1975) exposed in the diet for up to 2 years. The rats in the study by Carpy et al. (1975) received lower doses than the rats in the Hayes (1985) study. The possibility that myopia or other ocular effects may occur in humans exposed to disulfoton cannot be ruled out.

Body Weight Effects. Weight loss or decreased body weight gain were commonly observed in animals exposed to disulfoton for acute and intermediate durations (Costa et al. 1984, 1986; Fitzgerald and Costa 1992, 1993; Robinson et al. 1978; Schwab and Murphy 1981; Schwab et al. 1981, 1983; Stavinoha et al. 1969; Thyssen 1980). This decrease in weight gain is consistent with disulfoton neurological toxicosis since cholinergic signs (e.g., nausea, emesis, or diarrhea) will reduce food ingestion, absorption, and assimilation. Like the other overt signs of disulfoton toxicosis (see Neurological Effects below), the weight loss is usually transient. In a chronic feeding study, an 11-19s depression of body weight gain occurred in the rats exposed to the high concentration of disulfoton (Hayes 1985). The decrease in body weight gain was partially due to decreased food consumption, which was probably related to the general debilitation of the high dose rats. Intraperitoneal studies also have reported weight loss in animals injected with disulfoton (Costa and Murphy 1982; Costa et al. 1981; McDonald et al. 1988). Based on the results from animal studies, weight loss in humans following moderate to severe disulfoton exposure is possible.

Immunological and Lymphoreticular Effects. No studies were located regarding immunological effects in humans after exposure to disulfoton. However, down-regulation of cholinergic muscarinic receptors in T-lymphocytes and significantly inhibited acetylcholinesterase activity in T-lymphocytes were found in rats given 2 mg/kg/day disulfoton orally for l-2 weeks (Fitzgerald and Costa 1993). The inhibition of T-lymphocyte acetylcholinesterase activity paralleled that in the brain. Similar results were found in rats injected intraperitoneally with 2 mg/kg/day disulfoton for 2 weeks (Costa et al. 1990). The immunological significance of these neurological effects (see Section 2.2.2.4) is not known. Inflammatory changes throughout the respiratory tract, associated with bone marrow changes and low percentages of lymphocytes and high percentages of polymorphonuclear leukocytes in the differential leukocyte counts, were observed in rats exposed to

disulfoton by inhalation for intermediate duration (Thyssen 1980). Female rats exposed by inhalation to a high concentration in this study also had decreased spleen weights (Thyssen 1980), and rats given the high concentration of disulfoton in the diet for 2 years had a significantly increased incidence of plasma cell hyperplasia in the mandibular lymph nodes (males) and a significantly increased incidence of splenic lymphoid follicle depletion (females) (Hayes 1985). The plasma cell hyperplasia in the mandibular lymph nodes was probably a response to upper respiratory tract inflammation, which may have been due to aspiration of ingested food particles. In other inhalation (Shiotsuka 1989), dietary (Carpy et al. 1975; Hayes 1983; Hoffman et al. 1975; Klotzsche 1972; Rivett et al. 1972), and dermal (Flucke 1986) studies in animals exposed to disulfoton, histological examination of spleen, thymus, lymph nodes,‘and bone marrow revealed no treatment-related lesions. The relevance of the various lymphoreticular and possible immunological effects to humans exposed to disulfoton is not clear.

Neurological Effects. The neurological effects of disulfoton depend on its metabolism to active metabolites which, in turn, inhibit acetylcholinesterase activity at nerve synapses. Inhibition of acetylcholinesterase activity results in excessive accumulation of acetylcholine which, in turn, stimulates muscarinic cholinergic receptors located in various organ tissues. The response of these effector organs to overstimulation is recognized as a neurological effect, and the severity will depend on such factors as dose, sex, species, and age of the human or animal exposed. Despite mild depression of erythrocyte cholinesterase activity, neurological effects were not observed in pesticide-fertilizer mixers after inhalation exposure to 0.46-0.633 mg/ m^3 disulfoton and dermal exposure to 0.23 mg/kg/day disulfoton (Wolfe et al. 1978). One case of accidental ingestion of disulfoton resulted in cholinergic signs including miosis, salivation, masseteric spasms, monoplegia, and depressed serum cholinesterase activity (Yashiki et al. 1990). Weakness, fatigue, and cyanosis were observed in a farmer who, for several days, had worn gloves that were soaked in disulfoton (Savage et al. 1971).

Neurological effects observed in humans have also been demonstrated in animals after acute and intermediate oral exposure (Costa et al. 1984; Crawford and Anderson 1974; Mihail 1978; Schwab and Murphy 1981; Schwab et al. 1981, 1983; Sheets 1993a, 1993b), acute and intermediate inhalation exposure (Doull 1957; Shiotsuka 1989; Thyssen 1978, 1980), and acute dermal exposure (Flucke 1986; Mihail 1978) to disulfoton. Inhibition of brain acetylcholinesterase and other tissue acetylcholinesterase activities (Carpy et al. 1975; Christenson and Wahle 1993; Clark and Pearson 1973; Doull and Vaughn 1958; Flucke 1986; Hayes 1983, 1985; Hikita et al. 1973; Hixson and Hathaway 1986; Hoffman et al. 1975; Klotzsche 1972; Robinson et al. 1970, 1978; Schwab and

Murphy 1981; Schwab et al. 1981; Sheets 1993a, 1993b; Shiotsuka 1989; Thyssen 1978, 1980) also have been observed in animals in numerous studies regardless of the route, duration, or animal species. Female animals appear to be more sensitive than males to the cholinergic effects of disulfoton. Chronic oral exposure of animals to disulfoton has resulted in necrosis and atrophy of the optic nerve and retina (Hayes 1983; Uga et al. 1977) and unexplained changes in brain weight (Carpy et al. 1975). A 2-month dietary study in rats and mice found that disulfoton exposure increased the permeability of spinal cord and brain stem tissues (Clark and Stavinoha 1971).

Limited evidence indicates that disulfoton does not cause organophosphate-induced delayed neurotoxicity in chickens. This syndrome is caused by some phosphate, phosphonate, and phosphoramidate esters, which are not usually used as insecticides (Ecobichon 1990). Axonal degeneration followed by myelin degeneration in nerve fibers distal to the nerve cell body are common histopathological findings in hens with organophosphate-induced delayed neurotoxicity. However, oral doses of 0, 0.1, 0.6, or 1.5 mg/kg disulfoton for 30 days did not cause demyelination in White Leghorn hens (Taylor 1965b). Furthermore, White Leghorn hens given two 30 mg/kg oral doses of disulfoton 21 days apart displayed no signs of delayed neurotoxicity (Hixson 1983). While clinical signs of cholinergic intoxication (loss of equilibrium, decreased activity, diarrhea, and locomotor ataxia) were observed on the first treatment day, these signs disappeared within 5 days of treatment. Histological examination of the sciatic nerve, spinal cord, and brain revealed no treatment-related lesions. It should be noted that the disulfoton-treated hens in the Hixson (1983) study were also treated with 0.5 mg/kg atropine intramuscularly 10 minutes prior to disulfoton and with 12.5 mg/kg pralidoxime chloride (2-PAM) intramuscularly 30 minutes after disulfoton. These antidotes were given to prevent lethality, but 2-PAM also reverses cholinesterase inhibition, and atropine blocks the muscarinic and central nervous system toxicity (see Section 2.8.3). Therefore, the administration of these antidotes may have confounded the conclusion that disulfoton does not cause delayed neurotoxicity.

Numerous studies indicate that animals develop a tolerance to the cholinergic effects of disulfoton after a few days of oral exposure (Costa et al. 1981; Fitzgerald and Costa 1992, 1993; Schwab and Murphy 1981; Schwab et al. 1981, 1983). Animals exposed to disulfoton initially developed cholinergic signs of toxicity, but with subsequent dosing (5-10 days) these signs almost disappeared, even though cholinesterase activity remained depressed. Inhibition of brain, erythrocyte, or other tissue cholinesterase, and typical signs of cholinergic poisoning have also been reported in numerous studies

in which animals received intraperitoneal injections of disulfoton (Bombinski and DuBois 1958; Brodeur and DuBois 1963, 1964; Costa and Murphy 1982, 1983a, 1986; Costa et al. 1981, 1982a, 1986, 1990; Foley and McPhillips 1973; LeFever and Green 1975; Llorens et al. 1993; McDonald et al. 1988; McPhillips 1969a; McPhillips and Dar 1967; Perrine and McPhillips 1970; Schwab et al. 1981; Smith et al. 1968; Stavinoha et al. 1969; Westfall et al. 1974).

Animal studies suggest that disulfoton causes neurobehavioral changes; however, most of these changes do not appear to be adverse. Unexpected faster maze running times and fewer errors were made by rats fed disulfoton for 90 days, despite a significant depression in brain cholinesterase (Clark and Pearson 1973). Furthermore, an unexpected increase in exploratory behavior was observed in mice fed disulfoton (Clark et al. 1971). Disulfoton did not result in impaired memory (assessed by shock treatment sensitivity and passive avoidance retention) in mice made tolerant by intraperitoneal injections of disulfoton for 14 days (Costa and Murphy 1982). However, McDonald et al. (1988) hypothesized that a decrease in muscarinic receptor densities in brain tissues of disulfoton-tolerant rats was the reason for the observed impairment of spatial memory after intraperitoneal injection with disulfoton for 14 days. In rats given daily intraperitoneal injections of disulfoton for 30 days, reduced motor activity occurred at 2.0 mg/kg/day, but tolerance to this effect did not develop (Llorens et al. 1993). Disulfoton had no effect on acquisition or retention of passive avoidance, but impaired performance in a spatial memory water maze occurred at 2.0 mg/kg/day. This deficit in cognitive performance occurred at a time when tolerance to overt signs of cholinergic toxicity developed.

Data from the few available case reports and the numerous animal studies strongly suggest that disulfoton exposure may cause mild to severe neurological effects in humans. Although disulfoton-associated memory loss has not been reported in humans, there should be concern that occupational exposure and exposure at hazardous waste sites may interfere with the performance of cognitive and complex tasks.

Reproductive Effects. No studies were located regarding reproductive effects in humans after exposure to disulfoton. No effect on male fertility was found in mice treated orally in a dominant lethal study (Herbold 1980). A 3-generation study demonstrated that a disulfoton diet providing 0.5 mg/kg/day resulted in slightly reduced litter sizes in the third generation of rats (Taylor 1965a). In a study where males or females were fed disulfoton prior to and/or during mating, the failure of two of five females to become pregnant indicated that reproductive function in males as well as females may

have been severely affected (Ryan et al. 1970). A more extensive multigeneration feeding study in rats found decreased reproductive performance, evidenced by a decreased percentage of females placed for mating and decreased percentage of sperm-positive F_0, and F_1 parental females, decreased maternal weight of F_0 and F_1 dams during gestation and lactation, decreased litter counts, decreased viability and lactation indices, increased dead births and percentage of dead births in both generations, and decreases in F_{2b} litter counts and litter weights (Hixson and Hathaway 1986). Histological examination of male and female reproductive organs generally did not reveal any treatment-related lesions in rats (Carpy et al. 1975; Hayes 1985; Klotzsche 1972; Shiotsuka 1989; Thyssen 1980), mice (Hayes 1983; Rivett et al. 1972), dogs (Hoffman et al. 1975), or rabbits (Flucke 1986) exposed to disulfoton by any route. The only exception was uterine cystic hyperplasia in female rats fed the high dietary concentration of disulfoton for 2 years (Hayes 1985). Reproductive function was not assessed in these studies. Based on the reproductive studies in animals, the possibility for reproductive effects in humans exposed to disulfoton cannot be ruled out.

Developmental Effects. No studies were located regarding developmental effects in humans after exposure to disulfoton. Incomplete ossification of the parietal bones and the stemebrae, but no tissue malformations were observed in the offspring of rats fed disulfoton during gestation (Lamb and Hixson 1983). Erythrocyte acetylcholinesterase depression, cloudy swelling and fatty infiltration of the liver, mild nephropathy, and juvenile hypoplasia of the testes were found in F_{3b} litters in a threegeneration feeding study (Taylor 1965a). Brain cholinesterase depression was also observed in the fetuses of rat dams fed disulfoton (Ryan et al. 1970) and in the F_{1a} pups of male and female rats in a dietary reproduction study (Hixson and Hathaway 1986). Intraperitoneal injection of pregnant mice with disulfoton on gestation day 11 resulted in 71.4% fetal mortality and decreased litter size (Uzokwu 1974). No malformations were seen. Nevertheless, rabbit fetal survival, growth, and development was not affected by oral exposure of the dams to disulfoton during gestation (Tesh et al. 1982). Based on the animal data, the potential for disulfoton to cause fetotoxic and developmental effects in humans cannot be ruled out.

Genotoxic Effects. No studies were located regarding genotoxicity of disulfoton in humans after inhalation, oral, or dermal exposure or in animals after inhalation or dermal exposure. The results of in viva studies are summarized in Table 2-4. Disulfoton did not induce micronuclei in the erythrocytes of mice treated orally at 6 or 12 mg/kg/day for 2 days (Herbold 1981) or orally or intraperitoneally at 2, 4, or 8 mg/kg disulfoton (EPA 1984a; Sandhu et al. 1985). Disulfoton was also negative in a

TABLE 2-4. Genotoxicity of Disulfoton *In Vivo*

Species (test system)	End point	Results	Reference
Drosophila melanogaster	Sex-linked recessive lethal	–	EPA 1981a; Sandhu et al. 1985; Waters et al. 1981, 1982
Mouse (intraperitoneal or oral, not otherwise specified)	Induction of micronuclei	–	EPA 1984a; Sandhu et al. 1985; Waters et al. 1981, 1982
Mouse (oral)	Induction of micronuclei in bone marrow polychromatic erythrocytes	–	Herbold 1981
Mouse (oral	Dominant lethal	–	Herbold 1980

– = negative result

dominant lethal test in mice given a single oral dose of 5 mg/kg (Herbold 1980). Furthermore, disulfoton did not induce sex-linked recessive lethal mutations in *Drosophila melunogaster* (EPA 1981a; Sandhu et al. 1985; Waters et al. 1981, 1982).

Disulfoton has been tested in numerous types of *in vitro* assays, mainly with negative results (see Table 2-5). Disulfoton was negative in most assays for reverse mutation in most strains of *Salmonella typhimurium* with or without metabolic activation (EPA 1980a; Inukai and Iyatomi 1976; Moriya et al. 1983; Sandhu et al. 1985; Waters et al. 1981, 1982); but positive results were obtained in LT-2 strains (Hanna and Dyer 1975) and in one assay with strain TA1535 (Moriya et al. 1983; Shirasu et al. 1982, 1984) of *S. typhimurium* without activation. Results of reverse mutation assays in *Escherichia coli* were equivocal; positive results without activation in WP2 strains were reported in one study (Hanna and Dyer 1975), but negative results in WP2 uvrA with and without activation were found in another study (EPA 1980a, 1984a; Sandhu et al. 1985; Waters et al. 1981, 1982). Disulfoton was negative in assays of differential toxicity in *S. typhimurium, E. coli,* and *Bacillus subtilis* with or without activation (EPA 1980a; Herbold 1983; Inukai and Iyatomi 1976; Sandhu et al. 1985; Waters et al. 1981, 1982). Negative results were obtained in assays for reverse mutation, gene conversion, mitotic crossing over and recombinants, and for primary DNA damage in eukaryotic yeast, *Succhuromyces cerevisiue,* with and without activation (Brusick 1981; EPA 1984a; Jagannath 1981; Sandhu et al. 1985; Waters et al. 1981, 1982). However, positive results without activation were obtained in assays for chiasma frequency (genetic recombinants), mitotic index, chromosomal aberrations, and pollen fertility in barley *(Hordeurn vulgaris)* (Murty et al. 1983; Panda 1983; Singh et al. 1977). Some positive and some negative results have been obtained in cultured mammalian cells. Positive or weakly positive results were obtained for sister chromatid exchanges in Chinese hamster ovary cells with metabolic activation in two assays (EPA 1984a; Sandhu et al. 1985; Waters et al. 1981, 1982), but negative results were found in another study (Chen et al. 1981, 1982). Conversely, disulfoton induced sister chromatid exchanges in Chinese hamster ovary cells without metabolic activation, but not with metabolic activation, in another study (Putnam 1987). Disulfoton was negative for HGPRT mutations in Chinese hamster ovary cells with and without activation (Yang 1988). Positive results without activation were obtained for forward mutations in mouse lymphoma cells, for unscheduled DNA synthesis in human lung fibroblasts (EPA 1984a; Sandhu et al. 1985; Waters et al. 1981, 1982), and for growth inhibition and increased protein synthesis in human HeLa cells (Litterst et al. 1969). However, negative results were found for chromosomal aberrations in human hematopoietic cell lines (Huang 1973) and for alterations of DNA or RNA synthesis in human HeLa cells (Litterst et al. 1969).

TABLE 2-5. Genotoxicity of Disulfoton *In Vitro*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium LT-2 strains	Reverse mutation	No data	+	Hanna and Dyer 1975
S. typhimurium TA1535	Reverse mutation	No data	+	Moriya et al. 1983; Shirasu et al. 1982, 1984
S. typhimurium	Reverse mutation			Moriya et al. 1983
WP2hcr		No data	–	
TA100		No data	–	
TA1537		No data	–	
TA1538		No data	–	
TA98		No data	–	
S. typhimurium TA100	Reverse mutation	–	–	Sandhu et al. 1985
S. typhimurium	Reverse mutation			EPA 1980, 1984a; Waters et al. 1981, 1982
TA1535		–	–	
TA1537		–	–	
TA1538		–	–	
TA98		–	–	
TA100		–	–	
S. typhimurium	Reverse mutation			Inukai and Iyatomi 1976
TA1535		–	–	
TA1537		–	–	
TA98		–	–	
TA100		–	–	
S. typhimurium SL4525(rec$^+$)/ SL4700(rec$^-$)	Differential toxicity	No data	–	EPA 1984a; Waters et al. 1981, 1982
Escherichia coli WP2 strains	Reverse mutation	No data	+	Hanna and Dyer 1975

TABLE 2-5. Genotoxicity of Disulfoton *In Vitro* (continued)

Species (test system)	End point	Result: With activation	Result: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium LT-2 strains	Reverse mutation	No data	+	Hanna and Dyer 1975
E. coli WP2 uvrA	Reverse mutation	–	–	EPA 1980, 1984a; Sandhu et al. 1985; Waters et al. 1981, 1982
E. coli W3110/p3478	Differential toxicity	–	–	Herbold 1983
E. Coli W3110/p3478	Differential toxicity	No data	–	EPA 1980
Bacillus subtilis H17/MW5	Differential toxicity	No data	–	EPA 1980
B. subtilis NIG17/NIG45	Differential toxicity	No data	–	Inukai and Iyatomi 1976
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae D7	Reverse mutation	–	–	EPA 1984a; Sandhu et al. 1985; Waters et al. 1981, 1982
S. cerevisiae	Reverse mutation			
S138		–	–	Jagannath 1981
S211		–	–	
S. cerevisiae D7	Gene conversion and mitotic crossing-over	–	–	Sandhu et al. 1985; Waters et al. 1981, 1982
S. cerevisiae D3	Induction of mitiotic recombinants	–	–	EPA 1980; Sandhu et al. 1985
S. cerevisiae D3	Primary DNA damage	No data	–	Waters et al. 1981, 1982
S. cerevisiae D6	Mitotic non-disjunction	–	–	Brusick 1981

TABLE 2-5. Genotoxicity of Disulfoton *In Vitro* (continued)

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium LT-2 strains	Reverse mutation	No data	+	Hanna and Dyer 1975
Plants:				
Barley (*Hordeum vulgare*) seeds	Chiasma frequency (genetic recombinants)	No data	+	Murty et al. 1983
Barley (*H. vulgare*) seeds	Mitotic index	No data	+	Panda 1983
	Chromosomal aberrations in embryonic shoots and pollen mother cells	No data	+	Panda 1983
Barley (*H. vulgare*) seeds	Pollen fertility	No data	+	Singh et al. 1977
Barley (*H. vulgare*) seeds	Chromosomal aberrations	No data	+	Singh et al. 1977
Mammalian cells:				
Chinese hamster ovary cells	HGPRT mutation	–	–	Yang 1988
Chinese hamster ovary cells	Sister chromatid exchange	(+)	–	Sandhu et al. 1985
Chinese hamster ovary cells V79	Sister chromatid exchange	–	–	Chen et al. 1981, 1982
Chinese hamster ovary cells	Sister chromatid exchange	+	–	EPA 1984a; Waters et al. 1981, 1982
Chinese hamster ovary cells	Sister chromatid exchange	–	+	Putnam 1987
Mouse lymphoma cells L517874	Forward mutation	–	+	EPA 1984a; Sandhu et al. 1985
Mouse lumphoma cells L5T	Forward mutation	No data	+	Waters et al. 1981, 1982

TABLE 2-5. Genotoxicity of Disulfoton *In Vitro* (continued)

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium LT-2 strains	Reverse mutation	No data	+	Hanna and Dyer 1975
Human lung fibroblasts WI-38 cells	Unscheduled DNA synthesis	–	+	EPA 1980a, 1984a; Sandhu et al. 1985
Human hematopoietic cell lines	Chromosomal aberrations			Huang 1973
B411–4		No data	–	
RPMI–1788		No data	–	
RPMI–7191		No data	–	
Human HeLa cells	Growth inhibition	No data	+	Litterst et al. 1969
Human HeLa cells	DNA synthesis	No data	–	Litterst et al. 1969
Human HeLa cells	RNA synthesis	No data	–	Litterst et al. 1969
Human HeLa cells	Protein synthesis	No data	+	Litterst et al. 1969

DNA = Deoxyribonucleic acid; HGPRT = hypoxanthine–guanine phosphoribosyl transferase; RNA = Ribonucleic acid; – = negative result; + = positive result; (+) = weakly positive result

While many of the studies on genotoxicity of disulfoton were negative, the positive results indicate a potential for mutagenic and clastogenic effects in humans exposed to disulfoton.

Cancer. No studies were located regarding cancer in humans after exposure to disulfoton. Carcinogenic effects were not observed in rats (Carpy et al. 1975; Hayes 1985), mice (Hayes 1983), or dogs (Hoffman et al. 1975) fed disulfoton for 1.5-2.0 years. There is no reason to believe that disulfoton is carcinogenic.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers 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 or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to disulfoton are discussed in Section 2.5.1. 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 (NAUNRC 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 effects caused by disulfoton are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism3s 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 2.7, Populations That Are Unusually Susceptible.

2.5.1 Biomarkers Used to Identify or Quantify Exposure to Disulfoton

Disulfoton and its metabolites have been measured in various tissues and body fluids (blood, urine, feces, liver, kidney, and body fat) from humans or animals exposed to disulfoton (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. At ≈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). In both cases, the original 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 will result in detectable levels in the liver (Bull 1965; Puhl and Fredrickson 1975). However, monitoring of liver levels would require biopsy, which is not practical.

The presence of disulfoton and/or its metabolites in urine is usually a reliable biomarker of disulfoton exposure. Specimens of urine collected from 64 locations across the United States, comprising the sample areas of the Second Health and Nutrition Examination Survey (NHANES II), reported detection (detection limit 0.02 ppm) of DEP and DETP at a frequency of 6-7% and DEDPT at a frequency of <1% of those tested (Murphy et al. 1983). 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 postexposure (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). 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. The dialkyl phosphate metabolites are not only very sensitive indicators of disulfoton exposure, but their presence strongly suggests previous exposure to a diethyl organophosphate ester. One animal study demonstrated that a greater percentage of the disulfoton dose was eliminated as DEP (Bull 1965). This provides limited but supporting evidence that DEP is a more sensitive urinary biomarker than the other metabolites discussed.

A combination of neurological signs is usually a biomarker of organophosphate exposure. Neurological signs such as pupil miosis, muscular tremors, and increased salivation have been observed in humans accidentally exposed to disulfoton (Yashiki et al. 1990) and in animals given disulfoton (Schwab et al. 1981).

Inhibition of erythrocyte acetylcholinesterase activity or serum cholinesterase activity with or without concomitant neurological signs is usually a good indicator of organophosphate exposure. In addition, T-lymphocyte acetylcholinesterase activity was found to be rapidly and greatly depressed in rats during a 14-day daily exposure to disulfoton, but rapidly recovered after exposure (Fitzgerald and Costa 1993). Therefore, T-lymphocyte acetylcholinesterase activity could be used as a biomarker of exposure to organophosphorus pesticides during exposure, but not once exposure has ceased. The acetylcholinesterase activity recovered more slowly in erythrocytes than in lymphocytes, indicating the erythrocyte activity is a better biomarker of exposure once exposure has ceased. However, the severity of the signs and symptoms and the degree of cholinesterase depression are not always correlated. Standards for serum cholinesterase activity (175-440 IU) have been established for humans (Yashiki et

al. 1990), but there is a wide normal range due to the variation in the human population. A man had depressed blood cholinesterase activity (<10 IU for 5 days and <40 IU for 8 days after exposure) as well as cholinergic signs of toxicity following accidental ingestion of disulfoton (Yashiki et al. 1990). As demonstrated in this case, cholinesterase activity can remain depressed for at least a week after neurological signs have disappeared; therefore, other parameters (e.g., urine metabolites) may help with the diagnosis. Cholinesterase depression was not observed in 11 employees exposed to disulfoton for ≤2.5 weeks (Brokopp et al. 1981). The presence of urinary metabolites of disulfoton was the only biomarker of disulfoton exposure. Employees occupationally exposed to disulfoton for 9 weeks had marked depression of the erythrocyte acetylcholinesterase activity, but no neurological signs (Wolfe et al. 1978). Urinary metabolites were not reported; therefore, cholinesterase depression was the only biomarker of exposure. Because organophosphates other than disulfoton and carbamates can depress cholinesterase activity (Osweiler et al. 198.5) cholinesterase activity is not specific for disulfoton exposure. Furthermore, liver dysfunction, pregnancy, malnutrition, neoplasia, infection, and certain drugs such as codeine and morphine may lower plasma pseudocholinesterase activity, while hemoglobinopathies such as sickle cell disease and thalassemia and other anemias may lower erythrocyte cholinesterase activity (Goldfrank et al. 1990).

Urine catecholamines may also serve as biomarkers of disulfoton exposure. No human data are available to support this, but limited animal data provide some evidence of this. 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). Because organophosphates other than disulfoton can cause an accumulation of acetylcholine at nerve synapses, these chemical compounds may 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, but had no effect on NADPH cytochrome c reductase. Thus, the induction of the MFO system required repeated dosing with relatively high doses. Furthermore, 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.

2.5.2 Biomarkers Used to Characterize Effects Caused by Disulfoton

Disulfoton exposure results in cholinergic signs such as salivation, diarrhea, pupil constriction, muscle tremors, and weight loss. Ataxia, convulsions, coma, respiratory distress, and death are common signs associated with a more severe toxicosis. Nervous tissue is evidently the most sensitive target organ.

Because cholinesterase inhibition is a very sensitive biomarker for other chemicals, it is not always conclusive evidence of disulfoton exposure. However, depression of cholinesterase activity can alert a physician to the possibility of more serious neurological effects. Erythrocyte acetylcholinesterase 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 addition, a recent study showed that after rats received oral doses of disulfoton for 14 days, acetylcholinesterase levels in circulating lymphocytes correlated better with brain acetylcholinesterase activity than did erythrocyte cell cholinesterase activities during exposure (Fitzgerald and Costa 1993). However, recovery of the activity in lymphocytes was faster than the recovery of activity in the brain, which correlated better with the activity in erythrocytes. Animal studies have also demonstrated that brain acetylcholinesterase 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); however, the measurement of brain acetylcholinesterase in humans is too invasive to be practical.

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. This enzyme appears to be a useful biomarker of hepatic function in rats exposed to disulfoton, but may not be a useful biomarker in humans.

Increased levels of urinary catecholamines may also be associated with accumulation of acetylcholine that resulted from acetylcholinesterase 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).

Additional information regarding biomarkers for effects can be found in OTA (1990) and CDC/ATSDR (1990). A more detailed discussion of the health effects caused by disulfoton can be found in Section 2.2 of Chapter 2.

2.6 INTERACTIONS WITH OTHER SUBSTANCES

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 Green 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 P-450 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 P-450 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 P-450, or the content of cytochrome P-450. The 5-day treatment regimen resulted in increased activities of ethylmorphine N-demethylase and NADPH oxidase activities, as well as increased cytochrome P-450

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 P-450 activity (Sipes and Gandolfi 1986). Therefore, pretreatment with phenobarbital will not result in flavin monooxygenase-mediated activation of disulfoton to its active metabolite. Cytochrome P-450 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 P-450 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_{85} 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. The authors proposed that the different levels of protection were due to the induction of two different interconvertible forms of cytochrome P-450. 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 Type I substrate, 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). Nickel chloride, cobalt chloride, or cycloheximide decreased the levels of cytochrome b,, cytochrome c reductase, and total heme in rats (Fawade and Pawar 1983). These electron transport components were further decreased in rats pretreated with these inhibitors and given a single dose of disulfoton. Data from this study suggests an additive effect, since disulfoton also decreases the activities of these components. Evidence of an additive effect between disulfoton and these 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, these inhibitors decreased the activity of delta-aminolevulinic acid synthetase, but this decrease was reversed when disulfoton was administered.

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, and, therefore, resulted in an additive effect. 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). Because of this cross-tolerance, a benefit is derived as a result of this interaction. 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 nonpretreated animals. In another study, disulfoton-tolerant rats were tolerant to the cholinergic effects of octamethyl pyrophosphoramide (OMPA) but not parathion (McPhillips 1969a, 1969b). The authors were unable to explain why the insecticides OMPA and parathion caused different effects.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to disulfoton than will most persons exposed to the same level of disulfoton in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects or clearance rates and any resulting end product metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

No data were located that identify subpopulations of humans more susceptible to the toxic effects of disulfoton. Animal studies demonstrate that there are sex, age, and liver function differences in the susceptibility of animals to disulfoton toxicosis. Data from LC_{50} and LD_{50} studies suggest that female rats and mice are more sensitive than male rats and mice to disulfoton after inhalation (Thyssen 1978), oral (Bombinski and DuBois 1958; Crawford and Anderson 1974; Gaines 1969; Mihail 1978; Pawar and Fawade 1978), dermal (Gaines 1969; Mihail 1978), or intraperitoneal (Bombinski and DuBois 1958) exposure. Erythrocyte and brain cholinesterase activity was more depressed in female rats than in male rats (Klotzsche 1972; Ryan et al. 1970; Thyssen 1980). While absolute and relative brain weights generally increased in male rats exposed to disulfoton, the trend was reversed in female rats (Carpy et al. 1975). In the same study, liver, spleen, kidney, and pituitary weights were increased in male rats and decreased in female rats. The toxicological significance of these observations is unknown. Results from toxicokinetic studies showed that male rats eliminated disulfoton faster than female rats (Lee et al. 1985; Puhl and Fredrickson 1975). This apparent difference in the toxic responses of the sexes may have been due to differences in absorption, retention, metabolism, or a combination of factors.

Animal studies suggest that younger animals are more susceptible to disulfoton toxicosis than older animals. The intraperitoneal LD_{50} of disulfoton was lower in weanling rats than in adult rats (Brodeur and DuBois 1963). These investigators 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. 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).

Data suggest that animals pretreated with disulfoton or hepatic enzyme inducers were less susceptible to disulfoton toxicosis than those subpopulations that were not pretreated. Disulfoton-tolerant animal populations are generally less sensitive to subsequent disulfoton exposure than are nontolerant animals (Costa et al. 1984; Schwab and Murphy 1981; Schwab et al. 1981, 1983). In addition, animals pretreated with chemicals that induce MFO (e.g., phenobarbital) were less susceptible to disulfoton toxicosis (DuBois and Kinoshita 1968; Pawar and Fawade 1978).

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and experimental research concerning methods for reducing toxic effects of exposure to disulfoton. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposure to disulfoton. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

If disulfoton is inhaled, the victim should be removed to a fresh air environment. Artificial respiration and the use of oxygen has been recommended (Sittig 1991). Not much can be done to reduce absorption of disulfoton from the respiratory tract. If disulfoton is ingested, the stomach should be emptied by inducing emesis or by gastric lavage (Haddad and Winchester 1990). If the patient is unconscious, means to prevent aspiration are recommended prior to gastric lavage. Because most organophosphates contain hydrocarbon solvents, which are severe aspiration hazards, the use of emetics may be contraindicated (Ellenhom and Barceloux 1988). Activated charcoal and a cathartic should then be given to adsorb any of the remaining disulfoton. Repeated treatment with activated charcoal may be useful to prevent reabsorption following enterohepatic circulation. In the event of dermal exposure, contaminated clothing should be removed and the victim should be bathed with soap

and water in an attempt to decontaminate the skin. The eyes should be rinsed with water or physiological saline if disulfoton entered the eye.

2.8.2 Reducing Body Burden

No information is available regarding methods for specifically reducing the body burden of disulfoton. Diuresis, dialysis, and hemoperfusion have been used to reduce the body burden of other organophosphates in humans and animals (Ellenhorn and Barceloux 1988). Because disulfoton is rapidly metabolized to its active metabolites which bind to ubiquitous cholinesterases in nervous tissue, these methods may not be effective unless started immediately after exposure. However, repeated administration of activated charcoal/cathartics may prevent possible enterohepatic recirculation of disulfoton or its metabolites. Administration of oximes, such as 2-PAM, may also increase the excretion of disulfoton metabolites, since oximes accelerate the hydrolysis of phosphorylated cholinesterase (Ellenhorn and Barceloux 1988; Goldfrank et al. 1990; Haddad and Winchester 1990; Morgan 1982). However, “aging” of the phosphorylated enzyme may occur after l-2 days (Ecobichon 1990). Because 2-PAM is unable to reverse cholinesterase inhibition once “aging” has started, 2-PAM should be administered as soon as possible after exposure or any time following exposure when clinical signs or symptoms are present.

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism of action of disulfoton depends on active metabolites of disulfoton binding to acetylcholinesterase and causing an accumulation of acetylcholine at the synapse that results in muscarinic (e.g., salivation) and nicotinic (e.g., muscle tremors) effects. Atropine sulfate has been used as a therapeutic agent to reduce the severity of disulfoton toxicosis by binding to muscarinic receptors, thus blocking the muscarinic and central nervous system manifestations (Goldfrank et al. 1990). Atropine also reduces excessive bronchial secretions that can result in bronchopneumonia. This drug was also used successfully to treat a patient with severe signs of disulfoton toxicosis (Yashiki et al. 1990). Pretreatment of rats with atropine protected them against an intraperitoneal LD_{50} dose (Bombinski and DuBois 1958). Oxime derivatives (e.g., 2-PAM), accelerate the hydrolysis of phosphorylated cholinesterase and hence accelerate regeneration of the active cholinesterase (Ellenhorn and Barceloux 1988; Goldfrank et al. 1990; Haddad and Winchester 1990; Morgan 1982). The oximes usually reduce both the muscarinic and nicotinic effects of poisoning when administered within

36 hours after organophosphate exposure (Goldfrank et al. 1990; Morgan 1982). However, "aging" of the phosphorylated enzyme may occur after l-2 days (Ecobichon 1990). Because 2-PAM is unable to reverse cholinesterase inhibition once "aging" has started, 2-PAM should be administered as soon as possible after exposure. Atropine and 2-PAM synergistically alleviate the manifestations of cholinesterase inhibition when co-administered (Goldfrank et al. 1990). While atropine blocks the muscarinic effects, 2-PAM regenerates cholinesterase enzymes in sympathetic, parasympathetic, and central nervous system sites.

2.9 ADEQUACY OF THE DATABASE

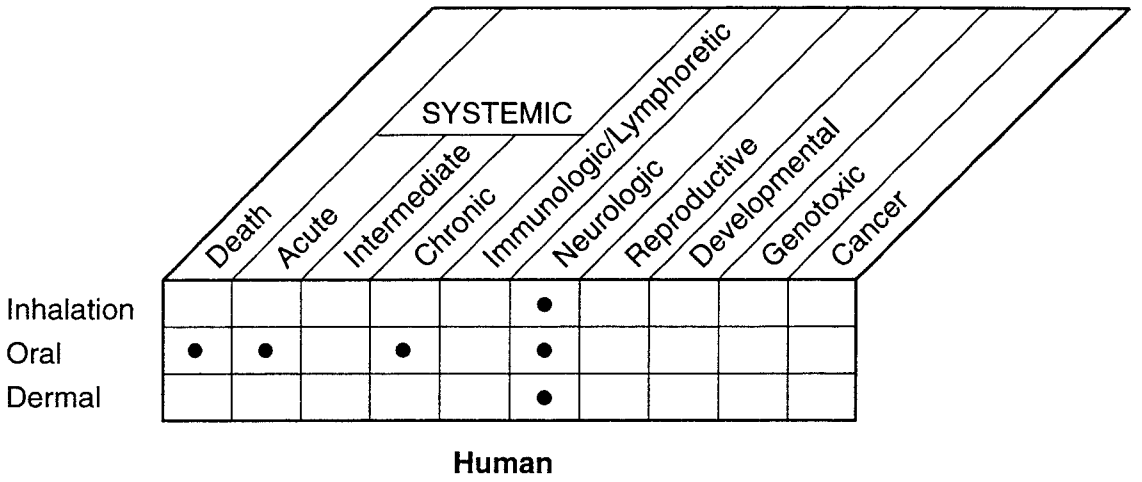
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of disulfoton is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of disulfoton.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

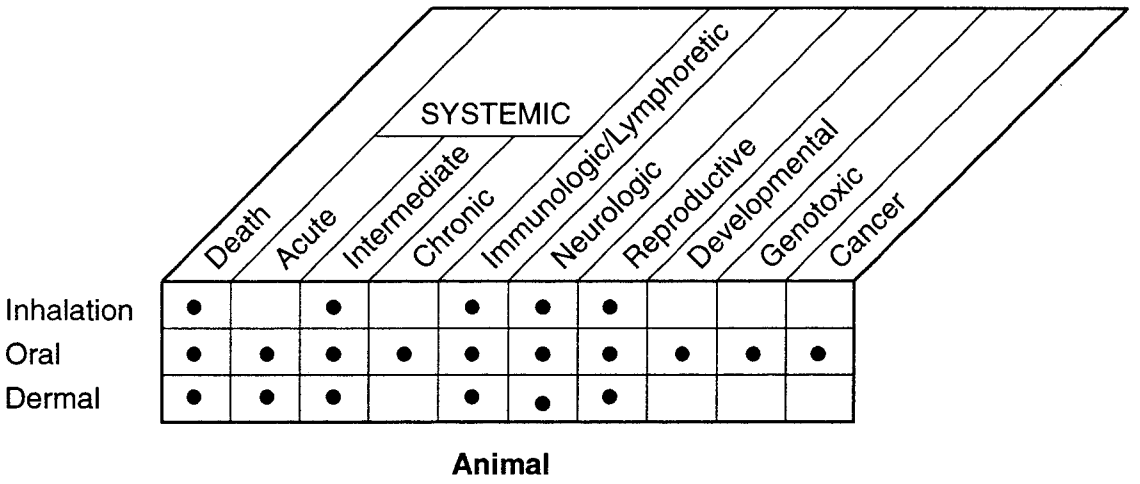
2.9.1 Existing Information on Health Effects of Disulfoton

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to disulfoton are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of disulfoton. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989) is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap

FIGURE 2-4. Existing Information on Health Effects of Disulfoton



Human



Animal

● Existing Studies

more broadly as any substance-specific information missing from the scientific literature.

As seen from Figure 2-4, data exist regarding neurological effects in humans after inhalation exposure to disulfoton. The data for neurological effects were derived from medical evaluations of workers at a pesticide-fertilizer mixing operation. Neurological effects consisted of >22.8% depression in erythrocyte cholinesterase activity after 9 weeks of exposure to disulfoton. Other severe neurological effects were not reported. Systemic and neurological effects were observed in humans after accidental or intentional ingestion of disulfoton. Respiratory and renal effects, more associated with postmortem changes, were reported in a man who died after ingesting disulfoton. Neurological effects consisted of miosis, increased salivation, masseteric spasms, and monoplegia. Epidemiological studies suggest that disulfoton causes myopia in children. Neurological effects were observed in humans after dermal exposure to disulfoton for acute or intermediate durations. Neurological effects after acute exposure included weakness, fatigue, and depressed cholinesterase activity. Intermediate dermal exposure resulted only in erythrocyte cholinesterase depression.

Death and neurological effects were observed in animals after acute- and intermediate-duration inhalation exposure to disulfoton. Intermediate-duration inhalation studies that investigated systemic end points, effects in lymphoreticular organs, and effects in reproductive organs were conducted in rats. Effects consisted of cholinergic signs of disulfoton toxicity, depressed erythrocyte and brain cholinesterase activity, inflammation in respiratory organs, increased adrenal weight, hematological effects, and bone marrow changes. Death, systemic effects of acute-, intermediate-, and chronicduration exposure, lymphoreticular, neurological, developmental, reproductive, genotoxic, and carcinogenic effects were investigated in animals after oral exposure to disulfoton. Systemic effects consisted of respiratory, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, and body weight effects. Ocular effects consisted of myopia development in dogs and cornea1 neovascularization in rats after chronic exposure to disulfoton. Neurological effects consisted of cholinergic signs of disulfoton toxicity, depressed erythrocyte and brain acetylcholinesterase activity, and pathology of the optic nerve and retina. Reproductive effects were observed in male and female rats, and fetotoxicity was reported in several developmental studies. Mostly negative results were obtained for genotoxicity, and negative results were found in carcinogenicity studies. Death and cholinergic toxicity were the only reliable effects reported in animal studies regarding acute dermal exposure to disulfoton. Rabbits that died showed gross effects in the lungs, stomach, liver, kidney,

and spleen upon necropsy. An intermediate-duration dermal study examined all systemic end points, effects in lymphoreticular and reproductive organs, and neurological effects.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. No studies were located regarding systemic effects in humans after inhalation or dermal exposure to disulfoton for acute durations. Acute oral ingestion by a man resulted in death, and respiratory and renal damage were found at autopsy performed at least 24 hours after death (Hattori et al. 1982). No studies were located regarding systemic effects in animals after inhalation exposure for acute durations. Information regarding systemic effects after dermal exposure is limited to reports of breathing difficulties in rats (Mihail 1978), and degenerative changes in heart, liver, kidney, and skin of rats, rabbits, and cats (Kundiev and Rappoport 1967). However, these studies were not very reliable. In addition, gross effects in the lungs, stomach, liver, kidney, and spleen were observed on necropsy of rabbits that died from cholinergic toxicity after a few dermal doses in a 3-week study (Flucke 1986). Acute inhalation lethality data are available for rats and mice (Doull 1957; DuBois 1971; Thyssen 1978, 1980); acute oral LD_{50} values are available for rats (Bombinski and DuBois 1958; Crawford and Anderson 1974; Gaines 1969; Mihail 1978; Pawar and Fawade 1978), mice (Mihail 1978; Pawar and Fawade 1978; Stevens et al. 1972a), and guinea pigs; (Bombinski and DuBois 1958; Crawford and Anderson 1973); acute dermal lethality data are available for rabbits (Flucke 1986); and dermal LD_{50} values are available for rats (DuBois 1957; Gaines 1969; Mihail 1969). Acute oral studies in animals have identified the liver as a possible target organ, evidenced by induction or inhibition of liver enzymes (Stevens et al. 1972a, 1972b, 1973) and lipid peroxidation (Fawade and Pawar 1978). Weight loss in animals also resulted from acute oral exposure to disulfoton (Costa et al. 1984, 1986; Schwab and Murphy 1981; Schwab et al. 1981, 1983). An acute inhalation MRL of 0.006 mg/ m^3 was derived based on a NOAEL for acetylcholinesterase inhibition in rats (Thyssen 1978). An acute oral MRL of 0.001 mg/kg/day was derived from animal data for acetylcholinesterase inhibition in rat dams exposed during gestation (Lamb and Hixson 1983). Acute-duration inhalation, oral, and dermal animal studies that perform comprehensive histological examination and look for other sensitive effects using several exposure concentrations or doses will provide dose-response data on possible end points other than neurological effects. Attempts to identify other target organs in animals after acute exposure will increase the weight of evidence that neurological effects (cholinesterase inhibition) are the most sensitive end points. This information is

important because there are populations surrounding hazardous waste sites that might be exposed to disulfoton for brief periods of time.

Intermediate-Duration Exposure. No studies were located regarding systemic effects in humans after inhalation, oral, or dermal exposure to disulfoton for intermediate durations. Intermediateduration inhalation studies in rats using several exposure concentrations and that included clinical chemistry, urinalysis, hematology, and comprehensive gross and histological examinations have been conducted (Shiotsuka 1989; Thyssen 1980). Inflammation in respiratory tissues, increased adrenal weight, decreased spleen weight, decreased lymphocytes, body weight gain reductions, bone marrow changes, and neurological effects were identified. An intermediate-duration inhalation MRL of $2x10^{-4}$ mg/ m^3 was derived based on a NOAEL for cholinergic effects and acetylcholinesterase inhibition in rats (Thyssen 1980). Intermediate-duration oral studies that used several dietary concentrations and conducted clinical chemistry, urinalysis, hematology, and performed comprehensive gross and histological examination in rats (Klotzsche 1972) and mice (Rivett et al. 1972) have been conducted. The liver was identified as a possible target organ, evidenced by a slight increase in liver weight in female mice (Rivett et al. 1972). Body weight gain reduction, in addition to neurological effects, also resulted from intermediate oral exposure in animals (Hixson and Hathaway 1986; Robinson et al. 1978; Schwab and Murphy 1981; Stavinoha et al. 1969). An intermediate-duration oral MRL of $9x10^{-5}$ mg/kg/day was derived based on a NOAEL for acetylcholinesterase inhibition in rat pups (Hixson and Hathaway 1986). A 3-week dermal study that used several dose levels and conducted clinical chemistry, urinalysis, hematology, and performed comprehensive gross and histological examination has been conducted on rabbits (Flucke 1986). No effects other than erythrocyte cholinesterase inhibition were found at 1.6 mg/kg/day, but the next higher dose of 6.5 mg/kg/day resulted in 100% deaths. A dermal study that uses doses between these extremes might provide dose-response information on systemic end points. Additional intermediate-duration animal studies by the oral and inhalation routes of exposure using doses intermediate between NOAEL values for effects other than cholinesterase inhibition and doses associated with death and severe neurotoxicity may be helpful for identifying thresholds for systemic effects. This information is important because there are populations surrounding hazardous waste sites that might be exposed to disulfoton for intermediate periods of time.

Chronic-Duration Exposure and Cancer. No studies were located regarding systemic effects in humans or animals after inhalation or dermal exposure to disulfoton for chronic durations. Results

from an epidemiological study suggest that oral exposure to disulfoton caused myopia in children (Ishikawa and Miyata 1980). Clinical evidence of myopia in dogs (Ishikawa and Miyata 1980; Suzuki and Ishikawa 1974) and histopathology of the ciliary muscles in the eyes of dogs (Ishikawa and Miyata 1980) were observed after chronic oral exposure. Another 2-year oral study was conducted in dogs, in which clinical chemistry, hematology, and gross and comprehensive histological examinations, including ophthalmology were performed; no effects for any systemic end points, including ocular, were found (Hoffman et al. 1975). The doses in this study were about 5 times lower than the doses that caused myopia and effects in the ciliary muscles. In addition to the 2-year oral studies in dogs, chronic oral studies have been conducted in rats (Carpy et al. 1975; Hayes 1985) and mice (Hayes 1983). These studies also used several dose levels and conducted clinical chemistry, hematology, urinalysis and gross and comprehensive histology. No systemic end points were found in the mice. One study in rats identified the liver and the kidney as possible target organs, suggested by an increase in liver and kidney weights in male rats (Carpy et al. 1975). The other study in rats found granulomatous and suppurative inflammation in the lungs, which may have been due to aspiration of the food particles; mucosal hyperplasia and chronic inflammation of the forestomach, skeletal muscle atrophy (due to debilitation); pancreatic atrophy; dermal lesions; and decreased body weight gain (Hayes 1985). In addition, ocular effects consisting of cystic degeneration of the Harderian gland and cornea1 neovascularization were observed. The chronic oral dietary studies in rats (Carpy et al. 1975; Hayes 1985), mice (Hayes 1983), and dogs (Hoffman et al. 1975) established LOAEL and NOAEL values for acetylcholinesterase inhibition. A chronic inhalation MRL was not derived because no studies were located. A chronic oral MRL of $6x10^{-5}$ mg/kg/day was derived based on a LOAEL for erythrocyte and brain cholinesterase inhibition in female rats in the study by Hayes (1985). Welldesigned chronic-duration inhalation and dermal studies in rats and mice, in which several dose levels are used and comprehensive end points are examined, might identify systemic target organs for these routes and establish dose-response relationships. Although cholinesterase inhibition is the most sensitive end point, additional chronic dietary studies in mice and dogs could be conducted that use doses to establish LOAEL and NOAEL values for end points other than cholinesterase inhibition, that is for systemic target organs. This information is important because there are populations surrounding hazardous waste sites that might be exposed to disulfoton for long periods of time.

No studies were located regarding cancer in humans after inhalation, oral, or dermal exposure to disulfoton or in animals after inhalation or dermal exposure. A 13-week inhalation study in rats reported that comprehensive histological examination of organs and tissues revealed no treatment-

related neoplastic lesions (Shiotsuka 1989); however, a chronic-duration inhalation study would be more appropriate to assess possible carcinogenicity. Carcinogenicity was not observed in rats (Carpy et al. 1975; Hayes 1985), mice (Hayes 1983) or dogs (Hoffman et al. 1975) that had been fed a diet of disulfoton for 1.5-2.0 years. Although there is little reason to suspect that disulfoton is carcinogenic, chronic inhalation, oral, or dermal studies should include comprehensive histology to confirm that disulfoton is not carcinogenic.

Genotoxicity. Disulfoton has been tested in numerous types of assays for genotoxicity. Disulfoton was positive for unscheduled DNA synthesis in human lung fibroblasts (EPA 1984a; Sandhu et al. 1985) and for growth inhibition in human HeLa cells (Litterst et al. 1969). Disulfoton was negative in assays for chromosomal aberrations in human hematopoietic cell lines (Huang 1973) and for alterations in DNA or RNA synthesis in human HeLa cells (Litterst et al. 1969). Disulfoton did not induce micronuclei in mice exposed orally or intraperitoneally (EPA 1984a; Herbold 1981; Sandhu et al. 1985), dominant lethality in mice exposed orally (Herbold 1980), or sex-linked recessive lethal mutation in *D. melunogaster* (EPA 1981a; Waters et al. 1981). Mostly negative results were obtained in in vitro tests in bacteria and yeast (Brusick 1981; EPA 1980a, 1984a; Herbold 1983; Jagannath 1981; Moriya et al. 1983; Sandhu et al. 1985; Waters et al. 1981, 1982), but a few positive results were obtained in some strains of *S. typhimurium* and *E. coli* (Hanna and Dyer 1975; Moriya et al. 1983; Shirasu et al. 1982, 1984). Disulfoton also produced positive results in several assays in barley (Murty et al. 1983; Panda 1983; Singh et al. 1977). Some positive and some negative results have been obtained in cultured mammalian cells. Positive results were found for sister chromatid exchange in Chinese hamster ovary cells in some studies (EPA 1984a; Putnam 1987; Sandhu et al. 1985; Waters et al. 1981, 1982), but negative results were obtained in another study (Chen et al. 1981, 1982). Negative results were also obtained for HGPRT mutations in Chinese hamster ovary cells (Yang 1988). Disulfoton was positive for forward mutation in mouse lymphoma cells (EPA 1984a; Sandhu et al. 1985; Waters et al. 1981, 1982). Although most assays were negative, the few positive results suggest a genotoxic potential. However, it is doubtful that additional testing would add meaningful data.

Reproductive Toxicity. No studies were located regarding reproductive effects in humans after inhalation, oral, or dermal exposure to disulfoton. Disulfoton did not affect male fertility in mice in an oral dominant lethal study (Herbold 1980). Slightly reduced litter sizes in third generations were the findings of a three-generation oral reproductive study in rats (Taylor 1965a). When males and females

were exposed to disulfoton for 60 days prior to and/or during mating, 2 of 5 females failed to become pregnant (Ryan et al. 1970). Although complete gross anatomical and histopathological examinations were lacking, the data do suggest that the male and/or female reproductive system may be affected. A more extensive multigeneration feeding study in rats found decreased reproductive performance of males and females; decreased maternal weight of F_0 and F_1 dams during gestation and lactation; decreased litter counts, viability index; and lactation index; increased dead births and percentage of dead births in both generations; and decreases in F_{2b} litter counts and litter weights (Hixson and Hathaway 1986). However, negative histopathological results were generally obtained from the examination of male and female reproductive systems in rats exposed by inhalation for 3 or 13 weeks (Shiotsuka 1989; Thyssen 1980); in rabbits treated dermally for 3 weeks (Flucke 1986); in rats (Klotzsche 1972) or mice (Rivett et al. 1972) fed disulfoton for 90 days; or in rats (Carpy et al. 1975; Hayes 1985), mice (Hayes 1983), or dogs (Hoffman et al. 1975) fed disulfoton for ≈2 years, with the exception of uterine cystic hyperplasia in female rats fed the high dietary concentration of disulfoton for 2 years (Hayes 1985). Studies on reproductive function in animals exposed by the inhalation or dermal routes will help clarify whether disulfoton affects reproduction by these routes as well as the oral route.

Developmental Toxicity. No studies were located regarding developmental effects in humans after inhalation, oral, or dermal exposure to disulfoton or in animals after inhalation or dermal exposure. Developmental effects have been found in animals after acute- and intermediate-duration oral exposure to disulfoton. Plasma and erythrocyte cholinesterase depression and increased incidences of incomplete ossified parietal bones and sternebrae were observed in fetuses from rats fed disulfoton on days 6-15 of gestation (Lamb and Hixson 1983). Bone and soft tissue malformations were not observed. Effects in fetuses or pups, such as depressed brain cholinesterase activity (Hixson and Hathaway 1986; Ryan et al. 1970), renal and hepatic pathology, and juvenile hypoplasia of testes (Taylor 1965a) were also observed in reproductive studies. However, disulfoton did not cause any fetotoxic effects in the fetuses from pregnant rabbits treated orally with disulfoton during gestation (Tesh et al. 1982). Additional developmental studies involving inhalation or dermal exposure of animals to disulfoton might indicate whether fetotoxic effects are route-dependent.

Immunotoxicity and Lymphoreticular Effects. No studies were located regarding immunological effects in humans after inhalation, oral, or dermal exposure to disulfoton. In two acute animal studies (Costa et al. 1990; Fitzgerald and Costa 1993), repeated intraperitoneal or oral doses of

disulfoton caused a down-regulation of cholinergic muscarinic receptors in lymphocytes. Although the effect on lymphocytes is regarded as a neurological effect, secondary effects due to neuroimmuno interactions are possible and warrant further investigation. After inhalation exposure of rats, inflammatory changes throughout the respiratory tract (associated with bone marrow changes and low percentages of lymphocytes and high percentages of polymorphonuclear leukocytes) and decreased spleen weight were observed (Thyssen 1980). In a chronic dietary study in rats, increased incidence of plasma cell hyperplasia in the mandibular lymph nodes and a significantly increased incidence of splenic lymphoid follicle depletion were observed (Hayes 1985). In other inhalation (Shiotsuka 1989), dietary (Carpy et al. 1975; Hayes 1983; Hoffman et al. 1975; Klotzsche 1972; Rivett et al. 1972), and dermal (Flucke 1986) studies in animals exposed to disulfoton, histological examination of lymphoreticular organs revealed no treatment-related lesions. However, immunological data collected from animals exposed to disulfoton by all three routes for acute, intermediate, or chronic durations might indicate whether disulfoton affects the immune system. A battery of immune function tests would clarify whether disulfoton is an immunotoxicant.

Neurotoxicity. Exposure to disulfoton by the inhalation, oral, or dermal routes has resulted in neurological effects in humans. Disulfoton can cause erythrocyte cholinesterase depression in humans after inhalation exposure without other overt neurological effects (Wolfe et al. 1978). The more overt neurological effects have been observed in humans after oral exposure to disulfoton (Hattori et al. 1982; Yashiki et al. 1990). The clinical signs consisted of muscle tremors and incoordination, increased salivation, pupil miosis, and even death. Weakness and fatigue (Savage et al. 1971) and depressed erythrocyte acetylcholinesterase activity (Wolfe et al. 1978) were observed in humans after dermal exposure to disulfoton. No significant depression in brain, serum, or submaxillary gland cholinesterase activity and no overt signs of neurotoxicity were observed in rats after acute inhalation (5-10 days) exposure (DuBois and Kinoshita 1971). Inhalation, oral, and dermal exposures of animals to disulfoton resulted in signs of cholinergic toxicity (Costa et al. 1984; Doull 1957; Flucke 1986; Mihail 1978; Schwab and Murphy 1981; Schwab et al. 1981, 1983; Shiotsuka 1989; Thyssen 1980). These cholinergic signs were similar to those observed in humans and are associated with inhibition of acetylcholinesterase. Inhibition of brain, erythrocyte, and other tissue acetylcholinesterase activities (Carpy et al. 1975; Christenson and Wahle 1993; Clark and Pearson 1973; Doull and Vaughn 1958; Flucke 1986; Hayes 1983, 1985; Hikita et al. 1973; Hixson and Hathaway 1986; Hoffman et al. 1975; Klotzsche 1972; Robinson et al. 1978; Ryan et al. 1970; Schwab and Murphy 1981; Schwab et al. 1981; Sheets 1993a, 1993b; Shiotsuka 1989; Thyssen 1978, 1980) have been observed in animals in

numerous studies regardless of the route, duration, or animal species. The acute and intermediate inhalation MRLs and the acute, intermediate, and chronic oral MRLs are based on cholinesterase inhibition. Tolerance was also observed in animals exposed to disulfoton for ≥5-10 days (Costa et al. 1984; Schwab and Murphy 1981; Schwab et al. 1981, 1983). Neurobehavioral changes were observed in rats (Clark and Pearson 1973) and mice (Clark et al. 1971) exposed orally for intermediate durations. Necrosis and atrophy of the optic nerve and retina in dogs (Uga et al. 1977) were observed after oral exposure. Based on the results of the human and animal studies, neurological effects (subtle and/or overt) may occur regardless of the route of exposure. Additional animal studies do not seem to be warranted at this time.

Epidemiological and Human Dosimetry Studies. Epidemiological studies are limited. A coincidental increase in the incidence of myopia was observed in young children thought to be orally exposed to disulfoton (Ishikawa and Miyata 1980). Although there is clinical and histopathological evidence from animal studies to support the association between myopia and disulfoton exposure, other neurological effects (i.e., depressed acetylcholinesterase activity) were not reported. Employees exposed to disulfoton by inhalation and dermal routes (Brokopp et al. 1981; Wolfe et al. 1978) did not show overt signs of toxicity, but disulfoton exposure was confirmed in part by depressed cholinesterase activity and/or urinary metabolite identification. These studies are limited because it is not clear whether inhalation or dermal exposure contributed the most to the observed effects. Future human epidemiological studies should look for subtle neurological indicators of exposure (i.e., erythrocyte or lymphocyte acetyl cholinesterase depression) in addition to the ocular effects already described. The data would be useful for establishing cause/effect relationships and for future monitoring of individuals living near hazardous waste sites.

Biomarkers of Exposure and Effect

Exposure. Disulfoton and its metabolites have been detected in the blood and the urine of humans exposed to disulfoton either accidentally or in the workplace (Brokopp et al. 1981; Hattori et al. 1982; Wolfe et al. 1978; Yashiki et al. 1990). Whereas, disulfoton and its metabolites were detected in the blood of humans who ingested unknown quantities of disulfoton (Hattori et al. 1982; Yashiki et al. 1990), urinary metabolites were a better indicator of occupational exposure to the pesticide (Brokopp et al. 1981; Wolfe et al. 1978). Although no animal studies reported the detection of disulfoton or its metabolites in blood, data from animal studies demonstrated that urinary metabolites were a reliable

indicator of disulfoton exposure (Bull 1965; Lee et al. 1985; Puhl and Fredrickson 1975). These animal studies also demonstrated that DEP was a more sensitive urinary biomarker than other metabolites. DEPs are specific for diethyl organophosphates such as disulfoton. A combination of neurological signs is usually a biomarker of disulfoton exposure, as demonstrated in humans (Yashiki et al. 1990) and animals (Schwab et al. 1981). Inhibition of serum cholinesterase and/or erythrocyte acetylcholinesterase are usually reliable biomarkers of exposure in humans (Wolfe et al. 1978; Yashiki et al. 1990). Since blood cholinesterase depression was not observed, but urinary metabolites were detected in exposed employees (Brokopp et al. 1981), urinary metabolites may be a more sensitive biomarker of disulfoton exposure. Urinary metabolites are generally eliminated within 2 weeks after the last exposure and are not usually detected beyond this period. However, cholinesterase depression may be a better biomarker, because the enzyme may remain inhibited for >2 weeks. A recent study showed that T-lymphocyte acetylcholinesterase activity was rapidly and greatly depressed in rats during a 14-day exposure to disulfoton, but rapidly recovered after exposure (Fitzgerald and Costa 1993). Therefore, T-lymphocyte acetylcholinesterase levels could be used as a biomarker of exposure to organophosphorus pesticides during exposure, and could be investigated for use in biomonitoring worker exposure. Animal studies indicate that nonspecific biomarkers of disulfoton exposure may include increased urinary levels of catecholamines (Brzezinski 1969) or their metabolite HMMA (Wysocka-Paruszewska 1971) and increased MFO enzymes (Stevens et al. 1973). No human data were located to support these findings. Although available biomarkers of exposure for disulfoton are nonspecific, it is doubtful that further research will identify a more useful and specific biomarker.

Effect. Disulfoton exposure in humans or animals causes characteristic cholinergic effects such as increased salivation, diarrhea, muscle tremors, and pupillary miosis (Costa et al. 1984; Schwab et al. 1981, 1983; Yashiki et al. 1990). These effects are also associated with exposure to other organophosphates and are, therefore, not specific to disulfoton. Inhibition of erythrocyte acetylcholinesterase can alert the physician to the possibility of more serious neurological effects. In rats, acetylcholinesterase levels in circulating lymphocytes correlated better with brain acetylcholinesterase activity than did erythrocyte cell cholinesterase activities during exposure, but not during recovery after exposure (Fitzgerald and Costa 1993). Thus, lymphocyte acetylcholinesterase activity may be a better biomarker of effect than erythrocyte acetylcholinesterase activity during exposure, but erythrocyte acetylcholinesterase probably remains the better sentinel for brain acetylcholinesterase activity after exposure has ceased. However, other organophosphates and also carbamates can cause this neurological effect. Although animal studies have demonstrated that brain

acetylcholinesterase inhibition is a sensitive indicator of a neurological effect (Carpy et al. 1975), this measurement is not practical in humans. Increased β-glucuronidase activity (Kikuchi et al. 1981) and increased urinary catecholamine levels (Brzezinski 1969) observed in animals may be useful nonspecific biomarkers of effects in humans. There does not appear to be a need for additional biomarkers of effect.

Absorption, Distribution, Metabolism, and Excretion. No studies were located regarding the absorption, distribution, metabolism, and excretion of disulfoton by humans or animals after inhalation or dermal exposure. Limited data exist regarding the absorption, distribution, and excretion after oral exposure to disulfoton. Data on levels of disulfoton and metabolites excreted in urine and expired air suggest that some almost complete absorption of an administered dose of disulfoton over 3-10 days (Lee et al. 1985; Puhl and Fredrickson 1975). The data are limited regarding the relative rate and extent of absorption. Animal data suggest that disulfoton and/or its metabolites are rapidly distributed to the liver, kidney, fat, skin, muscle, and brain, with peak levels occurring within 6 hours (Puhl and Fredrickson 1975). Elimination of disulfoton and metabolites occurs primarily in the urine, with >90% excreted in the urine in 3-10 days (Lee et al. 1985; Puhl and Fredrickson 1975). Evidence further suggests that male rats eliminate disulfoton at a faster rate than females. This difference may be due to differences in absorption, metabolism, retention, excretion, or a combination of factors. The metabolic pathways of disulfoton are relatively well understood based on data from animal studies (Bull 1965; Lee et al. 1985; March et al. 1957; Puhl and Fredrickson 1975). Similar metabolites have been detected in the urine and tissues from humans exposed to disulfoton (Brokopp et al. 1981; Yashiki et al. 1990). One study suggests that a greater percentage of disulfoton sulfoxide is oxidized to demeton S-sulfoxide, rather than disulfoton sulfone to form demeton S-sulfone (Bull 1965). Additional studies in animals, designed to measure the rate and extent of absorption, distribution, and excretion of disulfoton after inhalation or dermal exposure would be useful for predicting the toxicokinetics of disulfoton in humans at an occupational or hazardous waste site.

Comparative Toxicokinetics. The primary target organ for disulfoton in animals and humans is the nervous system. Other organs, such as the liver, are hardly affected. Since there have been no toxicokinetic studies in animals or humans exposed by inhalation or dermal routes, it is impossible to compare animals and humans by these two routes of exposure. Data from occupational studies suggest that disulfoton was absorbed via inhalation and/or dermal routes of exposure (Brokopp et al. 1981; Wolfe et al. 1978); however, the data from these studies on the rate and extent of absorption are

limited. No animal studies were available for comparison. Although the rate and extent of absorption was unknown, disulfoton was readily absorbed by two men as demonstrated in two separate studies (Hattori et al. 1982; Yashiki et al. 1990). In animals, toxicokinetic data are available only in rats exposed by the oral route (Lee et al. 1985; Puhl and Fredrickson 1975). No studies were located regarding the distribution of disulfoton following inhalation or dermal exposure in humans or animals. Although no studies were located regarding the distribution of disulfoton following oral exposure in humans, data from animal studies were located. Disulfoton and its metabolites were detected in the liver, kidney, adipose tissue, muscle, skin, and brain (Puhl and Fredrickson 1975). Data from human (Brokopp et al. 1981; Wolfe et al. 1978; Yashiki et al. 1990), rat (Bull 1965; Lee et al. 1985; Puhl and Fredrickson 1975), and mouse (March et al. 1957) studies indicate that similar metabolic pathways operate in humans and rodents. No studies were located regarding the rate or extent of excretion of disulfoton in humans or animals after inhalation or dermal exposure. Although no studies were located regarding the rate or extent of excretion of disulfoton after oral exposure in humans, limited data for animal studies were located. Data from the only animal study located suggested that most of the disulfoton was eliminated within 3-10 days of exposure and that male rats eliminated disulfoton at a faster rate than females (Lee et al. 1985; Puhl and Fredrickson 1975). With intraperitoneal administration, rats eliminated 28% of the original dose within 48 hours (Bull 1965), and mice eliminated 30-60% of the original dose within 96 hours (March et al. 1957). There appears to be insufficient toxicokinetic data to use as a basis for comparison of animals and humans. Additional studies comparing the rate and extent of absorption, distribution, and elimination in several different animal species after inhalation, oral, and dermal exposure to disulfoton could be useful.

Methods for Reducing Toxic Effects. No data were located regarding the mechanism of absorption via the respiratory tract, gastrointestinal tract, or the skin. Besides moving the exposed individual to a fresh air environment and/or administering oxygen, very little can be done if disulfoton is inhaled (Sittig 1991). Induction of emesis, gastric lavage, and administration of a saline cathartic immediately after oral exposure help reduce absorption. Since diuresis, dialysis, and hemoperfusion have been used to reduce the body burden of other organophosphates (Ellenhorn and Barceloux 1988), it seems reasonable that these methods may also be used to treat disulfoton intoxication. Repeated administration of activated charcoal and cathartics may prevent possible enterohepatic recirculation of the parent compound and its metabolites. The treatment protocol also includes interfering with the known mechanism of action of disulfoton. Atropine is used to block the muscarinic effects of disulfoton (Goldfrank et al. 1990; Yashiki et al. 1990), and an oxime (e.g., 2-PAM) is given to

accelerate the hydrolysis of phosphorylated cholinesterase, which accelerates regeneration of the active cholinesterase (Ellenhorn and Barceloux 1988; Goldfrank et al. 1990; Haddad and Winchester 1990; Morgan 1982). There are numerous treatment protocols that successfully use some or all of the methods described. Therefore, further studies regarding the reduction of toxic effects for disulfoton exposure may not be warranted.

2.9.3 Ongoing Studies

A study by S.C. Soderholm of the University of Rochester is in progress to relate the deposition efficiency of volatile aerosols of disulfoton in humans to physical properties of the airborne system, including exposure concentration, saturation vapor concentration, particle size, and blood/gas partition coefficient (CRISP Database 1994). Disposition efficiency will be determined from laboratory experiments and computer modelling. The results of computer models will be compared to measure disposition efficiencies of disulfoton aerosols in humans. With this information, the inhaled dose in humans can be estimated.

Studies on particular organophosphates are currently being conducted by EPA to substantiate the findings that these organophosphates, including disulfoton, are associated with the development of myopia in humans, as reported in Japanese populations (Dementi 1994).