CHAPTER 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 on 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. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

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 by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to disulfoton, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with exposure to substance x was also conducted; the results of this review are presented in Appendix C.

Animal inhalation studies are presented in Table 2-1 and Figure 2-2, and animal oral studies are presented in Table 2-2 and Figure 2-3; animal dermal data are presented in Table 2-3.

Levels of significant exposure (LSEs) 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 (SLOAELs) 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

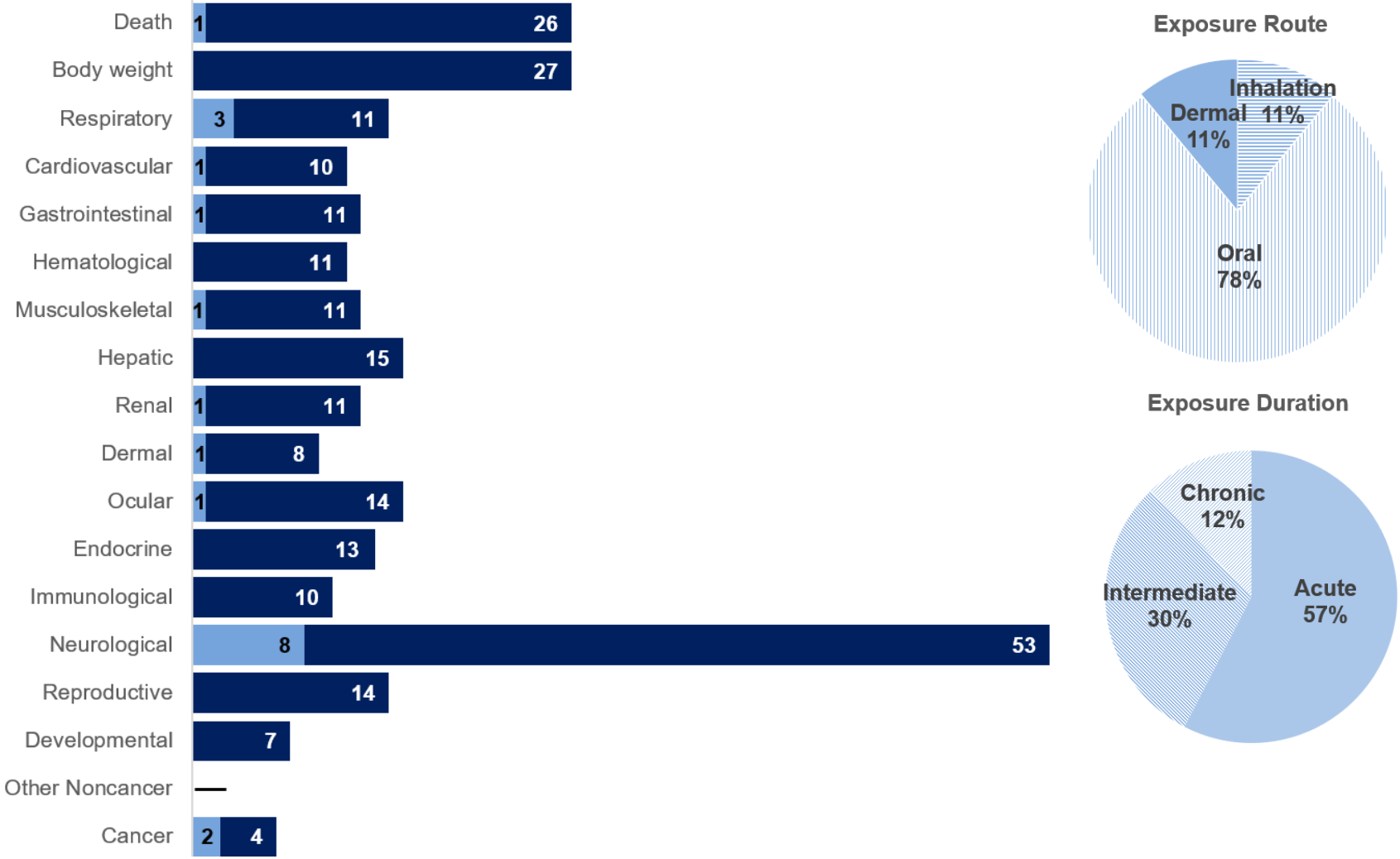
dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there 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 endpoints. 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.

A User's Guide has been provided at the end of this profile (see Appendix D]). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

The health effects of disulfoton have been evaluated in experimental animal studies and a few human occupational studies and case studies. As illustrated in Figure 2-1, most of the health effects data come from oral and inhalation studies in animals. Animal data are available for each exposure route and exposure duration category. Multiple studies evaluating the toxicity of disulfoton have evaluated a suite of endpoints. Neurological effects are the most examined in the literature, followed by death and body weight effects. The most common neurological effect noted is altered AChE activity; typically, ATSDR classifies AChE inhibition between 20 and 59% as a less serious LOAEL and >59% as a serious LOAEL. Cholinesterase inhibition classified as a less serious LOAEL and accompanied by clinical symptoms of cholinergic toxicity may be classified as a serious LOAEL. Human case reports and occupational studies have evaluated and reported the effect of disulfoton exposure on the central nervous system. The genotoxicity of disulfoton has also been examined.

Figure 2-1. Overview of the Number of Studies Examining Disulfoton Health Effects*

Most studies examined the potential neurological and body weight effects of disulfoton, in addition to mortality.
Fewer studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 112 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effects
ACUTE EXPOSURE									
Doull 1957									
1	Rat (Sprague-Dawley) 5 M	0.5–1 hour	65.1, 121.1, 195.1, 202.2, 279.5, 416.0, 433.4	CS LE	Death			202.2	3/5 died
					Neuro			65.1	Muscle twitching and fibrillation, ataxia, salivation, urination, defecation, lacrimation
DuBois and Kinoshita 1971									
2	Rat (Holtzman) 3 F	10 days 1 hour/day	0, 0.14, 0.35, 0.7	BI CS	Neuro	0.7			
DuBois and Kinoshita 1971									
3	Rat (Holtzman) 3 F	5 days 1 hour/day	0, 0.14, 0.35, 0.7	BI CS	Neuro	0.7			
Thyssen 1978									
4	Rat (Wistar) 10–20 M 10–20 F	1 hour	M: 133, 196, 256, 322, 660 F: 27, 33, 46, 58, 80, 133	BC BW CS LE	Death			63 F 290 M	Computed LC_{50}
					Neuro		27 F 133 M		Sluggishness, failure to groom, typical signs of cholinesterase inhibition, not otherwise described
Thyssen 1978									
5	Rat (Wistar) 10–20 M 20–40 F	4 hours	M: 34, 48, 51, 64, 78, 96 F: 3.4, 5, 7, 10, 13, 20	BC BW CS LE	Death			15 F 60 M	Computed LC_{50}
					Neuro	51 M	3.4 F 64 M		Sluggishness, failure to groom, typical signs of cholinesterase inhibition, not otherwise described

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effects
Thyssen 1978									
6	Rat (Wistar) 10 M, 10 F	5 days 4 hours/day	0, 0.5, 1.8, 9.8	BI BW GN LE	Death			9.8 F	9/10 died
					Bd wt	9.8			
					Neuro	0.5		1.8	19–26% depression in RBC AChE activity; unspecified behavioral disorders, sluggish, drowsy
Doull 1957									
7	Mouse (Carworth Farms) 10 F	1 hour	53.4, 58.2, 65.1, 121.1, 195.1	CS LE	Death			53.4	1/10 died
					Neuro			53.4	Muscular twitches and fibrillations, ataxia; salivation, urination, defecation, lacrimation
INTERMEDIATE EXPOSURE									
Shiotsuka 1988									
8	Rat (Fischer-344) 9–10 M, 10 F	3 weeks 5 days/week 6 hours/day	0, 0.006, 0.07, 0.7	BI BW CS LE	Bd wt	0.7			
					Neuro	0.7			
Shiotsuka 1989									
9	Rat (Fischer-344) 12 M, 12 F	13 weeks 5 days/week 6 hours/day	0, 0.018, 0.16, 1.4	BC BI BW CS FI GN HP LE HE OP OW UR	Bd wt	1.4			
					Resp	1.4 F			
						0.16 M	1.4 M		50% increased incidence of inflammation of the nasal turbinates
					Cardio	1.4			
					Gastro	1.4			
					Hemato	1.4			
					Musc/skel	1.4			
					Hepatic	1.4			
					Renal	1.4			
					Dermal	1.4			
					Ocular	1.4			

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effects
					Endocr	1.4			
					Immuno	1.4			
					Neuro	0.16	1.4		14–31% inhibition of plasma AChE, 22–34% inhibition of RBC AChE, 28–29% inhibition of brain ChE
Thyssen 1980									
10	Rat (Wistar TNO/W 74) 10 M	3 weeks 5 days/week 6 hours/day	0, 0.02	BC BI BW CS GN HE HP OW UR	Bd wt	0.02			
					Resp	0.02			
					Cardio	0.02			
					Gastro	0.02			
					Hemato	0.02			
					Hepatic	0.02			
					Renal	0.02			
					Endocr	0.02			
					Immuno	0.02			
					Neuro	0.02			
					Repro	0.02			
Thyssen 1980									
11	Rat (Wistar TNO/W 74) 10 M, 10 F	3 weeks 5 days/week 6 hours/day	0, 0.1, 0.5, 3.7		Death			3.7 F	5/10 died
					Bd wt	0.5 F	3.7 F		11–12% decreased body weight gain
						3.7 M			
					Resp	0.1 F	0.5 F	3.7 F	LOAEL: inflammatory changes in respiratory tract (larynx, trachea, emphysema, peribronchial red cell infiltrates, lungs perivascular red cell infiltrated); serious LOAEL: mottled distended lungs in the rats that died
						3.7 M			
					Cardio	3.7			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effects
					Gastro	0.5		3.7 F	Bloated gastrointestinal tract and ulcer-like foci in the glandular mucosa in rats that died
					Hemato	0.1	0.5		Minimal to definite bone marrow changes concurrent with respiratory inflammatory changes
					Hepatic	3.7			
					Renal	0.5 F	3.7 F		Pale discoloration of kidneys in dead rats
					Endocr	0.5 F	3.7 F		Increased absolute (14%) and relative (21%) adrenal weight
						3.7 M			
					Immuno	3.7			
					Neuro	0.1 F[b]	0.5 F		30% inhibition of brain AChE, lethargy by day 15
						0.5 M	3.7 M		24% inhibition of RBC AChE, 48% inhibition of brain AChE
					Repro	3.7			
Thyssen 1980									
12	Rat (Wistar TNO/W 74) 10–20 F	6 hours/day 5 days/week 3 weeks	0, 0.02, 3.1	BC BI BW CS GN HE HP OW UR	Death			3.1	3/20 died
					Bd wt	3.1			
					Resp	0.02		3.1	Increased inflammatory changes in the respiratory tract (larynx, trachea, emphysema, peribronchial red cell infiltrates, lungs perivascular red cell infiltrated); distended and dark discoloration in lungs of dead rats
					Cardio	3.1			
					Gastro	0.02	3.1		Reddened gastrointestinal tract in dead rat

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effects
					Hemato	0.02	3.1		Low lymphocytes percentage (35–60%) and high polymorpho-nuclear leukocytes percentage (40–62%) in 2/5 rats; reactive bone marrow changes accompanied by inflammatory changes in the respiratory tract
					Hepatic	0.02	3.1		Increased absolute (13%) and relative (20%) liver weight
					Renal	0.02	3.1		Increased relative kidney weight (14%)
					Endocr	0.02	3.1		Increased absolute (10%) and relative (18%) adrenal weight
					Immuno	0.02	3.1		Decreased absolute (18%) and relative (14%) spleen weight
					Neuro	0.02		3.1	Muscle tremors, convulsions, increased salivation, difficulty breathing
					Repro	3.1			

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an intermediate-duration inhalation MRL of 0.0006 mg/m^3; concentration adjusted for intermittent exposure, converted to a human equivalent concentration of 0.018 mg/m^3 and divided by a total uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability). This MRL was also adopted for the acute-duration inhalation MRL; see Appendix A for more detailed information regarding the MRL.

RBC and brain AChE activity are assessed by comparing the activity of exposed groups to study controls and assessing whether AChE was inhibited by the chemical of interest. ATSDR classifies a NOAEL as <20% inhibition; a LOAEL is classified as 20–59% inhibition; and a SLOAEL is classified as >59% inhibition. If AChE activity is inhibited by 20–59% but is accompanied with clinical signs of cholinergic toxicity, it may be classified as a SLOAEL.
Highlighted rows indicate an MRL principal study.

AChE = acetylcholinesterase; BC = blood chemistry; Bd wt or BW = body weight; BH = behavioral; BI = biochemical indices; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); FI = food intake; Gastro = gastrointestinal; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; LC_{50} = concentration producing 50% death; M = male(s); MRL = Minimal Risk Level; Musc/skel = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; OP = ophthalmology; OW = organ weight; RBC = red blood cell; Repro = reproductive; Resp = respiratory; SLOAEL = serious LOAEL; UR = urinalysis

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

Acute (≤14 days)

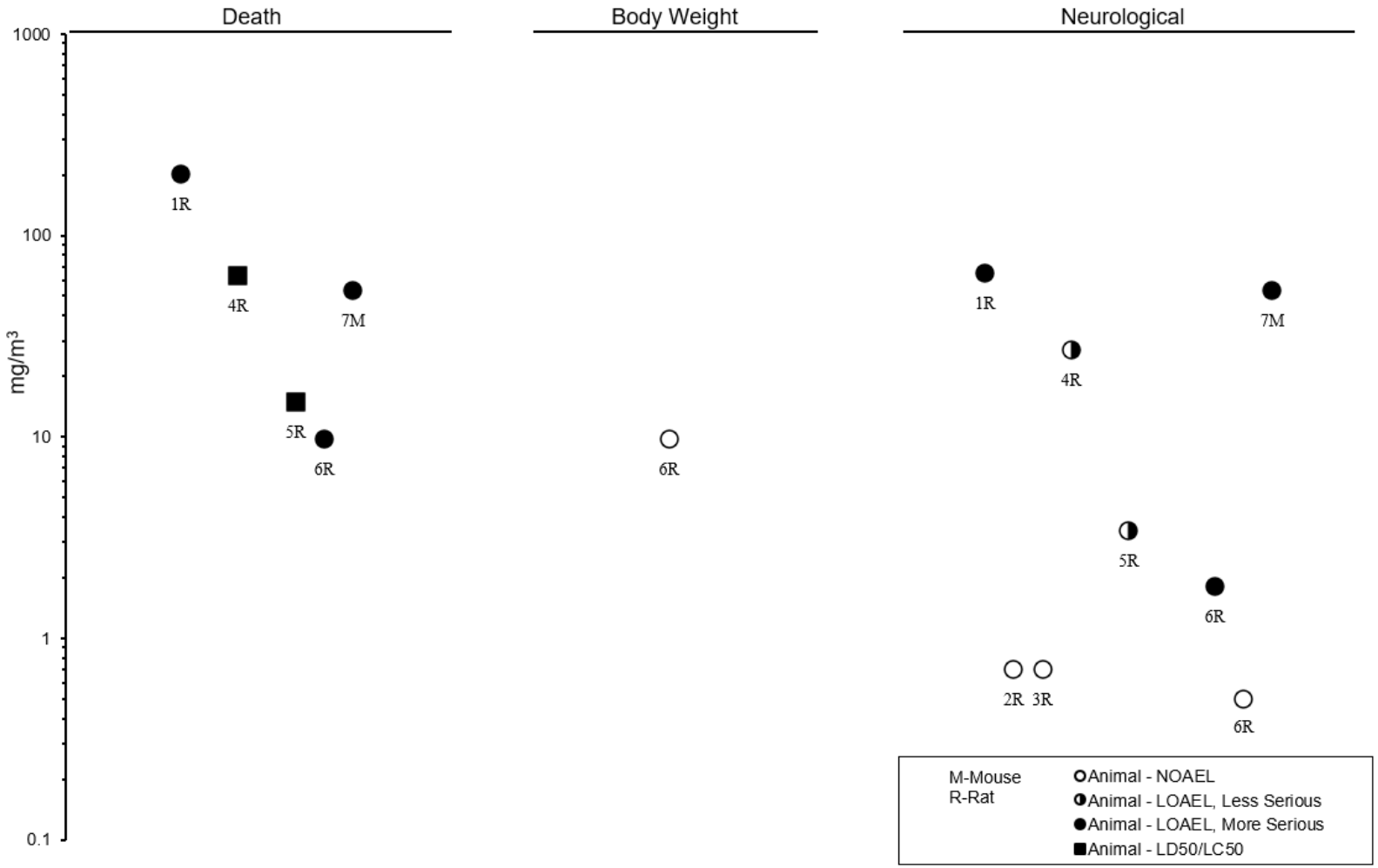

Figure 2-2. Levels of Significant Exposure to Disulfoton– Inhalation
Intermediate (15–364 days)

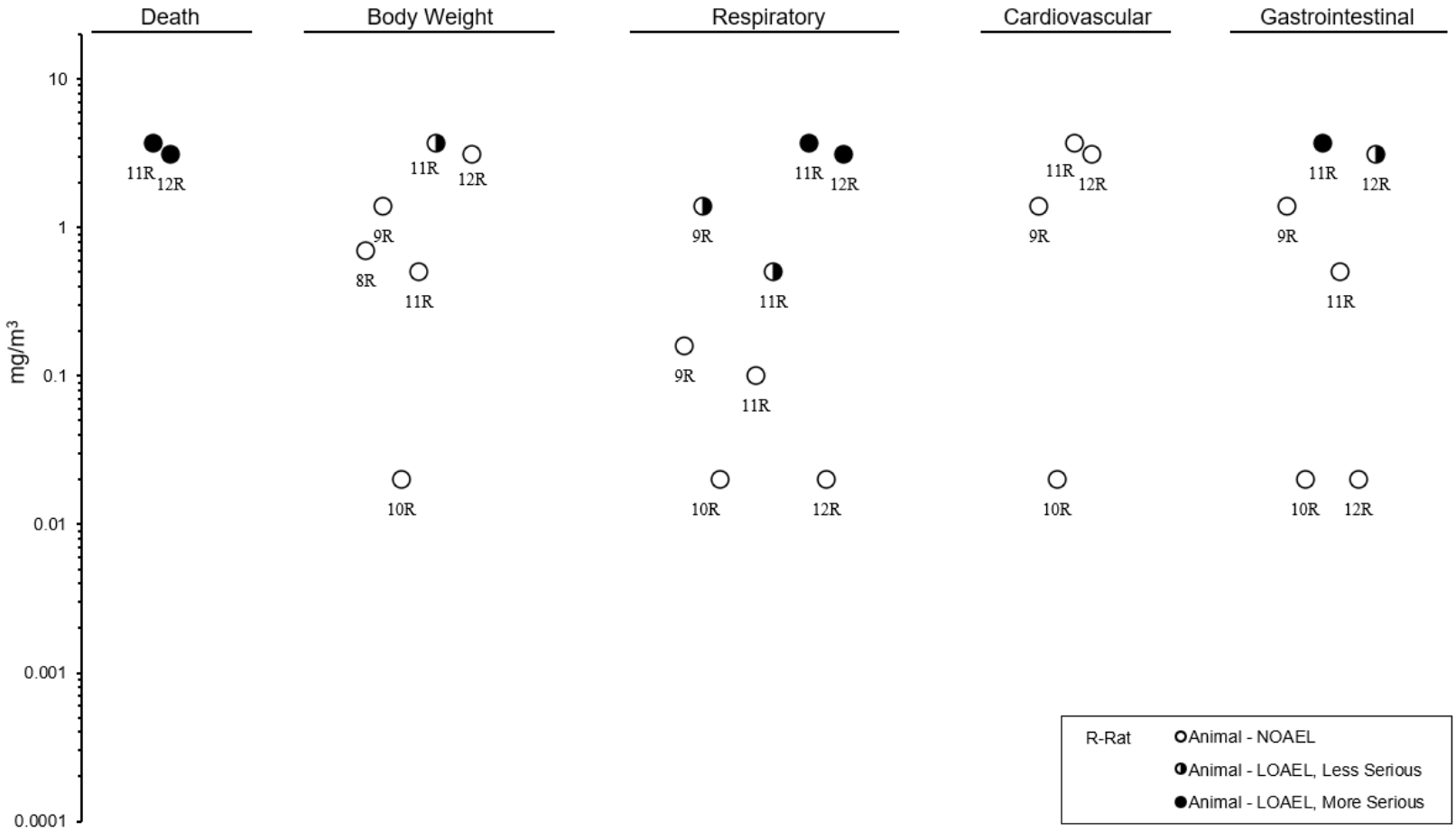

Figure 2-2. Levels of Significant Exposure to Disulfoton– Inhalation
Intermediate (15–364 days)

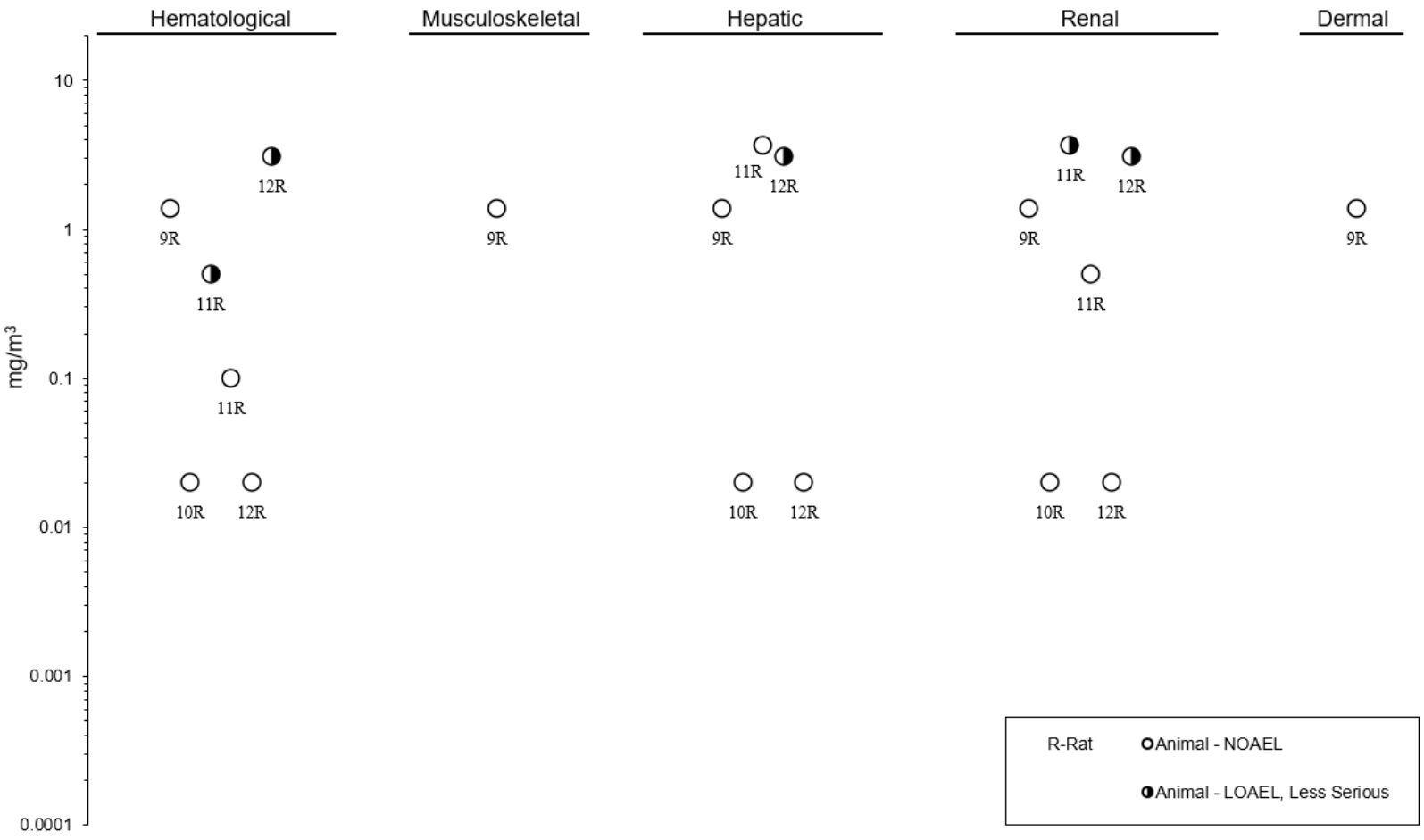

Figure 2-2. Levels of Significant Exposure to Disulfoton– Inhalation

Intermediate (15–364 days)

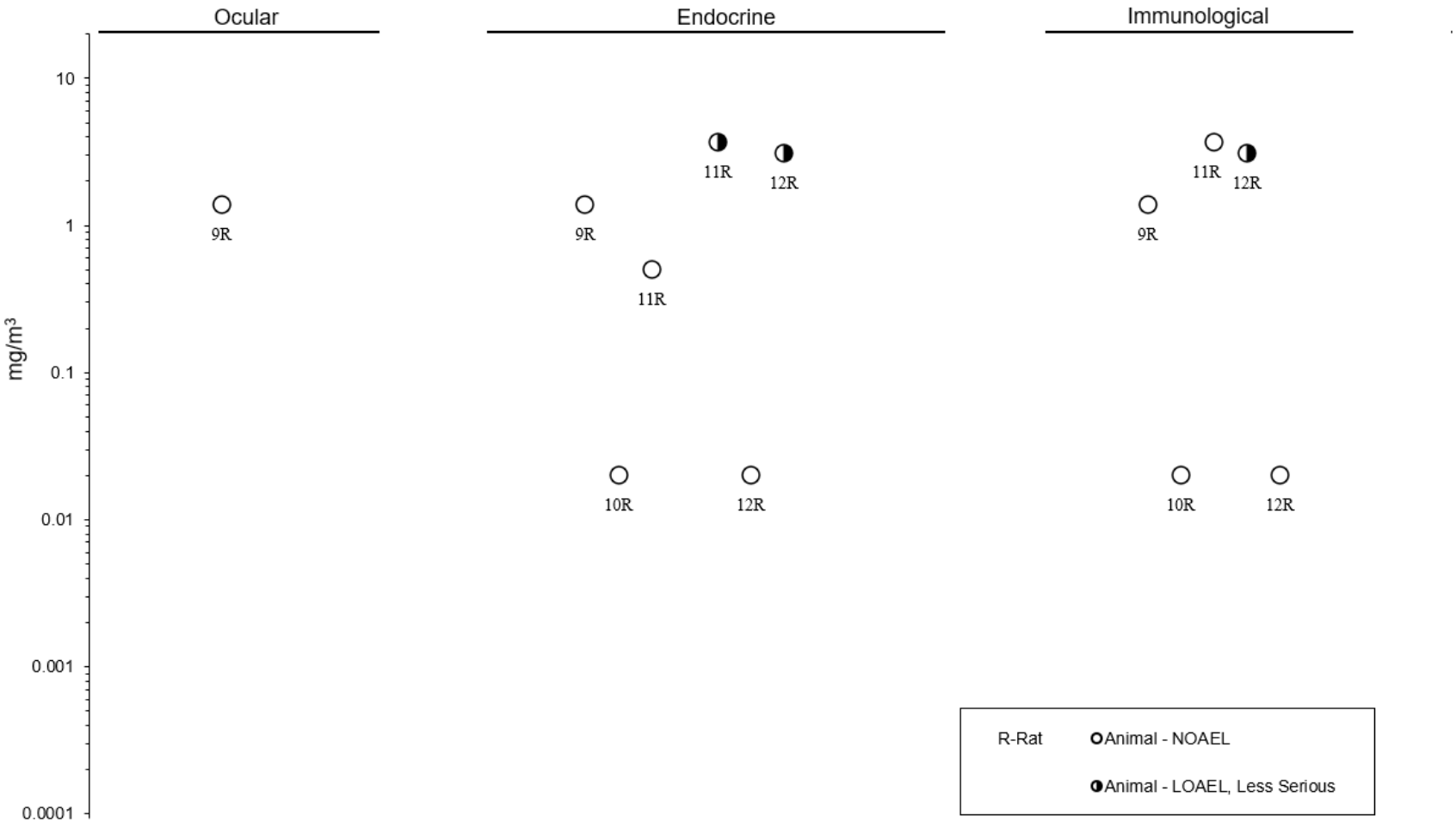

Figure 2-2. Levels of Significant Exposure to Disulfoton– Inhalation
Intermediate (15–364 days)

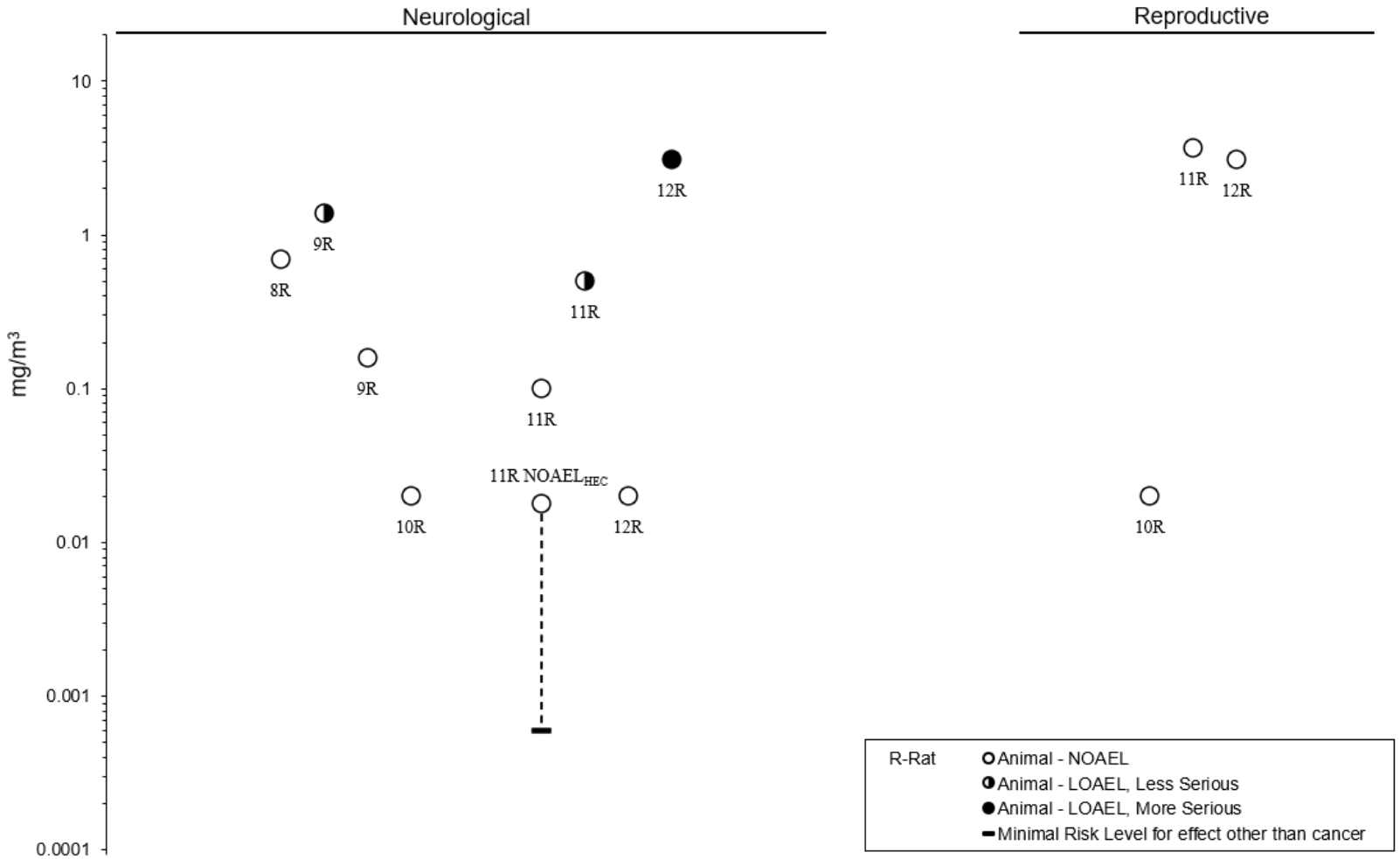

Table 2-2. Levels of Significant Exposure to Disulfoton – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE									
Brzezinski 1969									
1	Rat (Wistar) 5 B	Once (GO)	0, 6.25	BI	Endocr			6.25 F	173 and 313% increase in urinary noradrenaline and adrenaline levels, respectively
Brzezinski 1973									
2	Rat (Wistar) 14–25 M	Once (GO)	0, 5	BI	Endocr			5	Increase in urinary adrenaline (238%) and noradrenaline (61%) 1 day after exposure
Costa and Murphy 1983a									
3	Rat (Sprague-Dawley) 5–14 M	10 days (GO)	0, 2	BI	Neuro			2	89% inhibition of brain AChE activity
Costa et al. 1984									
4	Rat (Sprague-Dawley) 3–10 M	10 days (GO)	0, 2	BI BW CS OW	Bd wt			2	32% reduction in weight gain
					Neuro			2	50% reduction in pancreatic AChE activity, salivation, lacrimation, diarrhea
Costa et al. 1986									
5	Rat (Sprague-Dawley) 10 M	10 days (GO)	0, 2	BI BW	Bd wt			2	50% reduced body weight gain
					Neuro			2	Decreased density of muscarinic receptors in cerebral cortex; 84% inhibition of brain AChE
Crawford and Anderson 1974									
6	Rat (NS) 4 M, 4 F	Once (GW)	M: 2.0 F: 0.5, 1.0, 2.0, 4.0	CS LE	Death			2 F	2/4 died
					Neuro			0.5 F	Tremors

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
EPA 2007									
7	Rat (Wistar) 6 M, 6 F	Once (G)	M: 1.5 F: 0, 0.75	CS LE NX	Neuro			0.75 F	65% inhibition of RBC AChE 8 hours post-dosing
							1.5 M		40–51% inhibition of RBC AChE and 27–42% inhibition of brain AChE 4, 6, and 8 hours post-dosing
EPA 2007									
8	Rat (Wistar) 10 M, 10 F	Once (G)	0, 0.5	CS LE NX	Neuro		0.5		50–56% inhibition of RBC AChE and 26–56% inhibition of brain AChE activity 24 hours post-dosing
EPA 2007									
9	Rat (Wistar) 6 M, 6 F	Once (G)	0, 0.25, 0.75, 1.5 (M) 0, 0.25, 0.5, 0.75 (F)	CS LE NX	Neuro	0.25 F	0.5 F	0.75 F	34% inhibition RBC AChE activity at 0.5 mg/kg/day; 70% inhibition of RBC AChE activity at 0.75 mg/kg/day
						0.75 M	1.5 M		46% inhibition of RBC AChE activity and 32% inhibition of brain AChE activity
EPA 2007									
10	Rat (Wistar) 10 F, 10 M	Once (G)	0, 0.125, 0.25, 0.5	CS LE NX	Neuro	0.125 F	0.25 F		22% inhibition of RBC AChE activity and 19% inhibition of brain AChE
						0.25 M	0.5 M		53% inhibition of RBC AChE activity and 39% inhibition of brain AChE
Fawade and Pawar 1983									
11	Rat (Hindustan antibiotics) 8 M	Once (GO)	0, 2	BI	Hepatic		2		Increased lipid peroxidation (20–32%)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Fitzgerald and Costa 1992									
12	Rat (Long-Evans) 4 M	14 days (GO)	0, 2	BW CS	Bd wt		2		Temporary but significant (p<0.025) decreased body weight gain at day 3 with recovery by days 5–6
					Neuro			2	Decrease in muscarinic cholinergic receptors in cortex (22%), medulla pons (11%), hippocampus (17%), striatum (30%)
Fitzgerald and Costa 1993									
13	Rat (Long-Evans) 4–7 M	1–2 weeks 7 days/week (GO)	0, 2	BI BW CS	Bd wt		2		Significantly (p<0.025) reduced body weight gain beginning at day 3 with recovery by days 5–6
					Neuro			2	60–84% decrease in brain AChE activity, diarrhea, flaccidity, malaise
Klaus 2006a									
14	Rat (Wistar) 6 M, 6 F	11 days (G)	0, 0.25, 0.5, 1.0 (M); 0, 0.125, 0.25, 0.5 (F)	BX BW CS LE NX	Bd wt	0.5 F 1 M			
					Neuro	0.125 F	0.25 F	0.5 F	28% inhibition of RBC AChE activity and 33% inhibition of brain AChE activity at 0.25 mg/kg/day; 63% inhibition of RBC AChE activity and 70% inhibition of brain AChE activity at 0.5 mg/kg/day
						0.25 M	0.5 M	1 M	38% inhibition of RBC AChE activity and 39% inhibition of brain AChE activity at 0.5 mg/kg/day; 72% inhibition of RBC AChE activity and 70% inhibition of brain AChE activity at 1 mg/kg/day

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Klaus 2006b									
15	Rat (Wistar) 10 M, 10 F	11 days PNDs 11–21 (G)	0, 0.06, 0.125, 0.25	CS LE NX	Neuro		0.06 F[b]		29% inhibition of RBC AChE activity ($BMDL_{20RD}$=0.028 mg/kg/day)
						0.06 M	0.125 M		23% inhibition of brain AChE activity, 19% of RBC AChE activity
Lamb and Hixson 1983									
16	Rat (CD) 25 F	10 days GDs 6–15 (G)	0, 0.1, 0.3, 1.0	CS BI DX FX	Neuro	0.1	0.3	1	41% inhibition of plasma and RBC AChE activity in dams at 0.3 mg/kg/day; 82–90% inhibition of plasma and RBC AChE activity in dams at 1 mg/kg/day
					Develop	0.3	1		Delayed ossification of parietal bones and sternebrae
Matsuda et al. 2000									
17	Rat (Wistar albino) NR/M	Once (GO)	6	BC HP	Neuro			6	75 and 69% inhibition of AChE in whole blood and skeletal muscle, respectively
Mihail 1978									
18	Rat (Wistar) 15 M,15 F	Once (GO)	M: 1, 4, 4.5, 5, 6, 7.5, 9, 10 F: 0.5, 1, 1.25, 1.5, 2, 2.5, 5	CS GN LE	Death			1.9 F 6.2 M	Computed LD_{50}
					Resp	0.5 F	1		Dyspnea up to 8 days post-treatment
					Neuro	0.5 F 1 M		1 F 4 M	Muscle twitching cramps, salivation
Pawar and Fawade 1978									
19	Rat (Hindustan antibiotics) NS B	Once (G)	0, 1-12.3	CS LE	Death			3.2 F 7.2 M	LD_{50}

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Schwab and Murphy 1981									
20	Rat (Holtzman) 50 F	9 days (F)	0, 0.38, 1.0	BI BW CS	Bd wt	0.38	1		10–12% reduced body weight gain at all weighing times
					Neuro			0.38	60–65% inhibition of brain AChE after <15-day exposure
Schwab et al. 1981									
21	Rat (Sprague-Dawley) 5–10 M	3 days a time for 1–23 days (GO)	0, 2, 2.5, 3, 3.5	BI BW CS LE	Death			3.5	Three of five unpretreated rats died after three doses
					Bd wt			2	21% reduced body weight
					Neuro			2	Exophthalmia, salivation, excessive urination and defecation, tremors
Schwab et al. 1983									
22	Rat (Sprague-Dawley) 3–5 M	1–10 days (GO)	0, 2.0	BI BW CS	Bd wt		2		Weight loss not otherwise specified
					Neuro			2	Salivation, lacrimation, excessive urination and diarrhea, fasciculations, tremors, 15–51% inhibition of ileal AChE activity
Sheets 1993a									
23	Rat (Sprague-Dawley) 10 M, 9–10 F	Once (GO)	M: 0,0.24, 1.5, 5.2 F: 0, 0.24, 0.76, 1.5	BI BW CS GN HP LE OW	Death			1.5 F	1/10 died from acute cholinergic intoxication
					Musc/skel	5.2			
					Ocular	5.2			
					Neuro	0.24 F		0.76 F	Muscle fasciculations, decreased vocalization, minimal head or body movement, 53% decrease in RBC AChE activity
						0.24 M		1.5 M	Muscle fasciculations, tremors, minimal head or body movement

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Su et al. 1971									
24	Rat (Holtzman) 6–12 F	1 week (F)	0, 0.05, 0.25, 1.25	BI	Neuro	0.05	0.25		50% inhibition of brain AChE activity
Wysocka- Paruszewska 1971									
25	Rat (Wistar) 4 M, 4 F	Once (GO)	M: 0, 1.25, 2.5, 3.75, 5 F: 0, 0.26, 0.52, 0.78, 1.04	BI	Endocr	0.26 F	0.52 F		Increased excretion of 4-hydroxy-3-methoxy-mandelic acid in urine (27.8–32%)
							1.25 M		Increased excretion of 4-hydroxy-3-methoxy-mandelic acid in urine (51% after 2 days)
Yagle and Costa 1996									
26	Rat (Sprague-Dawley) 34 M	14 days (GO)	0, 2	BC BW CS HP	Bd wt		2		3-8% lower body weight
					Gastro		2		Diarrhea in 5/34 rats
					Neuro			2	81% decrease in AChE activity
Fawade and Pawar 1978									
27	Mouse (Hindustan antibiotics) 8 M	2–4 days (GO)	0, 0.5, 1, 1.5, 2	BI	Hepatic		0.5		Increased lipid peroxidation (9%)
Fawade and Pawar 1980									
28	Mouse (Hindustan antibiotics) 8 M	Daily 3 days (GO)	0, 1	BI	Hepatic		1		Increased lipid peroxidation (34–58%)
Herbold 1980									
29	Mouse (NMRI/ ORIG) 50 M	Once (GO)	0, 5	CS RX	Repro	5			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Mihail 1978									
30	Mouse (MMRI) 15 M,1 5F	Once (GO)	M: 2.5, 5, 6, 7, 8, 10; F: 2.5, 5, 6.5, 7.5, 10, 11	CS LE	Death			8.2 F 7 M	Computed LD_{50}
					Resp	2.5	5		Acute dyspnea
					Neuro	2.5		5	Muscle twitches, clonic cramps, salivation
Pawar and Fawade 1978									
31	Mouse (Hindustan antibiotics) NS B	Once (G)	0, 1–10	CS LE	Death			2.7 F 5.8 M	LD_{50}
Schafer and Bowles 1985									
32	Mouse (wild deer mouse) 1–6 NS	Once (G)	NR	CS LE	Death			18	Mortality of an unspecified number of mice
Stevens et al. 1972a									
33	Mouse (Swiss-Webster) NS M	Once (GO)	19.3	CS LE	Death			19.3	LD_{50}
Stevens et al. 1972b									
34	Mouse (Swiss) 6–20 M	1–10 days (GO)	0, 2.4, 4.9, 9.6	LE	Death			9.6	2/8 died
					Hepatic	4.9	9.6		Significant shortening of the hexobarbital sleeping time
Crawford and Anderson 1973									
35	Guinea pig (NS) 3M, 3F	Once (GW)	M: 2.5, 5, 10, 20; F: 4,0, 8, 16, 32	CS LE	Death			12.7 F 8.9 M	LD_{50}
Mihail 1978									
36	Dog (Beagle) 1–2 F	Once (GO)	1, 2, 5, 10	CS LE	Death			5	Computed LD_{50}

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Tesh et al. 1982									
37	Rabbit (New Zealand) 14–22 F	13 days GDs 6–18 (G)	0, 0.3, 1, 1.5, 2, 3	BW DX	Bd wt	3			
					Develop	1.5			
INTERMEDIATE EXPOSURE									
Christenson and Wahle 1993									
38	Rat (Fischer-344) 35 M, 35 F	6 months (F)	M: 0, 0.02, 0.03, 0.06 F: 0, 0.02, 0.03, 0.07	BI BW CS FI LE	Bd wt	0.07			
					Neuro	0.03 F	0.07 F		22–29% inhibition in RBC AChE activity
						0.06 M			
Clark and Pearson 1973									
39	Rat (Charles River) 10 M	3 months (F)	0, 0.5, 1.25, 2.5	CS NX	Neuro		0.5		59% inhibition of brain AChE activity
Clark and Stavinoha 1971									
40	Rat (NS) NS	2 months (F)	0, 2.5	HP	Neuro		2.5		Increased permeability of CNS tissue
Hayes 1985									
41	Rat (Fischer 344) 50 M, 50 F	3-6 months (F)	M: 0, 0.05, 0.18, 0.75; F: 0, 0.06, 0.21, 1.02	BC BI BW CS FI GN HE HP OW UR	Neuro		0.06 F	0.21 F	14–22% inhibition of RBC AChE at 0.06 mg/kg/day; 68–69% inhibition of RBC AChE at 0.21 mg/kg/day
						0.05 M	0.18 M	0.75 M	14–22% inhibition of RBC AChE at 0.18 mg/kg/day; 68–69% inhibition of RBC AChE at 0.75 mg/kg/day
Hixson and Hathaway 1986									
42	Rat (Sprague-Dawley) 26 M, 26 F	F0: 15 weeks premating; F1b: 13 weeks premating and through pregnancy (F)	0, 0.009, 0.03, 0.09	CS BI BW FI DX GN HP	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
					Neuro	0.03 F		0.09 F	Tremor in the F0 females during the production of the F1 generation
						0.03 M			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Repro	0.009	0.03	0.09	Decreased F2b litter live births (25%) compared to control and decreased litter weights through GD 21 (30% change, compared to control at 8%) at 0.03 mg/kg/day; decreased sperm-positive F0 (21–33%) and F1 females (22-33%); decreased maternal F1 weight during gestation (8–12%) and lactation (10–12%), and maternal F0 weight during lactation (4–8%) compared to control; decreased litter counts, viability and lactation indices and increased stillbirths among all litters at 0.09 mg/kg/day
					Develop	0.009[c]	0.03		24–32% inhibition of brain AChE activity in F1 generation pups
Klaus 2006c									
43	Rat (Wistar) 13 F	21 days GDs 0–20 (F)	0, 0.042, 0.168, 0.694	BC BW CS FI LE NX	Bd wt	0.694			
					Neuro	0.042	0.168	0.694	44% inhibition RBC AChE activity and 32% inhibition of brain AChE activity at 0.0168 mg/kg/day; 90% inhibition of RBC AChE activity and 85% inhibition of brain AChE activity at 0.694 mg/kg/day
Klaus 2006c									
44	Rat (Wistar) NS	Maternal exposure on GDs 0–20	0, 0.042, 0.168, 0.694	BC	Develop	0.042	0.168	0.694	20% inhibition of RBC AChE activity in pups at 0.168 mg/kg/day; 83% inhibition of RBC AChE activity in pups at 0.694 mg/kg/day

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Klotzsche 1972									
45	Rat (Wistar) 25 M, 25 F	90 days (F)	M:0, 0.01, 0.07, 0.34 F: 0, 0.02, 0.11, 0.55	BC BI BW CS FI GN HE HP UR	Bd wt	0.55 F			
						0.34 M			
					Resp	0.55 F			
						0.34 M			
					Cardio	0.55 F			
						0.34 M			
					Gastro	0.55 F			
						0.34 M			
					Hemato	0.55 F			
						0.34 M			
					Musc/skel	0.55 F			
						0.34 M			
					Hepatic	0.55 F			
						0.34 M			
					Renal	0.55 F			
						0.34 M			
					Dermal	0.55 F			
						0.34 M			
					Ocular	0.55 F			
						0.34 M			
					Endocr	0.55 F			
						0.34 M			
					Immuno	0.55 F			
						0.34 M			
					Neuro	0.11 F 0.07 M	0.55 F 0.34 M		30–40% inhibition of plasma AChE and RBC AChE

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Robinson et al. 1978									
46	Rat (Albino) 71–72 M	30 days (F)	0, 2.5	BI BW	Death			2.5	4/71 died
					Bd wt			2.5	29% reduced body weight gain
					Neuro			2.5	Inhibition of AChE activity of 77.2% in brain, 81.9% in the stomach, 70.3% in the diaphragm
Ryan et al. 1970									
47	Rat (Albino) 5 M, 5 F	60–95 days (F)	0, 0.5	BI CS DX NX RX	Neuro		0.5		48% and 18% inhibition of brain AChE in males and females, respectively
					Repro			0.5	2/5 females failed to become pregnant
					Develop		0.5		32.1% inhibition of fetal brain AChE activity
Schwab and Murphy 1981									
48	Rat (Holtzman) 50 F	30–62 days (F)	0, 0.38, 1	BI BW CS	Bd wt	0.38	1		10–12% reduced body weight gain at all weighing times
					Neuro			0.38	75–80% inhibition of brain AChE
Sheets 1993b									
49	Rat (Fischer-344) 12 M, 12 F	13 weeks (F)	M: 0, 0.063, 0.27, 1.08 F: 0, 0.071, 0.315, 1.31	BI BW CS FI GN HP LE OP	Death			1.31 F	1/12 died on day 48
					Bd wt	1.31 F			
						1.08 M			
					Musc/skel	1.31 F			
						1.08 M			
					Ocular	1.31 F			
						1.08 M			
					Neuro	0.071 F		0.315 F	Muscle fasciculations, urine stain, 79–80% inhibition of RBC AChE activity, 64% inhibition of brain AChE activity

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
						0.063 M		0.27 M	61–67% inhibition of RBC AChE activity; 35% inhibition of brain AChE activity
Sheets 2005									
50	Rat (Wistar) 19–30 F	6 weeks GDs 0–21 and LDs 0–21 (F)	0, 0.038, 0.156, 0.670 during gestation; 0, 0.102, 0.389, 1.714 during lactation	BW CS DX FI GN LE NX	Bd wt		1.714		8–9% body weight decrease on LDs 14–21 compared to controls
					Neuro		0.102	0.389	27% inhibition of RBC AChE activity at 0.102 mg/kg/day; 73% inhibition of RBC AChE activity and 65% inhibition of brain AChE activity at 0.389 mg/kg/day
					Repro	1.714			
Sheets 2005									
51	Rat (Wistar) 5–20 B	Maternal generational exposure	0, 0.038, 0.156, 0.670 during gestation; 0, 0.102, 0.389, 1.714 during lactation	BW CS DX FI GN LE NX OP RX	Ocular	1.714			
					Neuro	0.389	1.714		53-56% inhibition of RBC AChE activity and 30% inhibition of brain AChE
					Develop		0.389 F		Delayed mean age for attainment of vaginal opening
						0.389 M	1.714 M		16% decrease in pup weight by PND 21 compared to controls; 18% depressed body weight gain from birth to PND 21
Stavinoha et al. 1969									
52	Rat (Holtzman or Charles River) 4–5 F	141–178 days (F)	0, 0.5, 1.25, 2.5	BI BW	Bd wt	0.5		1.25	40% reduced body weight gain
					Neuro			0.5	72% inhibition of brain AChE activity

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Taylor 1965a									
53	Rat (Holtzman) 10 M, 20 F	3 generations (F)	0, 0.1, 0.25, 0.5	BI CS DX HP	Develop		0.1	0.5	30–40% inhibition of RBC AChE in F3b weanlings at 0.1 mg/kg/day; cloudy swelling and fatty livers, mild nephropathy, juvenile hypoplasia of testes in F3b weanlings at 0.5 mg/kg/day
Clark and Stavinoha 1971									
54	Mouse (NS) NS	2 months (F)	0, 19.5	HP	Neuro		19.5		Increased permeability of central nervous system tissue
Clark et al. 1971									
55	Mouse (Charles River) 40–48 B	4 weeks (F)	0, 26	CS	Death			26 F	5/25 died
Clark et al. 1971									
56	Mouse (Charles River) 80–96 B	4-12 weeks (F)	0, 21.7, 26	CS	Neuro		21.7		Increased exploratory behavior
Rivett et al. 1972									
57	Mouse (CF-LP) 12 M, 12 F	13 weeks (F)	M: 0, 0.02, 0.12, 0.63 F: 0, 0.03, 0.14, 0.71	BC BI BW CS FI GN HE HP UR	Bd wt	0.71			
					Resp	0.71			
					Cardio	0.71			
					Gastro	0.71			
					Hemato	0.71			
					Hepatic	0.71			
					Renal	0.71			
					Ocular	0.71			
					Endocr	0.71			
					Immuno	0.71			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Neuro	0.14 F	0.71 F		27–37% inhibition of RBC AChE and plasma AChE activity
						0.63 M			
Hikita et al. 1973									
58	Dog (Beagle) 2–4 NS	5 months 5 days/week 1 time/day (C)	0, 0.5, 1.0, 1.5	BC BI CS	Neuro			0.5	80% inhibition of RBC AChE
Hoffman and Welscher1975									
59	Dog (Beagle) 4 M, 4 F	40 weeks (F)	0, 0.06	BI	Neuro		0.06		22–50% inhibition of RBC AChE; 33–36% inhibition of plasma AChE
CHRONIC EXPOSURE									
Carpy et al. 1975									
60	Rat (Sprague-Dawley) 60 M, 60 F	1.5–2 years (F)	M: 0, 0.05, 0.06, 0.1 F: 0, 0.04, 0.09, 0.1	BC BI BW CS FI GN HE HP OW UR	Immuno	0.1			
					Neuro	0.09 F	0.1 F		21% inhibition of brain AChE
						0.05 M	0.06 M		26–37% inhibition of brain AChE
Carpy et al. 1975									
61	Rat (Sprague-Dawley) 60 M, 60 F	1.5–2 years (F)	M: 0, 0.05, 0.06, 0.1 F: 0, 0.04, 0.09, 0.1	BC BI BW CS FI GN HE HP OW UR	Bd wt	0.1			
					Resp	0.1			
					Cardio	0.1			
					Gastro	0.1			
					Hemato	0.1			
					Musc/skel	0.1			
					Hepatic	0.1			
					Renal	0.1			
					Dermal	0.1			
					Ocular	0.1			
					Endocr	0.1			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Hayes 1985									
62	Rat (Fischer 344) 50 M, 50 F	104–106 weeks (F)	M: 0, 0.05, 0.18, 0.75; F: 0, 0.06, 0.21, 1.02	BC BI BW CS FI GN HE HP OW UR	Death			1.02 F	20/50 died; increase in mortality compared to control
					Bd wt	0.21 F	1.02 F		11–19% decrease in body weight gain
						0.18 M	0.75 M		
					Resp	0.21 F	1.02 F		Granulomatous and suppurative inflammation of the lungs
						0.18 M	0.75 M		
					Cardio	1.02 F			
					Gastro	0.21 F	1.02 F		Mucosal hyperplasia and chronic inflammation of the forestomach
						0.75 M			
					Hemato	1.02 F			
					Musc/skel	0.21 F		1.02 F	Skeletal muscle atrophy corresponded to generalized debilitation at this dose
						0.75 M			
					Hepatic	1.02 F			
					Renal	1.02 F			
					Dermal	0.21 F	1.02 F		Acanthosis, hyperkeratosis, ulcer of the skin
						0.18 M	0.75 M		
					Ocular	0.06 F	0.21 F	1.02 F	Cystic degeneration of Harderian gland at 0.21 mg/kg/day; corneal neovascularization at 1.02 mg/kg/day
						0.18 M		0.75 M	Corneal neovascularization; inflammation of the eye (cornea)
					Endocr	1.02 F			
						0.18 M	0.75 M		Pancreatic atrophy
					Immuno	0.21 F	1.02 F		Splenic lymphoid follicle depletion

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
						0.18 M	0.75 M		Plasma cell hyperplasia in the mandibular lymph nodes
					Neuro		0.06 F[d]	0.21 F	24% inhibition of RBC AChE at 0.06 mg/kg/day; 57–77% inhibition of RBC AChE, 53% inhibition of brain AChE, optic nerve degeneration, rough fur coat at 0.21 mg/kg/day
						0.05 M		0.18 M	46–67% inhibition of RBC AChE, 53% inhibition of brain AChE, optic nerve degeneration
					Repro	0.21 F	1.02 F		Uterine cystic hyperplasia
						0.75 M			
Hayes 1983									
63	Mouse (CD-1) 50 M, 50 F	23 months (F)	M: 0, 0.11, 0.5, 2.13 F: 0, 0.14, 0.65, 2.53	BC BI BW CS FI HE OW	Bd wt	2.53 F			
						2.13 M			
					Resp	2.53 F			
						2.13 M			
					Cardio	2.53 F			
						2.13 M			
					Gastro	2.53 F			
						2.13 M			
					Hemato	2.53 F			
					Musc/skel	2.53 F			
					Hepatic	2.53 F			
					Renal	2.53 F			
					Dermal	2.53 F			
					Ocular	2.53 F			
					Endocr	2.53 F			
					Immuno	2.53 F			
						2.13 M			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Neuro	0.65 F		2.53 F	Significant inhibition of RBC AChE and brain AChE by 82 and 46%, respectively
						0.5 M	2.13 M		Significant inhibition of RBC AChE and brain AChE by 56 and 44%, respectively
Hoffman and Welscher 1975									
64	Dog (Beagle) 4 M, 4 F	2 years (F)	0, 0.02, 0.03, 0.14	BC BI BW CS FI GN HE HP OP OW	Bd wt	0.14			
					Resp	0.14			
					Cardio	0.14			
					Gastro	0.14			
					Hemato	0.14			
					Musc/skel	0.14			
					Hepatic	0.14			
					Renal	0.14			
					Ocular	0.14			
					Endocr	0.14			
					Immuno	0.14			
					Neuro	0.03	0.14		46–53% inhibition of RBC AChE; 34.4% inhibition of brain AChE in males

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Ishikawa and Miyata 1980									
65	Dog (Beagle) 1–10 NS	2 years 5 days/week 1 time/day (C)	0, 0.63, 1.25, 1.89	BI CS HP OP	Ocular			0.63	Myopia, astigmatism, severe degeneration of ciliary muscle cells
Jones et al. 1999									
66	Dog (Beagle) 4 M, 4 F	12 months (F)	M: 0, 0.015, 0.121, 0.321 F: 0, 0.013, 0.094, 0.283	BC CS HE NX OP UR	Hemato	0.013 F 0.015 M			
					Ocular	0.013 F		0.094 F	60% inhibition of cornea ChE
							0.015 M	0.321 M	33% inhibition of cornea ChE at 0.015 mg/kg/day; 67% inhibition of retina and cornea ChE at 0.321 mg/kg/day
					Neuro	0.013 F	0.094 F	0.283 F	22% inhibition of brain AChE at 0.094 mg/kg/day; >60% inhibition of RBC AChE at day 91 of exposure at 0.283 mg/kg/day
						0.015 M		0.321 M	>80% inhibition of RBC AChE on day 91 of exposure

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Uga et al. 1977									
67	Dog (Beagle) 1–2 NS	2 years 5 days/week 1 time/day (C)	0, 0.5, 1.0, 1.5	HP	Neuro			0.5	Necrosis and atrophy of optic nerve and retina

[a]The number corresponds to entries in Figure 2-3.
[b]Used to derive an acute oral MRL of 0.0003 mg/kg/day; the $BMDL_{20RD}$ of 0.028 mg/kg/day was 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 oral MRL of 0.00009 mg/kg/day; the NOAEL of 0.009 mg/kg/day was 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 oral MRL of 0.00006 mg/kg/day; the LOAEL of 0.06 mg/kg/day was divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). See Appendix A for details.

RBC and brain AChE activity are assessed by comparing the activity of exposed groups to study controls and assessing whether AChE was inhibited by the chemical of interest. ATSDR classifies a NOAEL as <20% inhibition; a LOAEL is classified as 20–59% inhibition; and SLOAEL is classified as >59% inhibition. If AChE activity is inhibited by 20–59% but is accompanied with clinical signs of cholinergic toxicity, it may be classified as a SLOAEL.
Highlighted rows indicate an MRL principal study.

AChE = acetylcholinesterase; B = both male(s) and female(s); BC = blood chemistry; Bd wt or BW = body weight; BH = behavioral; BI = biochemical indices; (C) = capsule; Cardio = cardiovascular; ChE = cholinesterase; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FX = fetotoxicity; (G) = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; (GO) = gavage in oil vehicle; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; LD = lactation day; LD_{50} = lethal dose, 50% kill; M = male(s); MRL = Minimal Risk Level; Musc/skel = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OP = ophthalmology; OW = organ weight; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; SLOAEL = serious LOAEL; UR = urinalysis

Figure 2-3. Levels of Significant Exposure to Disulfoton – Oral Acute (≤14 days)

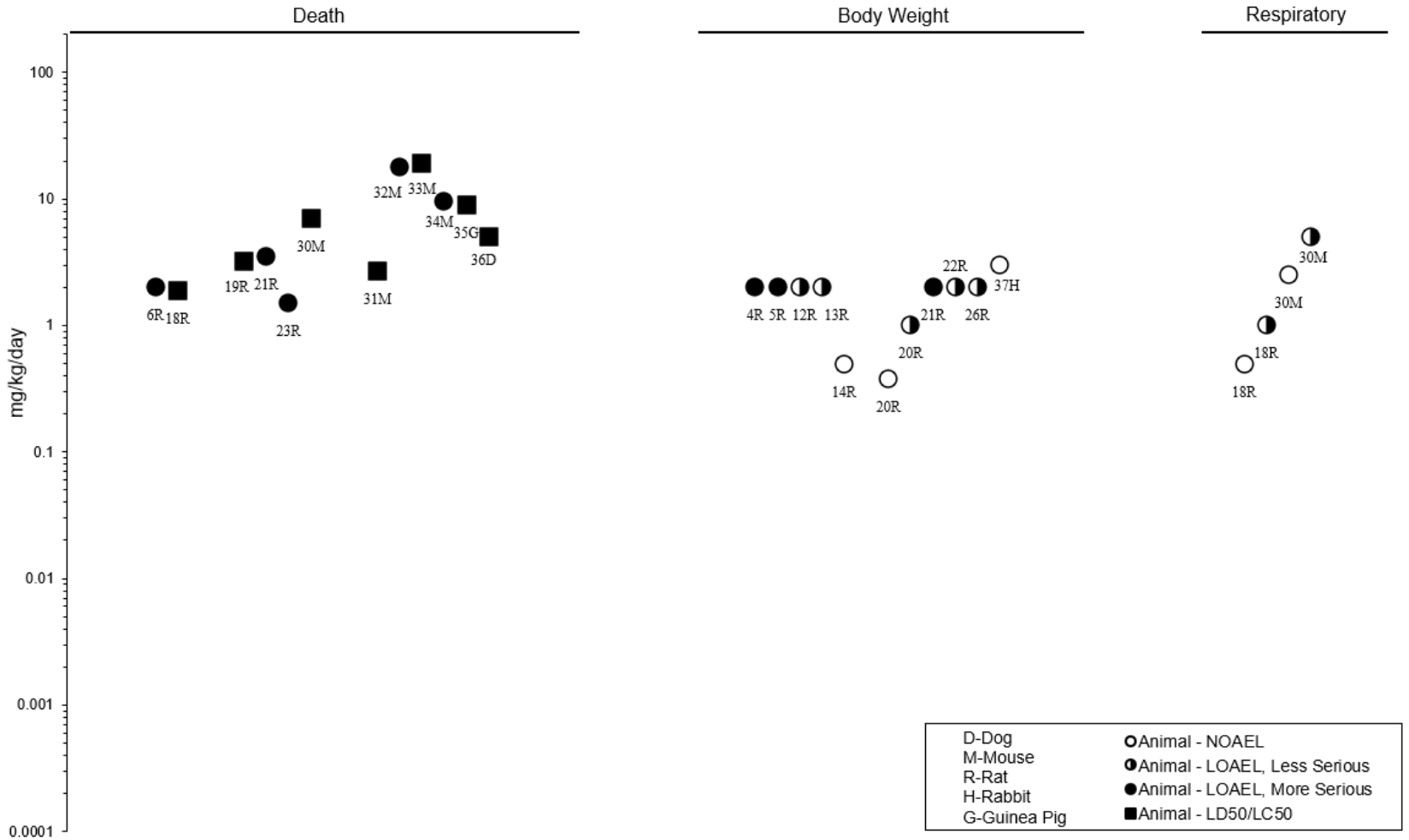

Figure 2-3. Levels of Significant Exposure to Disulfoton–Oral
Acute (≤14 days)

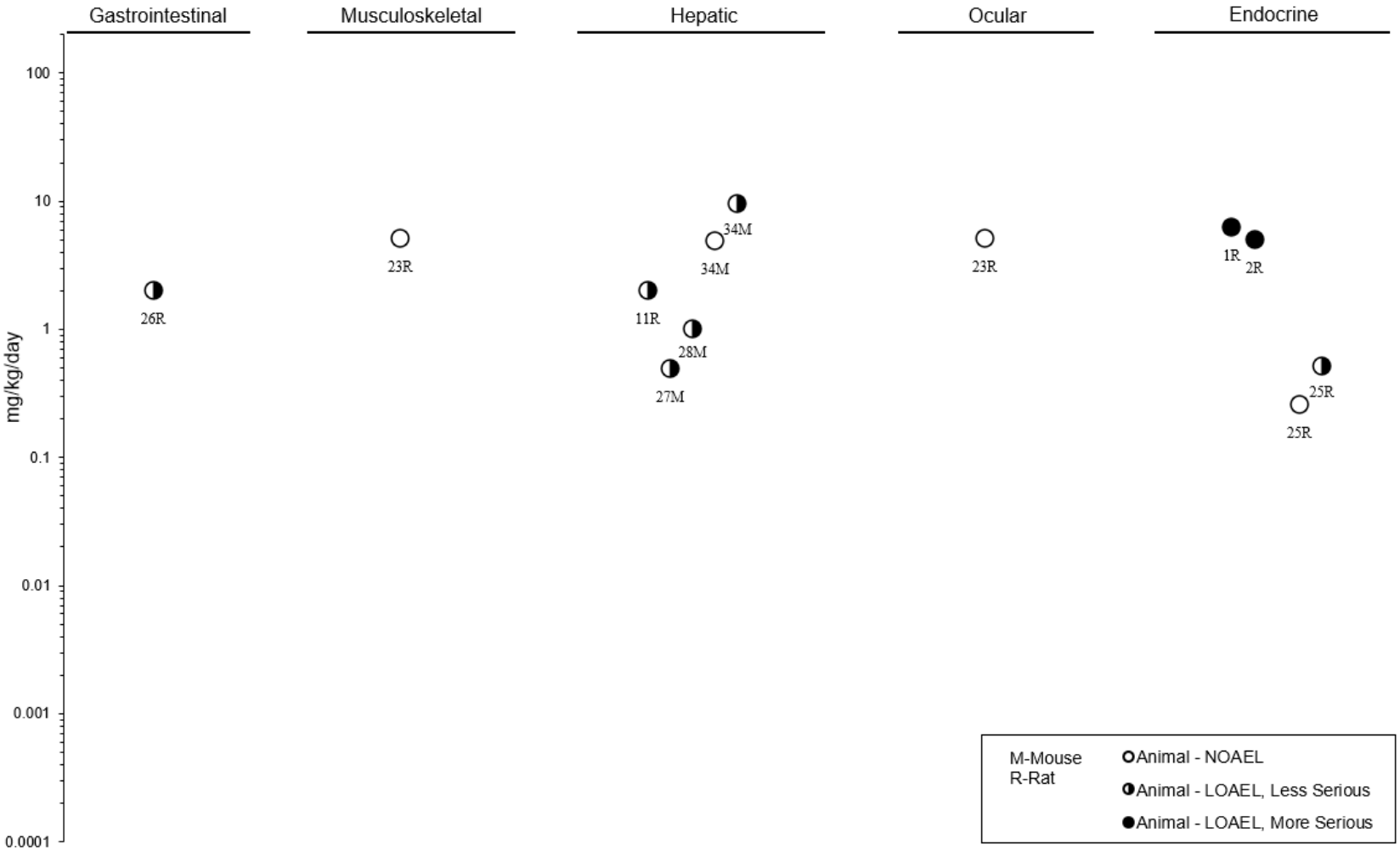

Figure 2-3. Levels of Significant Exposure to Disulfoton–Oral
Acute (≤14 days)

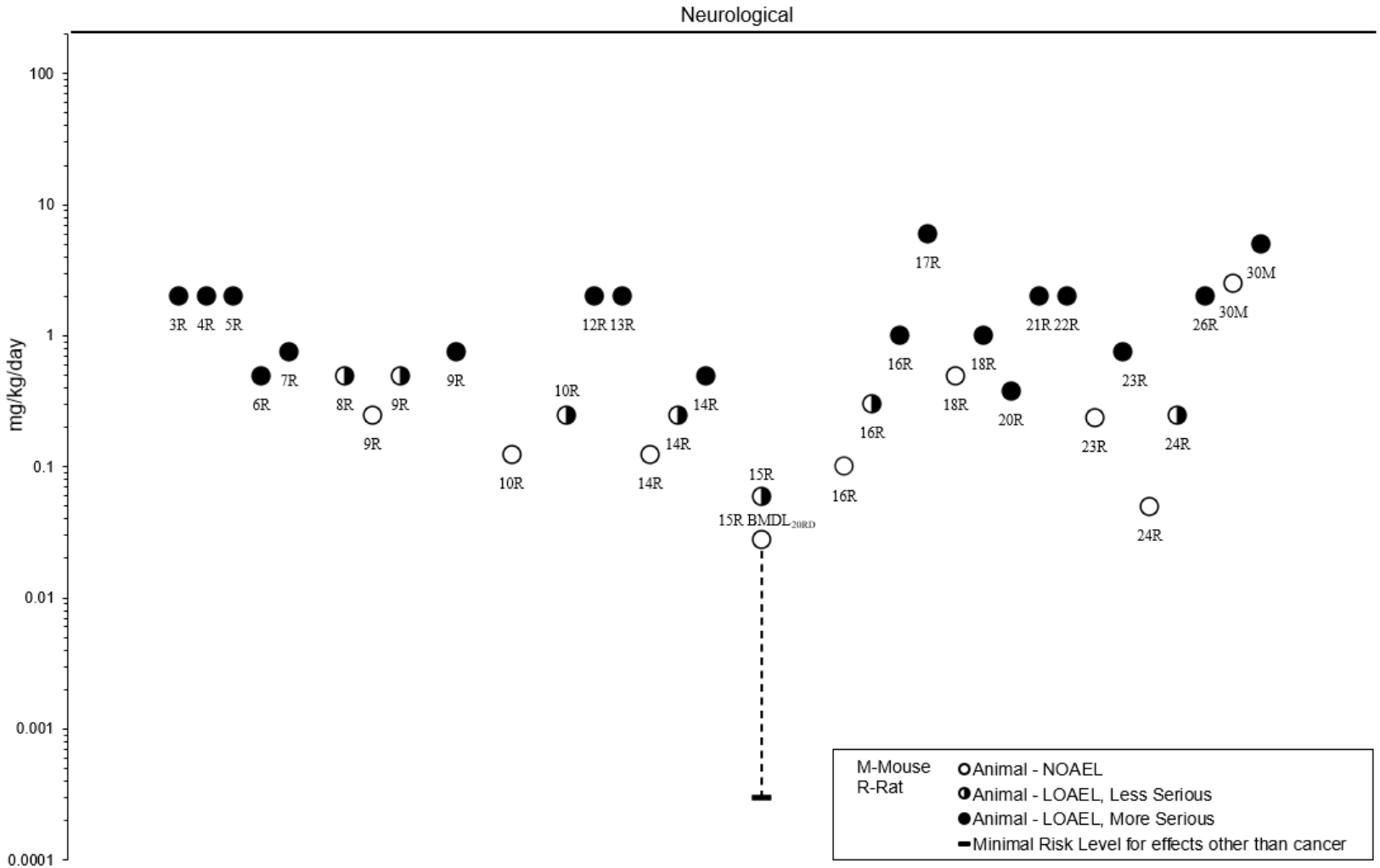

Figure 2-3. Levels of Significant Exposure to Disulfoton–Oral
Acute (≤14 days)

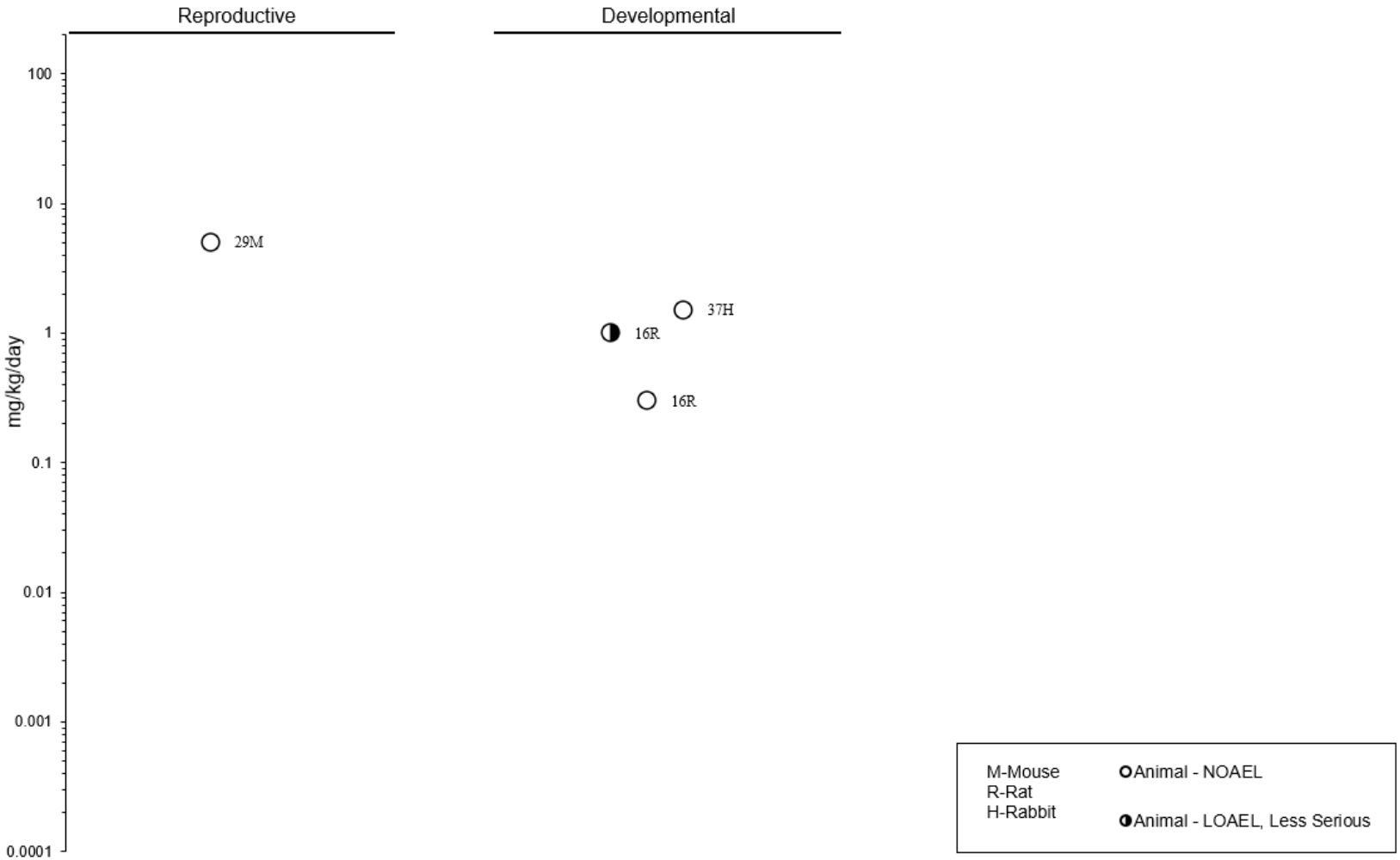

Figure 2-3. Levels of Significant Exposure to Disulfoton–Oral
Intermediate (15–364 days)

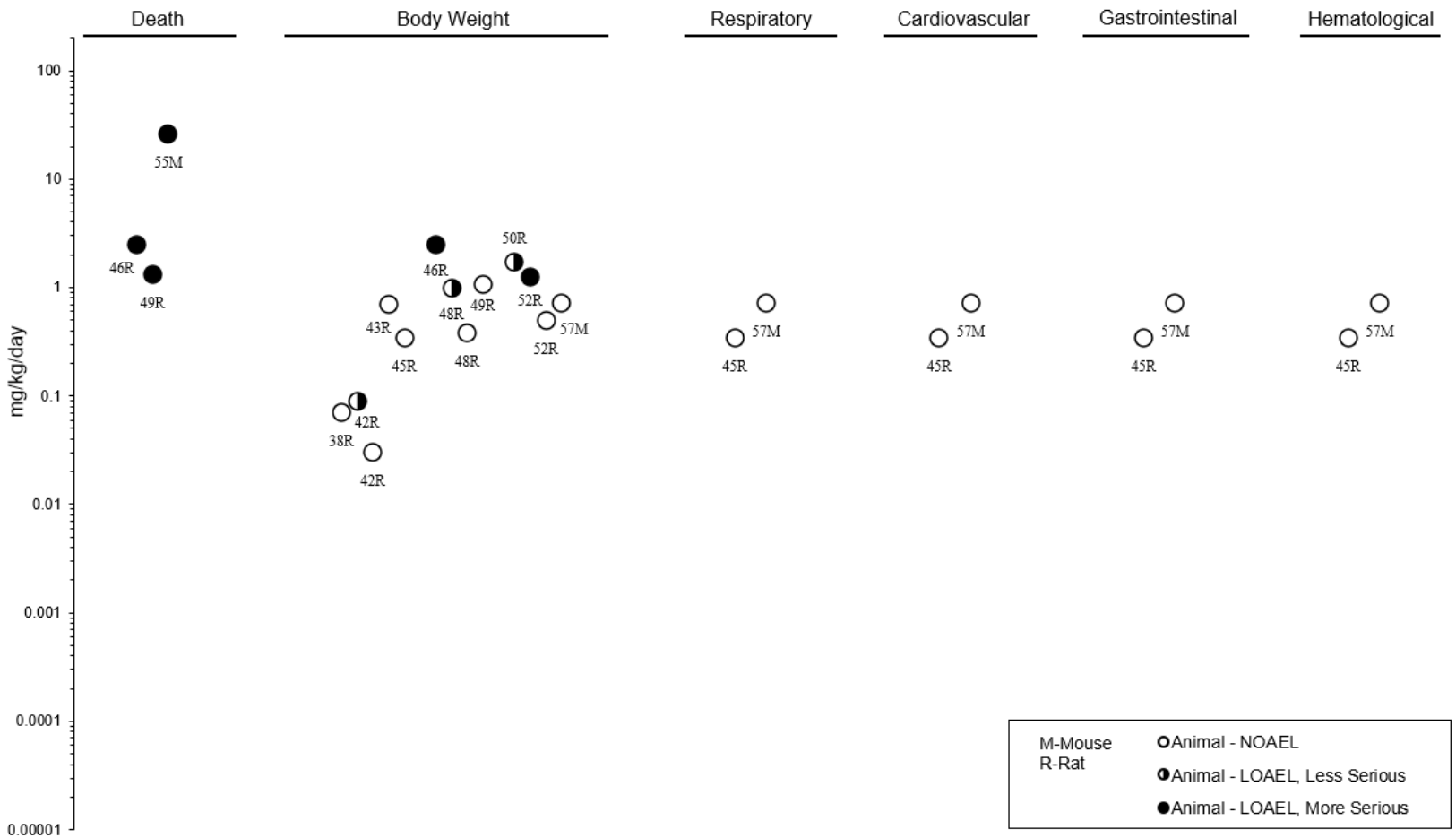

Figure 2-3. Levels of Significant Exposure to Disulfoton–Oral
Intermediate (15–364 days)

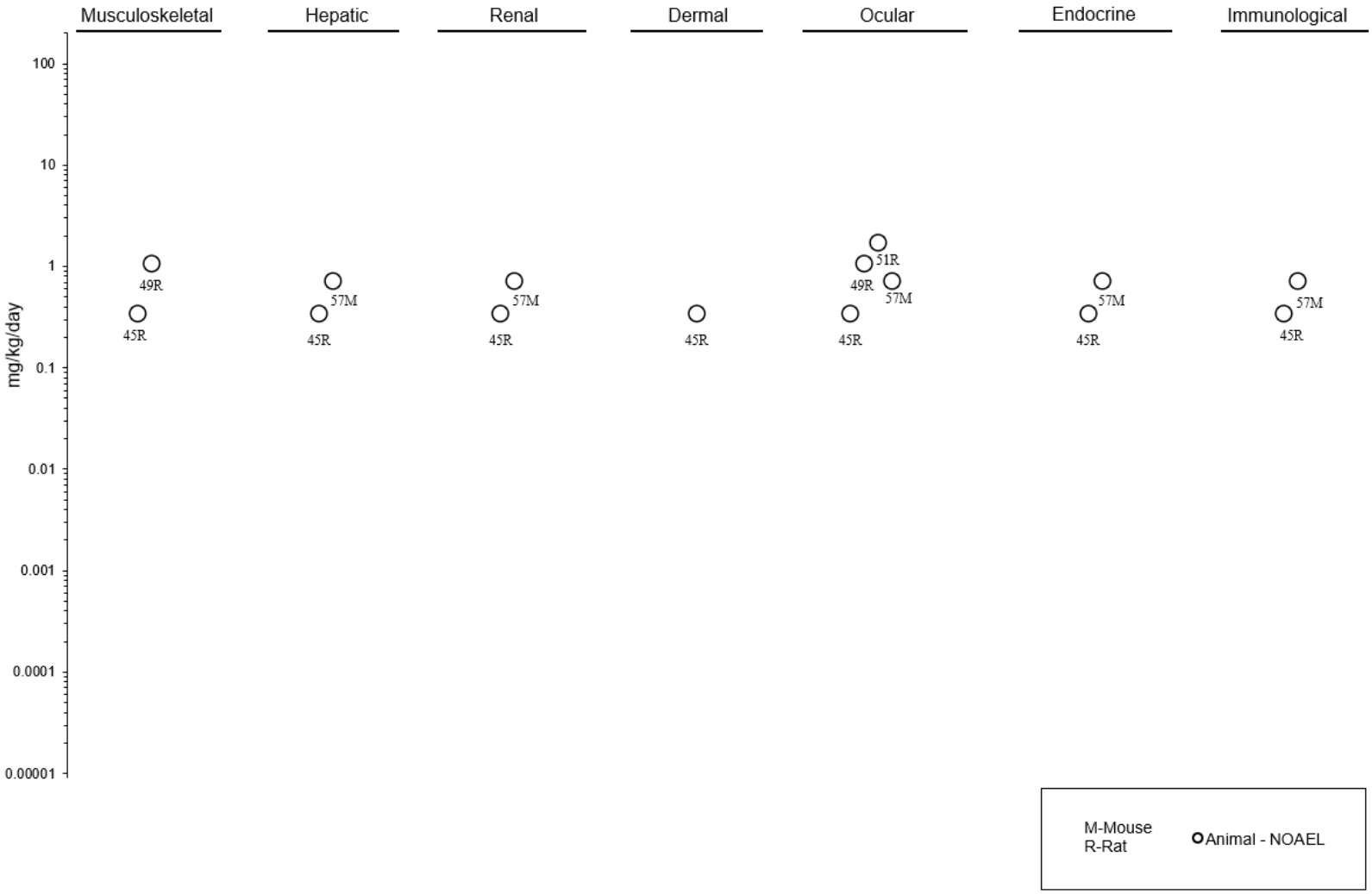

Figure 2-3. Levels of Significant Exposure to Disulfoton–Oral
Intermediate (15–364 days)

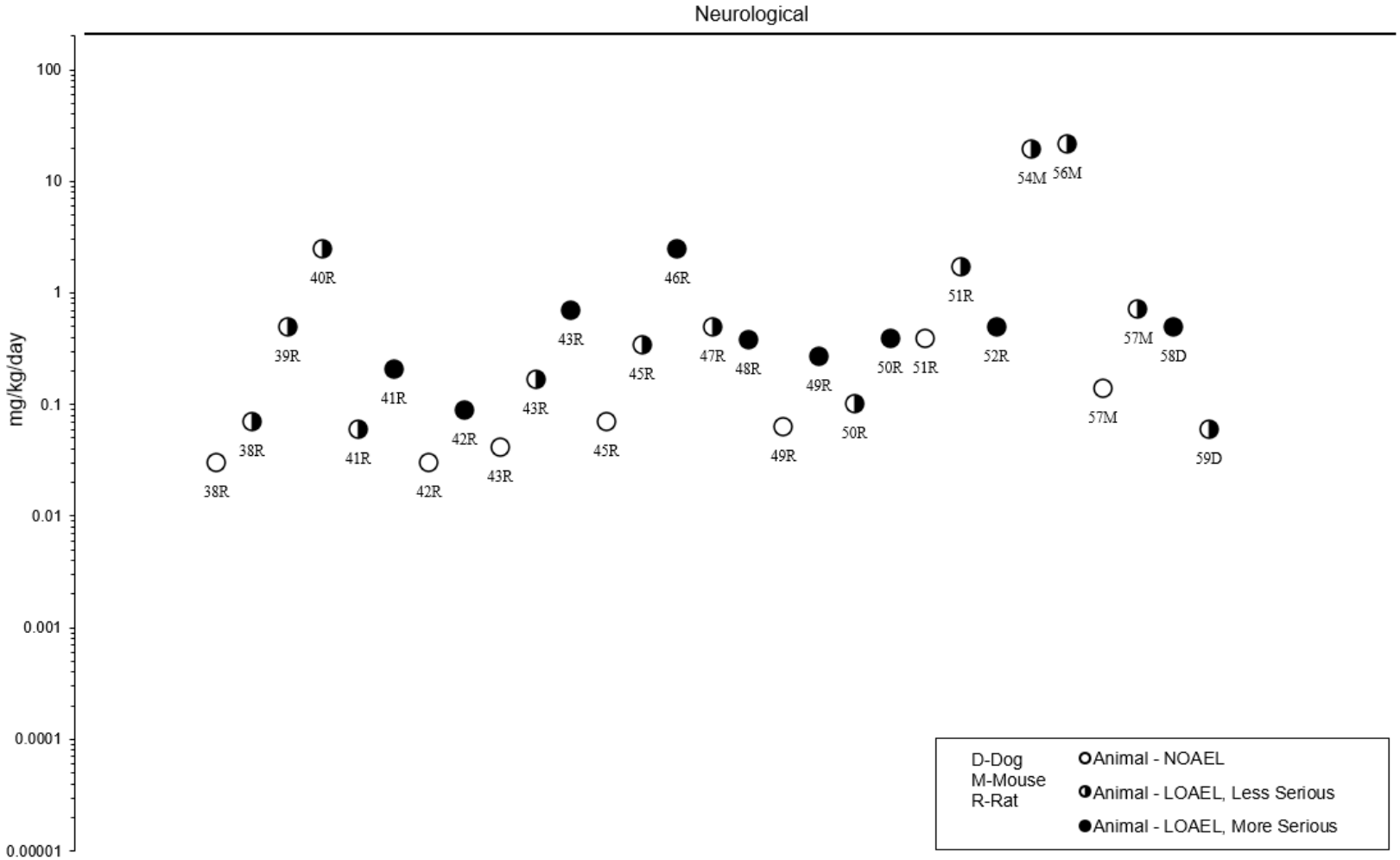

Figure 2-3. Levels of Significant Exposure to Disulfoton–Oral
Intermediate (15–364 days)

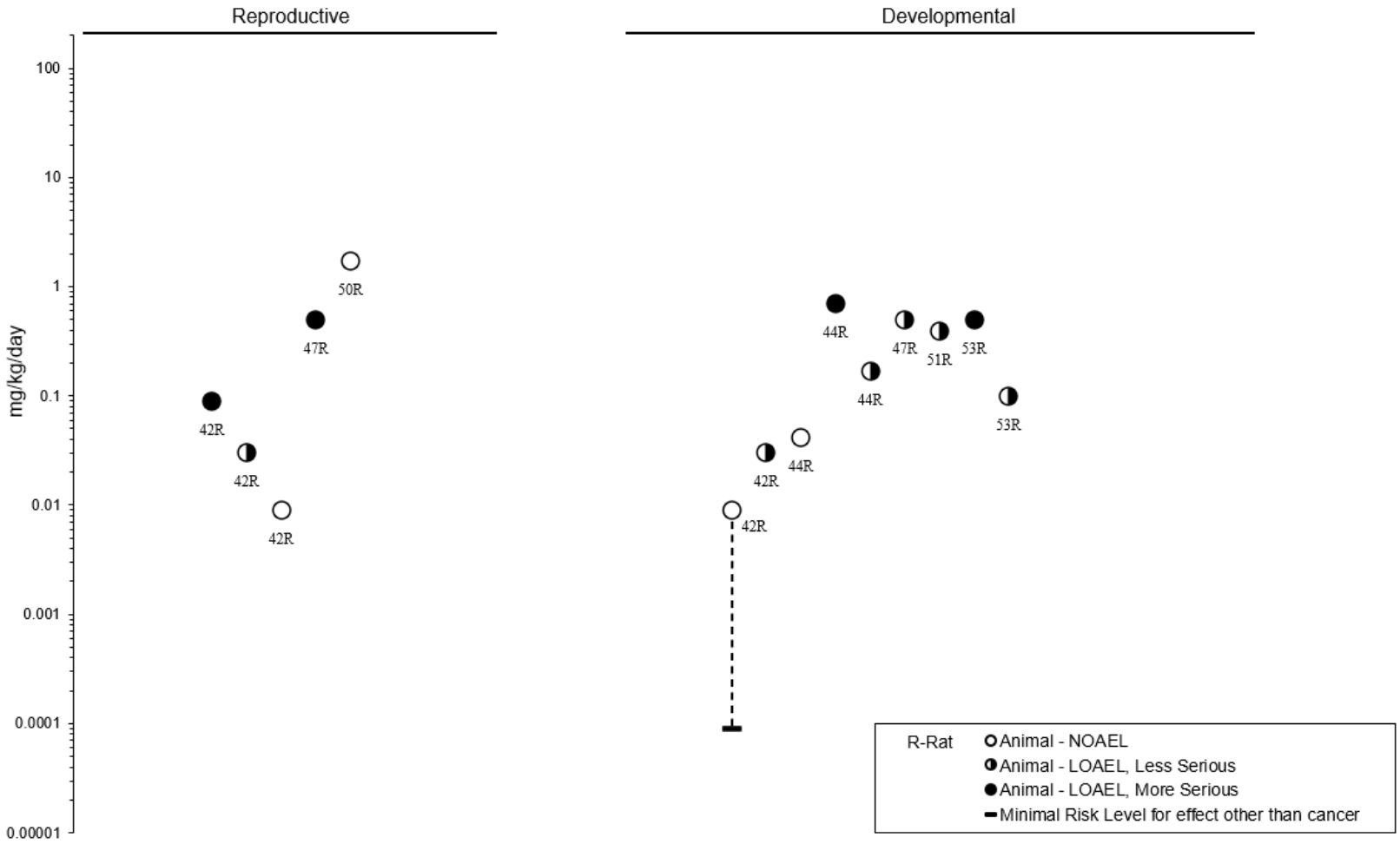

Figure 2-3. Levels of Significant Exposure to Disulfoton–Oral
Chronic (≥365 days)

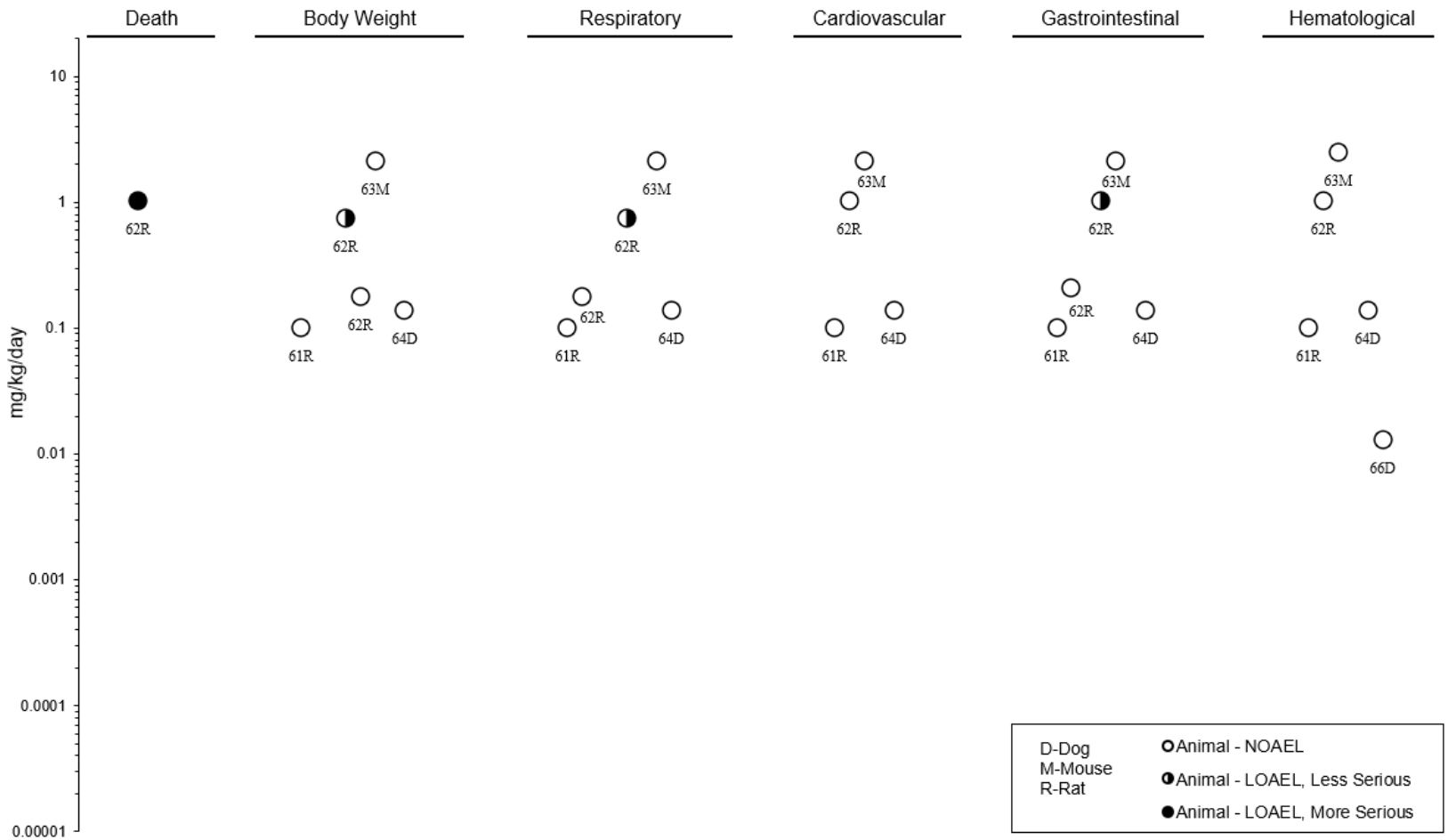

Figure 2-3. Levels of Significant Exposure to Disulfoton–Oral
Chronic (≥365 days)

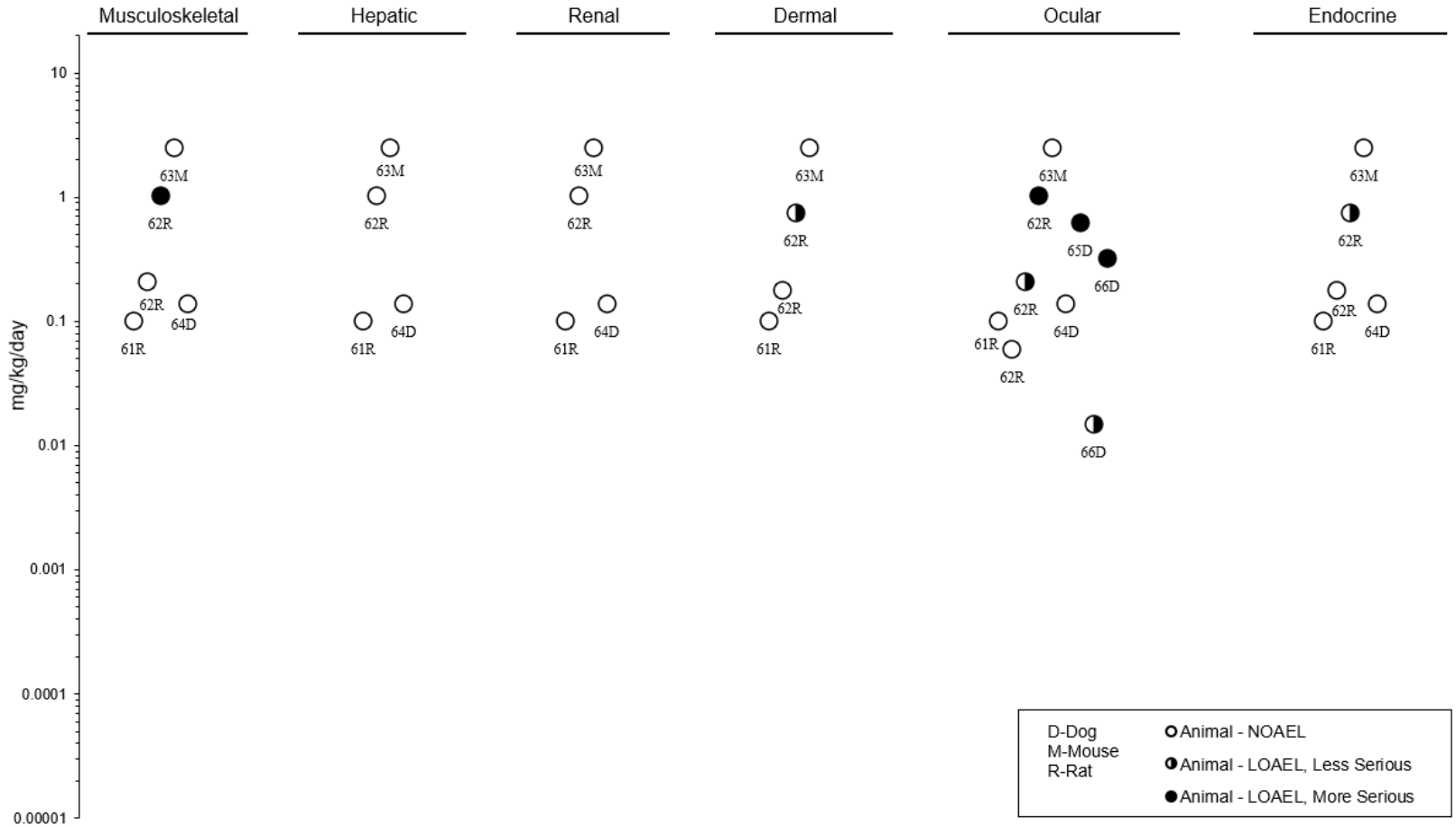

Figure 2-3. Levels of Significant Exposure to Disulfoton–Oral
Chronic (≥365 days)

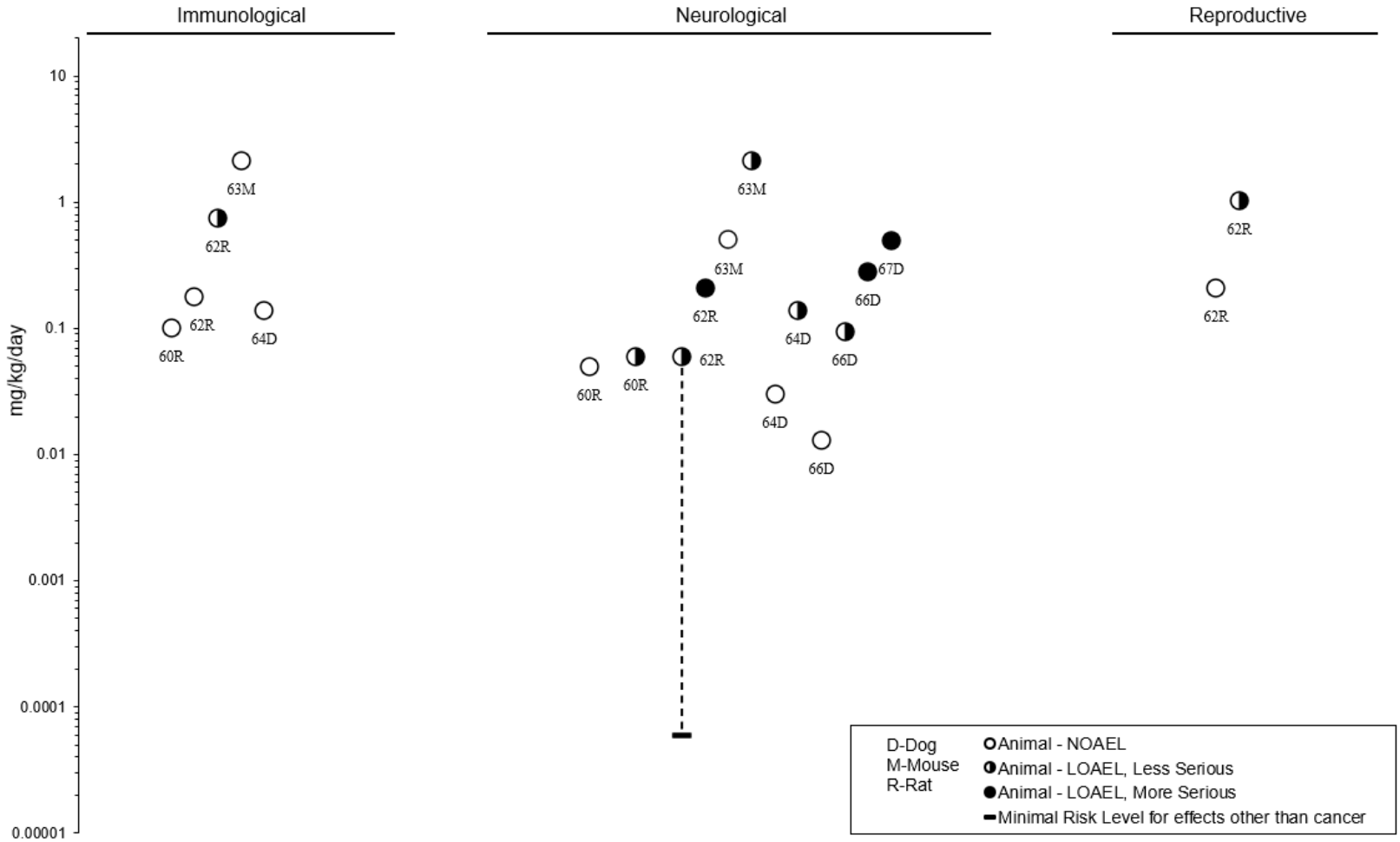

Table 2-3. Levels of Significant Exposure to Disulfoton – Dermal

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE									
Croutch and Sheets 2000									
1	Rat (Wistar) 5 M, 5 F	3 days	0, 50, 100, 200, 500	BW CS NX	Bd wt	500			
					Dermal	500			
					Neuro		50 F	200 F	39% inhibition of RBC AChE activity 24 hours after the third dose at 50 mg/kg/day; 62% inhibition of RBC AChE activity 24 hours after the third dose at 200 mg/kg/day
						100 M	200 M		21% inhibition of RBC AChE and brain AChE activity 24 hours after the third dose
DuBois 1957									
2	Rat (Sprague-Dawley) 35 M	Once	NR	LE	Death			20	LD_{50}
Mihail 1978									
3	Rat (Wistar) 5–10 M, 5–10 F	Once	M: 5, 10, 15, 17.5, 20 F: 2.5, 3, 3.5, 5, 10	LE	Death			3.6 F 15.9 M	Computed LD_{50}
Flucke 1986									
4	Rabbit (New Zealand) 2 NS	1–2 days 6 hours/day	0, 0.4, 2, 10	BI CS LE	Death			10	2/2 died
Flucke 1986									
5	Rabbit (New Zealand) 2 NS	1-5 days 6 hours/day	0, 0.4, 2, 10	BI CS LE	Neuro	2		10	2/2 rabbits exhibited unspecified cholinergic signs and died after one or two doses

Table 2-3. Levels of Significant Exposure to Disulfoton – Dermal

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Flucke 1986									
6	Rabbit (New Zealand) 5 M, 5 F	1–2 weeks 5 days/week 6 hours/day	0, 6.5	CS LE	Death			6.5	10/10 died
					Bd wt			6.5	Little or no feed intake and distinct weight loss up to time of death
					Resp			6.5	Distended, pale, mottled, fluid containing lungs in rabbits that died
					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
					Dermal	6.5			
					Immuno			6.5	Small pale spleen in rabbits that died
					Neuro			6.5	Muscle spasms, dyspnea, salivation after 1–2 days of exposure
INTERMEDIATE EXPOSURE									
Flucke 1986									
7	Rabbit (New Zealand) 5 M, 5 F	3 weeks 5 days/week 6 hours/day	0, 0.4, 1.6, 6.5	BI BC BW CS FI GN HE HP LE OW UR OW	Death			6.5	Females died after 1–6 treatments, males died after 3–10 treatments
					Bd wt	1.6			
					Resp	1.6			
					Cardio	1.6			
					Hemato	1.6			
					Hepatic	1.6			

Table 2-3. Levels of Significant Exposure to Disulfoton – Dermal

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Renal	1.6			
					Dermal	1.6			
					Endocr	1.6			
					Neuro	0.4			
							1.6 F		21–33% inhibition of RBC AChE activity
					Repro	1.6			
Flucke 1988									
8	Rabbit (New Zealand White) 5 M, 5 F	21 days 5 days/week 6 hours/day	0, 0.8, 1, 3	BC BW CS FI HE HP LE NX OW UR	Bd wt	1 F	3 F		Statistically significant 3% decrease in body weight
						3 M			
					Resp	1	3		Difficulty breathing observed in rabbits on days 17 and 21
					Gastro	1	3		Diarrhea observed in two rabbits on days 16 and 17
					Hemato	3			
					Neuro		0.8 F		20% inhibition of RBC AChE activity on day 21
						1 M		3 M	62% inhibition of RBC AChE activity on day 21

Red blood cell and brain AChE activity are assessed by comparing the activity of exposed groups to study controls and assessing whether AChE was inhibited by the chemical of interest. ATSDR classifies a NOAEL as <20% inhibition; a LOAEL is classified as 20–59% inhibition; and a SLOAEL is classified as >59% inhibition. If AChE activity is inhibited by 20–59% but is accompanied with clinical signs of cholinergic toxicity, it may be classified as a SLOAEL.

AChE = acetylcholinesterase; BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical indices; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); FI = food intake; Gastro = gastrointestinal; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; LD_{50} = lethal dose, 50% kill; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NR = not reported; NS = not specified; OW = organ weight; RBC = red blood cell; Resp = respiratory; SLOAEL = serious LOAEL; UR = urinalysis

2.2 DEATH

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

In an acute inhalation study, 1-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 1-hour exposure resulted in death of three of six males at 180.1 mg/m^3 and two of six females at 87.6 mg/m^3 (DuBois and Kinoshita 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 1–8 days after exposure. No deaths occurred in either sex at ≤1.8 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 disulfoton 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. Additionally, female rats may be more susceptible to the lethality of disulfoton than male rats. In female mice, a 1-hour exposure 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.

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 and 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 glomerulus, and congestion of most organs. Analysis of urine and blood samples confirmed that disulfoton was responsible for the death (Hattori et al. 1982).

The dose of disulfoton associated with death following acute oral exposure in animals depends on the sex, species, and duration of exposure. Female rats and mice are generally more sensitive than male rats and mice, and rats generally appear to be more sensitive than mice following oral exposure to disulfoton. LD_{50} values are 1.9–3.2 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 an 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 1974), 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 four of six female rats given a dose of 2.5 mg/kg by gavage and in one of nine 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), one of five male rats died after receiving one dose of 3.5 mg/kg disulfoton, while two more rats died after receiving the same dose for 3 consecutive days (Schwab et al. 1981). In the same study, one of eight rats died after receiving either 2.5 or 3.5 mg/kg/day 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 of 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 1.31 mg/kg/day disulfoton in the diet was found dead on day 48 due to cholinergic effects (tremor, muscle fasciculations) (Sheets 1993b). In addition, 4 of 71 male rats died when given a diet providing 2.5 mg/kg/day disulfoton 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).

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 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.

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 likely related to the different formulations of disulfoton. In a range-finding study, two of two 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 five of five females after 1–6 treatments and of five of five 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 (Flucke 1986). The rabbits that died in these experiments exhibited persistent cholinergic signs of intoxication (muscle spasms, dyspnea, and salivation) before death. One of five male rabbits exposed to 3 mg/kg/day of disulfoton died after 17 days of exposure with clinical signs of cholinesterase depression (Flucke 1988).

2.3 BODY WEIGHT

No studies were located regarding effects on body weight in humans after inhalation, oral, or dermal exposure to disulfoton.

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).

Weight loss or decreased body weight gain is commonly observed in animals after acute exposure to disulfoton following oral exposure and is one of the typical signs of cholinergic toxicity of cholinesterase inhibitors (see Section 2.15). 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 et al. 1981; Schwab and Murphy 1981). Rats treated with 2.0 or 2.5 mg/kg/day disulfoton by gavage for 1–10 days initially exhibited a 20–50% reduction in weight gain (Costa et al. 1984, 1986; Schwab et al. 1981; Schwab et al. 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). Similarly, rats exhibited significantly lower body weight, 92–97% of control animals, on the third day of exposure to 2 mg/kg/day disulfoton; the difference was no longer significant after a 28 day recovery period (Yagle and Costa 1996). However, no treatment-related effects on body weight were seen in rats given ≤0.5 mg/kg/day (female) or ≤1 mg/kg/day (males) for 11 days (Klaus 2006a). When effects were seen, 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 acute exposure 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 exposure studies. Rats given 2.5 mg/kg/day disulfoton for 30 days gained 29% less body weight 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. 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 F1 parental females and 9–11% in F1 parental males receiving 0.09 mg/kg/day disulfoton in the diet during the premating period of 13 weeks (Hixson and Hathaway 1986). In a developmental study, body weight gain was depressed by 17–18% in offspring of dams exposed to 8 ppm in feed (0.67 mg/kg/day during gestation; 1.714 mg/kg/day during lactation), compared to controls by postnatal day (PND) 21 (Sheets 2005). In other intermediate-duration dietary studies, no effects on body weight gain were observed in rats given ≤1.31 mg/kg/day (Christenson and Wahle 1993; Klaus 2006c; Klotzsche 1972; Sheets 1993b, 2005) or in mice given ≤0.71 mg/kg/day (Rivett et al. 1972).

Hayes (1985) reported that in rats given disulfoton in the diet for 2 years, body weight gain was decreased by 11–19% 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. 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 and Welscher 1975).

In a 3-day study, disulfoton was applied to shaved skin of rats, occupying 10% of body surface and left for 6 hours, 5 days/week (Croutch and Sheets 2000). No effects on body weight were observed in rats treated with ≤500 mg/kg/day (5 mg a.i./kg/day). 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 and treated with the highest dose of 6.5 mg/kg/day (Flucke 1986). No effects on body weight were found in rabbits treated with ≤1.6 mg/kg/day for 3 weeks. In a similar study, significantly decreased body weight was reported for female rabbits exposed to 3 mg/kg/day for 3 weeks; however, the difference was only 3% from controls (Flucke 1988). At this dose, slight, but nonsignificant, decreases in body weight were seen in male rabbits, and decreased body weight gain was seen in both sexes.

2.4 RESPIRATORY

One study examined the association of pesticide use among male farm workers and both non-allergic and allergic wheeze (Hoppin et al. 2017). Current use of disulfoton was inversely associated with non-allergic wheeze (odds ratio [OR] 0.63; 95% confidence interval [CI] 0.42, 0.95), and no association was found with allergic wheeze (OR1.17; 95% CI 0.73, 1.87). No possible explanation for the inverse association was provided, and the study was limited, as the use of pesticide and symptoms were self-reported and no exposure levels were measured (Hoppin et al. 2017). Another study of 22 female and 8 male floriculturists who sprayed a mixture of pesticides including di-syxtox (containing disulfoton) for 10 and 1.5 years, respectively, found no respiratory disturbances following medical examination (Gómez-Arroyo et al. 2000). Among females exposed to the mixture of pesticides, occasional nasal mucosa irritations, along with headache and skin irritations, were reported; however, exposure levels, including specific to disulfoton were not measured.

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 2.14), which were minimal in male rats and

significant 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.

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. Moreover, no lesions were found in the lungs of 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 and Welscher 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.

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 rabbits treated with the high dose 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. Two of ten rabbits exposed to 3 mg/kg/day of disulfoton for 3 weeks showed difficulty breathing, with one rabbit dying after 17 days of exposure (Flucke 1988).

2.5 CARDIOVASCULAR

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

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).

Following ingestion of Di-Syston granules (5% disulfoton), a 75-year-old woman developed cardiac arrhythmias along with confusion and severe miosis approximately 5 hours after ingestion (Futagami et al. 1995). Cholinesterase activity was also inhibited in this patient (see Section 2.15). Inhibition of cholinesterase activity (and associated clinical symptoms) persisted for 19 days, but the patient showed almost complete recovery 28 days after hospital admission (no further details regarding recovery were reported).

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 and Welscher 1975) in the diet for up to 2 years.

No studies were located regarding cardiovascular 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 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).

2.6 GASTROINTESTINAL

No gastrointestinal disturbances were noted upon medical examination of 30 pesticide workers who worked with mixtures of several chemicals, including disulfoton (Gómez-Arroyo et al. 2000). Among the

22 female workers, nausea was reported when in contact with pesticides; however, this effect cannot be solely attributed to disulfoton exposure.

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 observed 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 observed. Likewise, no gastrointestinal tract lesions were seen in male or female rats exposed intermittently to ≤1.4 mg/m^3 for 13 weeks (Shiotsuka 1989).

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 and Welscher 1975) in the diet for up to 2 years. However, Hayes (1985) reported increased incidences of mucosal hyperplasia and chronic inflammation of the forestomach in female rats given 1.02 mg/kg/day disulfoton in the diet for 2 years. It was also noted that mucosal hyperplasia was usually diffuse; sometimes more locally severe; and accompanied by inflammation, fibrosis, and ulceration. Forestomach lesions were not observed in male rats at 0.75 mg/kg/day or in females at 0.21 mg/kg/day (Hayes 1985). Diarrhea was reported in 5 of 34 rats given a single oral dose of 2 mg/kg/day but only lasted 3–4 days after exposure, which indicated the development of tolerance (Yagle and Costa 1996).

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 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 (when one part of the intestine slides inside another part) 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. Diarrhea was seen in 2 of 10 rabbits treated with 3 mg/kg/day, with 1 rabbit dying after 17 days of exposure (Flucke 1988).

2.7 HEMATOLOGICAL

No studies were located regarding hematological effects in humans after inhalation, oral, or dermal exposure to disulfoton.

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 reported. 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 2.14). 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).

Limited information from animal studies suggests that intermediate- or chronic-duration exposure to disulfoton was 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 and Welscher 1975). No hematological effects were observed in female and male dogs following 1-year exposure to 0.013–0.321 mg/kg/day disulfoton in feed (Jones et al. 1999).

No hematological effects were found in two 3-week studies of rabbits exposed to ≤3 mg/kg/day of disulfoton, applied to the shorn, unabraded skin of rabbits and left for 6 hours, 5 days/week (Flucke 1986, 1988).

2.8 MUSCULOSKELETAL

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

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).

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 Section 2.12) 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 observed 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 and Welscher 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.

2.9 HEPATIC

No studies were located regarding hepatic effects in humans after inhalation, oral, or dermal exposure to disulfoton.

Clinical chemistry tests and histological examination of livers revealed no 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).

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, duration of dosing, and time between dosing and enzyme assays. Microsomal enzyme induction was considered not to be adverse

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 P450 content, was observed in mice given 9.6 mg/kg/day disulfoton for 3 days and sacrificed 24 hours later for 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 1–4 days (Fawade and Pawar 1978, 1980, 1983). Fawade and Pawar (1978) noted disulfoton significantly increased ascorbate-promoted lipid peroxidation and NADPH-driven lipid peroxidation by 13 and 14%, respectively, in mice orally dosed with 0.5, 1, 1.5, or 2 mg/kg/day for 2 days, then all to 1 mg/kg/day for 2 additional days. The study authors suggested that disulfoton or its oxygenated metabolite may have changed the conformation of heme protein thus enhancing lipid peroxidation. Fawade and Pawar (1978) also reported that "hepatic microsomal electron transport elements," defined as cytochrome P450 and cytochrome b_5, decreased as dose decreased (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 et 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 and Welscher 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.

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 exposed to the highest dose (6.5 mg/kg/day) died within 2 weeks during treatment. Necropsy of these rabbits revealed an enhanced lobular pattern in the liver; however, the study authors did not conclude whether this observation is associated with potential health effects (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 (Flucke 1986).

2.10 RENAL

No studies were located regarding renal effects in humans after inhalation or dermal exposure to disulfoton.

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).

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. Urinary stains (indicative of urine leakage) were observed in female rats fed 0.32 mg/kg/day disulfoton for 13 weeks (Sheets 1993b). No further renal effects were recorded. 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 and Welscher 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 female 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. The number of kidneys with malignant lymphoma among exposed mice did not significantly differ from controls, indicating they were not related to disulfoton exposure; the toxicological significance of the increased kidney weight is not clear.

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 exposed to the highest dose (6.5 mg/kg/day) died within 2 weeks during treatment. Necropsy of these rabbits 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.

2.11 DERMAL

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

Skin irritations were reported by 22 female floriculturist workers with occupational exposure to a mixture of sprayed pesticides, including disulfoton (Gómez-Arroyo et al. 2000). No further information was provided on dermal effects, and this effect cannot be directly attributed to disulfoton exposure.

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).

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.

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 (Flucke 1986). No treatment-related skin changes were seen in male and female rabbits treated with ≤3 mg/kg/day of disulfoton left on skin for 6 hours/day, 5 days/week for 21 days (Flucke 1988).

2.12 OCULAR

No studies were located regarding ocular effects in humans after inhalation or dermal exposure to disulfoton.

No ophthalmological evidence of ocular effects was 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).

The only information regarding ocular effects in humans comes from an epidemiological study in which a marked increase of myopia was observed in children aged 4–16 years living in areas where insecticides were used on a large-scale when compared with a control group of children. This observation coincided with an increased use of disulfoton in combination with other organophosphates to treat food crops (Ishikawa and Miyata 1980). 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 offspring of rat dams exposed to ≤1.174 mg/kg/day through gestation and 21 days of lactation, no treatment-related ocular effects were seen, including pupil constriction (Sheets 2005). 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 and Welscher 1975) in the diet for up to 2 years.

In Beagle dogs fed 0.015, 0.121, 0.321 mg/kg/day (males) or 0.013, 0.094, 0.283 mg/kg/day (females) disulfoton in the diet for 1 year, there was no significant inhibition of cholinesterase levels in lateral or dorsal rectus muscles (Jones et al. 1999). In male dogs, 33% inhibition of cornea cholinesterase was observed at 0.5 ppm. Cornea cholinesterase was 50–67% inhibited in female and male dogs exposed to 4 and 12 ppm, respectively. In both sexes, retina cholinesterase inhibition was 25–67% in both the 4 and 12 ppm exposure groups and ciliary body cholinesterase inhibition was 18–54% (Jones et al. 1999). Tissue cholinesterase inhibition did not appear to cause histological changes, gross pathology changes, nor alter ocular physiologic function. Additionally, no other findings, including tracking, refractivity, intraocular pressure, and pachymetry, indicated adverse ophthalmologic effects in dogs following chronic exposure to disulfoton (Jones et al. 1999).

2.13 ENDOCRINE

No studies were located regarding endocrine effects in humans after inhalation, oral, or dermal exposure to disulfoton.

No histological lesions were found in the thyroid or adrenal glands of male rats exposed intermittently 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 thyroid, parathyroids, pituitary, or pancreas were observed in rats exposed intermittently to ≤1.4 mg/m^3 for 13 weeks (Shiotsuka 1989).

Disulfoton exposure altered catecholamine levels in animals, and this hormonal imbalance may be associated with elevated acetylcholine levels (Brzezinski 1969, 1973; 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.

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 doses ≤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 was observed in females at doses ≤1.02 mg/kg/day, and no histopathological lesions in the adrenal, pituitary, thyroid, or parathyroids 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, parathyroids, 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 and Welscher 1975). The Hoffman study also found no changes or histopathological lesions in the parotid glands in dogs.

In a 3-week study in which disulfoton was applied to the shorn, unabraded skin of rabbits and left for 6 hours/day, 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).

2.14 IMMUNOLOGICAL

No studies were located regarding immunological effects in humans after inhalation, oral, or dermal 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, 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 treatment-related 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).

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 study 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 nodes or thymus revealed no treatment-related lesions in either sex at any dose. In other chronic dietary studies, no treatment-related 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 and Welscher 1975).

Down-regulation of cholinergic muscarinic receptors in T-lymphocytes and significantly inhibited AChE activity in T-lymphocytes were found in rats given 2 mg/kg/day disulfoton by gavage for 1–2 weeks (Fitzgerald and Costa 1993). The inhibition of T-lymphocyte AChE activity paralleled that in the brain. The immunological significance of these neurological effects (see Section 2.15) is not known.

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 exposed to the highest dose (6.5 mg/kg/day) died within 2 weeks during treatment. Necropsy of these rabbits 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.

2.15 NEUROLOGICAL

The neurologic system is the most sensitive target with the highest level of evidence associated with disulfoton exposure. AChE inhibition is the most sensitive neurological endpoint following exposure to disulfoton via inhalation or oral exposure, and acetylcholine is the primary neurotransmitter of the parasympathetic nervous systems. Brain AChE inhibition is the more toxicologically significant endpoint; however, since it can only be measured post-mortem, red blood cell AChE activity is used as a surrogate, as it is expected to correlate with brain AChE activity (EPA 2000). Disulfoton exposure results in inhibition of cholinesterase activity in blood and at nerve synapses of muscles, secretory organs, and nervous tissues such as the brain and spinal cord (Murphy 1986). The resulting acetylcholine accumulation results in central nervous system, nicotinic, and muscarinic effects. Brain and red blood cell AChE are considered significant if activity is inhibited by ≥20% following exposure, when compared to study controls.

The highest NOAEL values and all the LOAEL values for neurological effects in rats and mice for each duration category are recorded in Tables 2-1, 2-2, and 2-3, and plotted in Figures 2-2 and 2-3. Typically, ATSDR classifies AChE inhibition between 20–59% as a less serious LOAEL, and >59% as a serious LOAEL. Cholinesterase inhibition classified as a less serious LOAEL and accompanied by clinical symptoms of cholinergic toxicity may be classified as a serious LOAEL.

Nervous system effects may occur in humans after occupational exposure to disulfoton (Wolfe et al. 1978). Workers at a three pesticide-fertilizer mixing operations were exposed to mean disulfoton concentrations of 0.06–0.633 mg/m^3 in air, in addition to dermal exposure. Following 9 weeks of exposure, workers of dry mixing operations had a 22.8% depression in red blood cell AChE activity, but there were no reports of adverse clinical signs due to disulfoton exposure. The study was limited in that

baseline blood AChE activities were measured 2 weeks after the initial exposure and were compared with cholinesterase activities at 9 weeks, and it is unclear if workers were wearing respirators or not (Wolfe et al. 1978). Occasional headaches and nausea were reported by 22 female floriculturists following occupational exposure to a mixture of pesticides, including disulfoton, when spraying in greenhouses without any protection over a 10-year period (Gómez-Arroyo et al. 2000). Hearing loss has been associated with use of some organophosphate pesticides in agricultural workers (Crawford et al. 2008); however, data regarding potential associations between hearing loss and disulfoton exposure were not identified.

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 disulfoton 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) (Thyssen 1978). These effects were transient, lasting for about 24 hours after exposure. In an experiment designed to measure cholinesterase activity in rats exposed to 0.5, 1.8, or 9.8 mg/m^3 for 4 hours/day for 5 days, red blood cell AChE 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 (Thyssen 1978). In addition, all rats were reported to display unspecified behavioral disorders at 1.8 mg/m^3 and also unspecified signs of cholinergic toxicity at 9.8 mg/m^3. No inhibition of red blood cell AChE activity and no signs of cholinergic toxicity were observed at 0.5 mg/m^3 (Thyssen 1978). 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). Red blood cell AChE activity was inhibited in males by 24–28% and in females by 27–32% at 3.7 mg/m^3. Brain AChE 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 in Wistar male and female rats, no clinical signs of neurological effects and no effects on plasma, red blood cell, or brain AChE were observed at 0.02 mg/m^3 (Thyssen 1980). Female rats exposed to 3.1 mg/m^3 had muscle tremors, convulsions, increased salivation, and dyspnea, confirming the results of the first experiment. Male rats were not exposed to 3.1 mg/m^3 in the second experiment (Thyssen 1980). In Fischer rats exposed intermittently to 1.4 mg/m^3 for 13 weeks, red blood cell AChE activity was inhibited by 22–28% in males and 26–34% in females, and brain AChE activity was inhibited by 29% in males and 28% in females (Shiotsuka 1989). Cholinesterase activities were not affected at ≤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 AChE activities were found at 0.006–0.7 mg/m^3 disulfoton (Shiotsuka 1988). Red blood cell AChE activity was statistically consistently decreased at 0.7 mg/m^3, but the decrease was never greater than 17% of control levels.

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 (Hattori et al. 1982). Severe signs and symptoms of disulfoton toxicosis (miosis, salivation, masseter muscle spasms, and monoplegia) were observed in a 75-year old man within 2–3 hours of consuming 3 to 4 heaping tablespoons of Di-Syston (Yashiki et al. 1990). Serum cholinesterase activity was depressed below 10 IU for 5 days after admission, and below 40 IU at 8 days following admission (normal activity range is 175–440 IU). The occurrence of severe clinical signs and the measured concentrations of disulfoton in the patient's blood suggests a severe level of disulfoton intoxication occurred, but the patient recovered with medical intervention (Yashiki et al. 1990). A case-study of a non-occupationally exposed 75-year-old female, who ingested a "large quantity of Di-Syston granules," had markedly depressed red blood cell and plasma AChE activity 3.5 hours after ingestion (Futagami et al. 1995). Vomiting, nausea, and muscle fasciculations were also observed, and 5 hours after ingestion, confusion, miosis, and cardiac arrhythmias were noted. Twenty-four hours after ingestion, red blood cell AChE activity recovered from 3,524 IU/L to 8,688 IU/L (normal range considered 10,000–14,000 IU/L). By the next day red blood cell AChE activity depressed again, and remained low for 19 days after ingestion, which the study authors attributed

to absorption from residual Di-Syston and/or the more toxic compounds metabolized in the liver (Futagami et al. 1995). The patient showed almost complete recovery 28 days after hospital admission (no further details regarding recovery were reported).

Several studies in rats observed significant depression of brain AChE activity following oral exposure to disulfoton for 7–10 days (Costa and Murphy 1983a; Costa et al. 1986; Schwab and Murphy 1981; Su et al. 1971). Signs of cholinergic toxicity like 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 rat pups of both sexes, peak time of effect (red blood cell and brain AChE activity inhibition) was estimated at 24 hours post-dosing of 0.5 mg/kg (EPA 2007). A follow-up study tested multiple doses in both sexes and at the peak time of effect, red blood cell AChE activity was inhibited by 22–53%, and brain AChE by 19–39% in both sexes; cholinesterase inhibition increased with dose (EPA 2007). In a similarly designed study, the peak time of effects (cholinesterase inhibition) was estimated at 8 hours in females and 6 hours in males, after a single oral dose of 0.75 or 1.5 mg/kg in females and males, respectively (EPA 2007). When exposed to single varying doses and observed at the peak time of effect, females exhibited >34% red blood cell AChE inhibition at >0.5 mg/kg, and males exhibited 32 and 46% red blood cell and brain AChE activity inhibition, respectively, at 1.5 mg/kg (EPA 2007). In an extensive neurotoxicity screening study, rats were given single gavage doses of disulfoton (0.24, 1.5, and 5.2 mg/kg for males; 0.24, 0.76, and 1.5 mg/kg for females) (Sheets 1993a). The study reported clinical signs of cholinergic intoxication including muscle fasciculations, tremors, ataxia, oral stain (drooling), urine leakage, diarrhea, and decreased activity in the high-dose males (5.2 mg/kg) and high-dose females (1.5 mg/kg), and muscle fasciculations 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 (Sheets 1993a). These effects included muscle fasciculations, 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 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. Red blood cell AChE activity was inhibited by 21% in high-dose males, 75% in high-dose females, and 53% in mid-dose females (Sheets 1993a). 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 a separate study, AChE mRNA levels in

soleus muscle and sciatic nerve of male rats significantly decreased by 53% (compare to controls) 12 hours after administration of a single 6 mg/kg dose of disulfoton by gavage (Matsuda et al. 2000). This down-regulation persisted 30 days after the dose suggesting alterations at the transcriptional level. The nicotinic acetylcholine receptor in the soleus muscle also decreased 6 hours after the dose but recovered to control levels after 30 days. Gamma-enolase mRNA in sciatic nerve increased by 200% 2 hours after the dose and exceeded a 250% increase after 30 days; up-regulation of gamma-enolase mRNA was suggested as a marker of nervous system abnormality following disulfoton exposure, although the function of this gamma-enolase mRNA is not clear (Matsuda et al. 2000). Additionally, 75 and 69% inhibition of AChE was seen in whole blood and skeletal muscles of rats, respectively, 12 hours after the dose, but activity recovered for both, though more slowly in whole blood (30 days). The study authors suggested that down-regulation of AChE in skeletal muscle is associated with structural and functional alterations of the neuromuscular junction (Matsuda et al. 2000). No clinical observations were made as part of the study protocol.

Animals exposed to disulfoton develop typical signs of cholinergic toxicity associated with inhibition of brain AChE 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 diminished, while cholinesterase remained inhibited, indicating a tolerance to disulfoton developed. 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; Yagle and Costa 1996). Similar effects were also observed in female rats after 3 days on a diet that was mixed with 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 AChE 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 AChE 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 AChE 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(s) associated with disulfoton toxicity

was not impaired, despite the disappearance of overt neurological signs of toxicity following repeated doses of disulfoton. Cholinesterase activity depression has been seen in acute oral studies where clinical signs of cholinergic toxicity were absent (Klaus 2006a, 2006b). Klaus (2006a) treated Wistar rats by gavage for 11 days, and cholinesterase activity inhibition was dose-dependent in both sexes. Red blood cell and brain AChE were both significantly inhibited 1 hour after final dosing in males exposed to ≥0.5 mg/kg/day and in females exposed to ≥0.25 mg/kg/day (Klaus 2006a). Lower doses were tested in rat pups exposed to 0, 0.06, 0.125, or 0.25 mg/kg/day for 11 days beginning on PND 11 (Klaus 2006b). Significant red blood cell AChE inhibition began at ≥0.25 mg/kg/day in male pups. Female pups were more sensitive as significant red blood cell AChE depression (29% inhibition) began at 0.06 mg/kg/day and was dose-dependent (Klaus 2006b).

In rats given 2 mg/kg/day disulfoton for 14 days, there was 81 and 28% inhibition of AChE and [^{3}H] quinuclidinyl benzilate binding in the cerebral cortex, respectively (Yagle and Costa 1996). After a 28-day recovery period, activity and binding restored to nearly that of controls. The study primarily examined the loss of muscarinic receptors (MR) corresponding to changes in mRNA levels, focusing on m1, m2, and m3 subtypes (Yagle and Costa 1996). The m1 and m3 subtypes activate phosphoinositide hydrolysis, and the m2 subtype is negatively coupled to adenylyl cyclase. In the hippocampus, m1 mRNA and m2 RNA levels decreased by 23 and 24%, respectively; m2 mRNA decreased by 19% in the medulla, and m3 mRNA levels in the cortex lowered by 10%. All decreases were significant, but all m1 and m3 mRNA levels recovered in all tissues following a 4 week recovery, while m2 RNA in the hippocampus remained decreased. The study concluded that disulfoton may lead to protein and mRNA down-regulation in only certain brain tissues that express a particular combination of MR subtypes (Yagle and Costa 1996).

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) F0 female rats, but not the mid-dose (0.03 mg/kg/day) F0 females, during the production of the F1 generation (Hixson and Hathaway 1986). Pregnant rats given disulfoton during gestation had significantly inhibited plasma and red blood cell AChE 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).

An intermediate-duration extensive neurotoxicity screening was conducted for 13 weeks in rats fed disulfoton in the diet that provided doses of 0.06, 0.27, or 1.08 mg/kg/day for males and 0.07, 0.32, or

1.3 mg/kg/day for females (Sheets 1993b). Clinical signs of cholinergic intoxication seen in males exposed to 1.08 mg/kg/day included muscle fasciculations, diarrhea, tremors, ataxia, urine stain (urine leakage), oral stain (indicating excess salivation), and red nasal stain (known as red tears), and females exposed to 1.3 mg/kg/day showed similar signs and decreased movement. Mid-dose females (0.27 mg/kg/day) showed muscle fasciculations only. A battery of functional observational tests revealed effects in high-dose males and mid and high-dose females, and these effects included tremors, increased defecation, decreased forelimb grip strength, and decreased movement. 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. Red blood cell AChE activity was inhibited by 95–100% in high-dose rats and 67–80% in mid-dose rats. Brain AChE 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.

Neurotoxicity has been observed in pregnant female rats exposed to disulfoton in feed (Klaus 2006c; Sheets 2005). In an extensive study, females were exposed on gestation days (GDs) 0–21 to 0, 0.038, 0.156, or 0.67 mg/kg/day, and then exposed to corresponding doses of 0, 0.102, 0.389, or 1.714 mg/kg/day on lactation days 0–21 (Sheets 2005). Red blood cell AChE was inhibited by 27% in the lowest dose group, and significantly inhibited by 73% in the mid-dose groups, which also showed a 65% inhibition of brain AChE. Clinical signs suggesting neurotoxicity were only seen in the highest dose group during lactation, including repetitive chewing movements, muscle fasciculations, and jerking movements (Sheets 2005). In a separate study, pregnant female rats were continuously exposed to 0, 0.042, 0.168, or 0.694 mg/kg/day from GD 0 to 20 (Klaus 2006c). While no clinical signs of neurotoxicity were observed, at 0.168 mg/kg/day, red blood cell AChE activity was inhibited by 44% and brain AChE activity was inhibited by 32%; inhibition was dose-dependent (Klaus 2006c).

Intermediate-duration studies in animals indicate tolerance to disulfoton is developed over time. Clinical signs of cholinergic toxicity appear initially and diminish while cholinesterase activity remains inhibited, which is characteristically observed in organophosphate pesticides (Costa and Murphy 1982). 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 diminished 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 AChE 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 red blood cells 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; 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, red blood cell AChE 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 red blood cell AChE activity and a 33–36% inhibition of plasma AChE activity was found in dogs given diets containing disulfoton at a dose of 0.06 mg/kg/day for 40 weeks (Hoffman and Welscher 1975). In Beagle dogs given disulfoton in feed for 1 year, there was 31–65% inhibition of plasma AChE beginning 1 week after exposure to 0.12 mg/kg/day (males) and 0.09 mg/kg/day (females) (Jones et al. 1999). In the same study, red blood cell AChE in both sexes exposed to ≥09 mg/kg/day was inhibited by 48–90% at 3 months of exposure and continued to decrease to 40–85% by the end of the study period. Additionally, significant brain AChE inhibition of 22–33% was observed in female Beagle dogs, and overall cholinesterase inhibition was more marked in female dogs compared to males in the lowest dose group (Jones et al. 1999). Neural examination of the dogs did not find any functional abnormalities in reflexes or task performance and no clinical neurological findings related to disulfoton administration.

Disulfoton has also been studied for behavioral effects. Rats fed ≥0.5 mg/kg/day disulfoton for 90 days had significantly depressed brain AChE 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 result (improved learning) at reduced brain AChE 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). This was

evaluated by the permeability of stained brain tissue slices to copper ferricyanide complex. 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 are not clear. In the same study, plasma, red blood cell, and brain AChE activity were significantly inhibited in both male and female rats. A dose of 0.1 mg/kg/day resulted in a 21% inhibition of brain AChE activity in female rats. At 0.05 mg/kg/day, brain AChE 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, red blood cell AChE 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 AChE 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. Significant depression of red blood cell, plasma, and brain AChE 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 and Welscher 1975). However, significant depression of plasma, red blood cell, and brain AChE activity occurred at 0.14 mg/kg/day 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 were observed in dogs given disulfoton (0.5–1.5 mg/kg/day) for 2 years (Uga et al. 1977). The study authors regarded the pathological changes in the retina as mild; however, the nerve fibers in the optic nerve were reduced in number.

A farmer who had worn disulfoton contaminated 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 AChE activity was severely depressed, it was 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, red blood cell AChE 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 AChE activity was observed in workers involved with wet mix operations (0.013 mg/kg/day) (Wolfe et al. 1978). No significant reductions in plasma or red blood cell AChE 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 a 39% inhibition of red blood cell AChE in female rats applied 50 mg/kg (0.5 mg a.i./kg) of disulfoton on 10% of body surface and left for 6 hours/day for 3 days (Croutch and Sheets 2000). Inhibition was less significant at 100 mg/kg (0.1 mg a.i./kg). Brain and red blood cell AChE increased dose dependently starting at 100 mg/kg in both male and female rats. Cholinesterase activity was measured 24 hours after the final third day exposure was applied. Females were more sensitive to disulfoton exposure, as maximum brain and red blood cell AChE inhibition were 72–74%, while only 32–42% in males (Croutch and Sheets 2000). No clinical signs of cholinesterase depression were seen at any dose. 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. In a range-finding study, two of two 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 five of five females after 1–6 treatments and five of five males after 3–10 treatments with 6.5 mg/kg/day (Flucke 1986). Persistent cholinergic signs (muscle spasm, dyspnea, and salivation) were observed in the high-dose females after 1 or two treatments and in high-dose males after two treatments. No clinical signs of cholinergic intoxication were seen in the rabbits treated with 0.4 or 1.6 mg/kg/day, but red blood cell AChE activity was inhibited by 21–33% in the female rabbits treated with 1.6 mg/kg/day (Flucke 1986). In a similar study, one male rabbit died exhibiting clinical signs of cholinesterase depression following exposure to 3 mg/kg/day disulfoton 6 hours/day, 5 days/weeks for 3 weeks (Flucke 1988). In male and female rabbits, red blood cell AChE inhibition increased dose-dependently after day 21, and was significant in males at 3 mg/kg/day (62% inhibition) and in females at ≥0.8 mg/kg/day (20–51%). Brain AChE activity

was measured at study terminations and was only significant for males and females exposed to 3 mg/kg/day, with 55% and 27% inhibition, respectively (Flucke 1988).

In a study investigating the mechanism of neurological effects, disulfoton was added to the brain tissue of Wistar rats (Smulders et al. 2004). Competition binding experiments showed that disulfoton resulted in inhibitory effects on nicotinic acetylcholine receptors, which may account for neurotoxic signs not explained by AChE inhibition. Disulfoton did not bind to agonist-recognition sites of rat neuronal α4β2 nicotinic acetylcholine receptors indicating a noncompetitive two-step mechanism. Inhibition may result from rapidly reversible association and dissociation of disulfoton binding to a separate binding site, followed by receptor desensitization (Smulders et al. 2004).

2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans after inhalation, oral, or dermal 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 the epididymides, prostate, seminal vesicles, testicles, cervix, mammary glands, ovaries, or uterus were found in rats exposed intermittently to ≤1.4 mg/m3 for 13 weeks (Shiotsuka 1989).

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 multi-generation study was conducted in male and female rats exposed to disulfoton in the diet at doses of 0.009, 0.03, and 0.09 mg/kg/day (Hixson and Hathaway 1986). At the 0.09 mg/kg/day dose, decreased reproductive performance occurred, evidenced by a decreased percentage of females placed for mating and decreased percentage of sperm-positive F0 and F1 parental females. In addition, decreased maternal weight of F0 and F1 dams during gestation and lactation, decreased litter counts, viability index, and lactation index, and increased stillbirths and percentage of stillbirths occurred in both generations at 0.09 mg/kg/day. A decrease in F2b litter counts and litter weights occurred at 0.03 mg/kg/day. Gross and

histological examination of the ovaries, vagina, uterus, testes, epididymides, seminal vesicles, and prostate of the F0 and F1 parents revealed no treatment-related lesions. In an intermediate-duration study, male and female rats given 0.5 mg/kg/day disulfoton in their diets 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). In pregnant rats exposed during GDs 0–21 and then lactation days 0–21, no treatment-related effects on fertility, gestation indices, or gestation length were observed at exposures to ≤1.714 mg/kg/day of disulfoton in feed (Sheets 2005). 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 and Welscher 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 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. Effects on reproductive function was not evaluated.

2.17 DEVELOPMENTAL

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

Pregnant rats given disulfoton on GDs 6–15 had decreased plasma and red blood cell AChE activity at ≥0.3 mg/kg/day (Lamb and Hixson 1983). Fetotoxic effects included increased incidences of incomplete ossified parietal bones and sternebrae 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, no effects on fetal survival, growth, or development were reported in this study. 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 given 0.5 mg/kg/day disulfoton in the diet for 60 days prior to mating and/or during mating resulted in a 32.1% depression in fetal brain AChE activity (Ryan et al. 1970).

There were no treatment-related effects on number of litters, live births, stillbirths, or viability in pregnant rats after exposure to 0, 0.038, 0.156, or 0.67 mg/kg/day during gestation days 0–21 (corresponding to doses of 0, 0.102, 0.389, or 1.714 mg/kg/day) during lactation (Sheets 2005). Additionally, offspring showed no treatment-related effects in the functional observational battery assessment or in learning and memory testing. In female offspring from mid- and high-dose dams, delayed vaginal opening was observed, and in high-dose female offspring, 56% inhibition of red blood cell AChE and 30% inhibition of brain AChE activity were noted (Sheets 2005). Similarly, in male offspring, significant inhibition was seen in the high-dose group, with red blood cell and brain AChE activity inhibited by 53 and 30%, respectively. A 17–18% depression in body weight gain was also seen in both sexes of offspring born to dams in the high-dose group, when compared to controls (Sheets 2005). In a 3-generation reproductive study in rats, cloudy swelling and fatty infiltration of the liver, mild nephropathy (females), and juvenile hypoplasia of the testes were observed in F3b litters (Taylor 1965a). These litters also had significantly depressed red blood cell AChE activities. In another multi-generation study in rats, brain AChE activity was inhibited by 24 and 32% in male and female F1a pups, respectively, at 0.03 mg/kg/day, and by 50% and 59% in male and female F1a pups, respectively, at 0.09 mg/kg/day (Hixson and Hathaway 1986). No inhibition of brain AChE was found in the F1a pups at 0.009 mg/kg/day, and no grossly observable developmental abnormalities were observed in any pups in the F1 or F2 generation. In offspring of dams exposed to 0, 0.042, 0.168, or 0.694 mg/kg/day in feed, red blood cell AChE inhibition was inhibited by 20% for mid-dose offspring, and by 83% in high-dose offspring; no clinical signs were noted (Klaus 2006c).

2.18 OTHER NONCANCER

No studies were located regarding other cancer effects in humans or animals after oral, inhalation, or dermal exposure to disulfoton.

2.19 CANCER

No studies were located regarding cancer effects in humans after oral exposure to disulfoton, and no studies were located regarding cancer effects in humans or experimental animals after dermal exposure to disulfoton. The EPA has classified disulfoton in Group E, indicating evidence of noncarcinogenicity for humans (EPA 2021). The National Toxicology Program (NTP) and the International Agency for Research on Cancer (IARC) have not assessed the carcinogenicity of disulfoton (IARC 2018; NTP 2016).

Two occupational exposure studies have not observed associations between occupational exposure to disulfoton and cancer in pesticide applicators; exposure in these studies assumed to be primarily inhalation. One study evaluated the risk of aggressive prostate cancer in a cohort of 20,923 licensed private pesticide applicators in Iowa and North Carolina from the Agricultural Health Study cohort (Pardo et al. 2020). In this cohort, the risk of aggressive prostate cancer was not increased in subjects previously exposed to disulfoton ("ever used;" n=1,776) compared to unexposed subjects ("never used;" n=19,147); hazard ratio (HR) 0.96, 95% CI 0.74–1.26. Major limitations of this study include self-reported historical use of pesticides and lack of measured exposure levels. In a much smaller study, medical examination of 30 floriculturist workers occupationally exposed to various pesticides, including disulfoton, found no evidence of increased cancer (Gómez-Arroyo et al. 2000). Female workers had been occupationally exposed for 10 years, and males for 1.5 years; however, exposure to disulfoton was not measured. Given the small sample size, these results cannot be generalized to other populations.

In a 13-week study in rats exposed intermittently to ≤1.4 mg/m^3, Shiotsuka (1989) reported that comprehensive histological examination of organs and tissues revealed no treatment-related neoplastic lesions. Chronic-duration inhalation studies, which would be more appropriate to assess possible carcinogenicity, were not located for disulfoton.

No histological evidence of a carcinogenic effect was observed in Sprague-Dawley rats fed ≤0.1 mg/kg/day disulfoton for 1.5–2.0 years (Carpy et al. 1975), in F344 rats fed ≤1.02 mg/kg/day disulfoton for 2 years (Hayes 1985), or in CD-1 mice fed in diet ≤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 and Welscher 1975). No further carcinogenicity studies were located.

2.20 GENOTOXICITY

No studies were located regarding genotoxicity of disulfoton in humans after oral or dermal exposure or in experimental animals after inhalation or dermal exposure.

Cytogenetic testing in epithelial cells found significant differences between 30 floriculturist workers (8 men exposed for 1.5 years and 22 women exposed for 10 years) reportedly occupationally exposed to disulfoton and other pesticides (organochlorines, carbamates and other organophosphates) compared to control workers (Gómez-Arroyo et al. 2000). The study showed that pesticide exposure induced alterations on cell proliferation kinetics as M2 cells decreased and M3 cells increased significantly compared to controls suggesting acceleration of the cell cycle. Additionally, the mitotic index was significantly higher in the exposed group. Higher frequency of micronuclei/100 cells in the exposed group (1.01; 0.38 in controls) suggested that pesticide exposure increased genetic damage (Gómez-Arroyo et al. 2000). The study authors reasoned that tissue is damaged at the chromosomal level and also underwent nuclear abnormalities of pyknosis (shrunken nuclei), karyolysis (nuclear dissolution), and karyorrhexis (nuclei disintegrated), which were confirmed in the study. In the same study, higher sister chromatid exchange (SCE) frequency in lymphocytes of peripheral blood was observed among exposed workers (mean SCE 7.1±0.17) compared to controls (mean SCE 4.0±0.1); no significant difference was seen between exposed males and females. Study results were concluded to not be affected by alcohol consumption or smoking status. However, the study was limited due to differential exposure between males and females in years of exposure and nature of agricultural work (Gómez-Arroyo et al. 2000). Additionally, workers were exposed to complex mixtures of chemicals; thus, it is not clear that study findings can be attributed to disulfoton exposure alone.

The results of *in vivo* studies are summarized in Table 2-4. Disulfoton did not induce micronuclei in the red blood cells 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 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 melanogaster* (EPA 1981; Sandhu et al. 1985; Waters et al. 1981, 1982).

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

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

– = negative results

Disulfoton has been tested in numerous types of *in vitro* assays mainly with negative results; all results are summarized in Table 2-5. Disulfoton was negative in most assays for reverse mutation in most strains of *Salmonella typhimurium* with or without metabolic activation (EPA 1980; 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 1980, 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 1980; Herbold 1983; Inukai and Iyatomi 1976; Sandhu et al. 1985; Waters et al. 1981, 1982).

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

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Prokaryotic organisms				
Salmonella typhimurium LT-2 strains	Reverse mutation	No data	+	Hanna and Dyer 1975
S. typhimurium TA 1535	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, EPA 1984a; Waters et al. 1981, 1982
TA1535		–	–	
TA1537		–	–	
TA1538		–	–	
TA98		–	–	
TA100		–	–	
S. typhimurium	Reverse mutation			Inukai and Iyatomi 1976
TA1535		–	–	
TA1537		–	–	
TA98		–	–	
TA100		–	–	
Escherichia coli WP2 strains	Reverse mutation	No data	+	Hanna and Dyer 1975
E. coli WP2 uvrA	Reverse mutation	–	–	EPA 1980, EPA 1984a; Sandhu et al. 1985; Waters et al. 1981, 1982
S. typhimurium SL4525(rec+)/ SL4700(rec–)	Differential toxicity	No data	–	EPA 1984a; 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

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

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Eukaryotic organisms				
Fungi:				
Saccharomyces cerevisiae D7	Reverse mutation	–	–	EPA 1984a; Sandhu et al. 1985; Waters et al. 1981, 1982
S. cerevisiae S138 S211	Reverse mutation	–	–	Jagannath 1981
S. cerevisiae D7	Gene conversion and mitotic crossing-over	–	–	Sandhu et al. 1985; Waters et al. 1981, 1982
S. cerevisiae D3	Induction of mitotic recombinants	–	–	EPA 1980; Sandhu et al. 1985
S. cerevisiae D3	Primary DNA damage	No data	–	Waters et al. 1981, 1982
S. cerevisiae D3	Mitotic non-disjunction	–	–	Brusick 1981
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	Chromosomal aberrations	+	–	Lynch et al. 2008
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 lymphoma cells L5T	Forward mutation	No data	–	Waters et al. 1981, 1982

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

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Human lung fibroblasts WI-38 cells	Unscheduled DNA synthesis	–	+	EPA 1980, EPA 1984a; Sandhu et al. 1985
Human hematopoietic cells 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

– = negative result; + = positive results; (+) = weakly positive result; DNA = deoxyribonucleic acid; HGPRT = hypoxanthine-guanine phosphoribosyl transferase; RNA = ribonucleic acid

Negative results were obtained in assays for reverse mutation, gene conversion, mitotic crossing over and recombinants, and primary deoxyribonucleic acid (DNA) damage in eukaryotic yeast, *Saccharomyces cerevisiae*, with and without activation (Brusick 1981; EPA 1984a; Jagannath 1981; Sandhu et al. 1985; Waters et al. 1981, 1982). On the other hand, 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. Chromosomal aberrations were positive in Chinese hamster ovary cells with metabolic activation; however, they were negative without activation (Lynch et al. 2008). Positive or weakly positive results were obtained for SCEs 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 SCEs 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, unscheduled DNA synthesis in human lung fibroblasts (EPA 1984a; Sandhu et al. 1985; Waters et al. 1981, 1982), and 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 alterations of DNA or ribonucleic acid (RNA) synthesis in human HeLa cells (Litterst et al. 1969).

The genotoxicity of disulfoton has been reviewed (Brusick 1981; EPA 1980, 1984a; Herbold 1983; Inukai and Iyatomi 1976; Jagannath 1981; Moriya et al. 1983; Sandhu et al. 1985; Waters et al. 1981, 1982), and results are primarily negative in numerous tests for reverse mutation, and differential toxicity in bacteria, with or without activation, assays for mutagenic activity, gene conversion, mitotic crossing, and DNA damage in the yeast were also negative with or without activation. There is no evidence of chromosomal aberrations in different human cell lines. In mammalian cells, the test results were mixed; forward mutations for mouse lymphoma cells without but not with activation. Overall, the current database suggests that disulfoton has little to no genotoxicity potency.