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 diisopropyl methylphosphonate. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

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

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

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

effects and “serious” effects is considered to be important because it helps 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 anti/or duration, and place into perspective the possible significance of these effects to human health.

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

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

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

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

2.2.1 Inhalation Exposure

No studies were located regarding the following health effects in humans or animals after inhalation exposure to diisopropyl methylphosphonate.

2.2.1.I Death

2.2.1.2 Systemic Effects

2.2.1.3 Immunological and Lymphoreticular Effects

2.2.1.4 Neurological Effects

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

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

Genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

No studies were located regarding carcinogenic effects in bumans or animals after inhalation exposure to diisopropyl methylphosphonate.

2.2.2 Oral Exposure

No data are available regarding health effects in humans after oral exposure to diisopropyl methyl-phosphonate. However, data are available regarding animals.

Results of these studies are discussed below and presented in Table 2-1 and Figure 2- 1.

TABLE 2-1. Levels of Significant Exposure to Diisopropyl Methylphosphonate - Oral

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Rat (Sprague-Dawley)	once (GW)				1125 M (LD_{50})	Hart 1976
						826 F (LD_{50})	
2	Rat (Fischer- 344)	once (G)				1000 M (3/5 died)	DOD 1991b
3	Rat (Fischer- 344)	3 d 1x/d (G)				800 M (3/7 died)	DOD 1991b
4	Mouse (Swiss-Webster)	once (GW)				1041 M (LD_{50})	Hart 1976
						1363 F (LD_{50})	
5	Mouse (B6C3F1)	3 d 1x/d (G)				2000 M (5/5 died)	DOD 1991a
6	Mink	once (G)				503 F (LD_{50})	Aulerich et al. 1979
7	Cow	once (C)				1000 (2/2 died)	Cysewski et al. 1981; Palmer et al. 1979
8	Duck (Mallard)	once (G)				1500 (3/12 died)	Jones et al. 1992
9	Duck (Mallard)	once (G)				1490 (LD_{50})	Aulerich et al. 1979

TABLE 2-1. Levels of Significant Exposure to Diisopropyl Methylphosphonate - Oral (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)		LOAEL (effect) Serious (mg/kg/day)		Reference
	Systemic								
10	Rat (Charles River COBS CD [SD] BR)	4 d (F)	Hepatic		150	(induction of microsomal enzymes leading to decreased hexobarbital sleeping time)			Hart 1976
			Bd Wt	150					
11	Rat (Sprague-Dawley)	10 d Gd 6-15 (F)	Bd Wt	300					Hart 1980
12	Rat (Sprague-Dawley)	once (GW)	Resp	632			928	(hyperemia of the lungs)	Hart 1976
13	Mouse (Swiss-Webster)	once (GW)	Resp	2000					Hart 1976
14	Dog (Beagle)	14 d (F)	Resp	38					Hart 1976
			Cardio	38					
			Hemato	38					
			Hepatic	38					
			Renal	38					
			Bd Wt	38					

TABLE 2-1. Levels of Significant Exposure to Diisopropyl Methylphosphonate - Oral (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
15	Cow	once (C)	Resp	500		1000 (pulmonary emphysema)	Cysewski et al. 1981; Palmer et al. 1979
			Cardio	500		1000 (petechiae in the coronary grooves)	
			Gastro	500		1000 (acute gastroenteritis; ecchymotic hemorrhaging)	
			Renal	63	500 (mild congestion of the renal cortex)		
			Bd Wt	1000			
16	Duck (Mallard)	once (G)	Cardio		1500 (decrease in blood pressure)		Jones et al. 1992
17	Duck (Mallard)	once (G)	Bd Wt	1700	1800 (weight loss of 14.8%)		Aulerich et al. 1979
18	Duck (Mallard)	8 d (F)	Bd Wt	1007		1796 (57% decrease in body weight gain)	Aulerich et al. 1979
	Neurological						
19	Rat (Sprague-Dawley)	once (GW)				430 (ataxia; decreased activity; prostration)	Hart 1976
20	Mouse (Swiss-Webster)	once (GW)				430 (decreased activity; prostration)	Hart 1976
21	Mouse (B6C3F1)	3 d 1x/d (G)				1000 M (coma)	DOD 1991a
22	Mink	once (G)		150 F		300 F (salivation; lethargy; immobilization)	Aulerich et al. 1979

TABLE 2-1. Levels of Significant Exposure to Diisopropyl Methylphosphonate - Oral (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)		Reference
23	Cow	once (C)		500		1000	(tympanitis; ataxia; depression; prostration; engorgement of meningeal vessels; excess fluid in cerebral ventricles)	Cysewski et al. 1981; Palmer et al. 1979
	INTERMEDIATE EXPOSURE							
	Systemic							
24	Rat (Sprague-Dawley)	90 d (F)	Cardio	300				Hart 1976
			Hemato	300				
			Hepatic	300				
			Renal	300				
			Ocular	300				
			Bd Wt	300				
25	Rat (Sprague-Dawley)	30 wk (3 generations) (F)	Bd Wt	300				Hart 1980
26	Mouse (Swiss-Albino)	90 d (F)	Resp	273				Hart 1976
			Hepatic	273				
			Renal	273				
			Bd Wt	273				
27	Dog (Beagle)	90 d (F)	Cardio	75				Hart 1980
			Hemato	75[b]				
			Hepatic	75				
			Renal	75				
			Ocular	75				
			Bd Wt	75				

TABLE 2-1. Levels of Significant Exposure to Diisopropyl Methylphosphonate - Oral (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)		LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
28	Mink	21 d	Resp	17		201 M (decreased lung weights)		Aulerich et al. 1979
		(F)	Cardio	201		1852 M (decreased heart weights)		
			Hemato	17		201 (decreased lymphocytes)		
			Hepatic	201		1852 M (decreased liver weights)		
			Renal	201		1852 M (decreased kidney weights)		
			Bd Wt	1852				
29	Mink	49 wk	Resp	95				Aulerich et al. 1979
		(F)	Cardio	95				
			Gastro	95				
			Hemato	95				
			Musc/skel	95				
			Hepatic	95				
			Renal	95				
			Bd Wt	95				
30	Mink (Ranch Wild)	90 d (F)	Hemato	345	M	747 M (shortened RBC survival; increased Heinz bodies; increased reticulocytes; reduced RBC counts)		Bucci et al. 1992, 1994
				455	F	907 F (shortened RBC survival; increased Heinz bodies; increased reticulocytes; reduced RBC counts)		
			Hepatic	1009	M			
				1264	F			
			Ocular	1009	M			
				1264	F			
			Bd Wt	1009	M			
				1264	F			

TABLE 2-1. Levels of Significant Exposure to Diisopropyl Methylphosphonate - Oral (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)		LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
31	Mink (Ranch Wild)	1-11 mo ad lib (F)	Hemato	45	M	262 M (increased Heinz body counts)		Bucci et al. 1997
				57	F	330 F (decreased RBC; increased reticulocytes, MCV, Heinz bodies)		
			Bd Wt	262	M			
				330	F			
	Neurological							
32	Rat (Sprague-Dawley)	90 d (F)		300				Hart 1976
33	Dog (Beagle)	13 wk 7d/wk (F)		75				Hart 1980
34	Mink	21 d (F)		1852				Aulerich et al. 1979
35	Mink	49 wk (F)		95				Aulerich et al. 1979
36	Mink (Ranch Wild)	1-11 mo ad lib (F)		262	M			Bucci et al. 1997
				330	F			
	Reproductive							
37	Rat (Sprague-Dawley)	30 wk (3 generations) (F)		300				Hart 1980
38	Mink	49 wk (F)		95				Aulerich et al. 1979
39	Mink (Ranch Wild)	1-11 mo ad lib (F)		262	M			Bucci et al. 1997
				330	F			

TABLE 2-1. Levels of Significant Exposure to Diisopropyl Methylphosphonate - Oral (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)		LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
	Developmental							
40	Rat (Sprague-Dawley)	Gd 6-15 (F)		300				Hart 1980
41	Mink (Ranch Wild)	1-11 mo ad lib (F)		45 330	M F	262 M	(decreased RBC in F2 male kits at 6 weeks)	Bucci et al. 1997
	Immuno/Lymphor							
42	Mink	21 d (F)		1852				Aulerich et al. 1979
43	Mink	49 wk (F)		95				Aulerich et al. 1979
44	Mink (Ranch Wild)	1-11 mo ad lib (F)		262 330	M F			Bucci et al. 1997
	CHRONIC EXPOSURE							
	Systemic							
45	Mink (Ranch Wild)	13 mo ad lib (F)	Hemato Bd Wt	57 [c] 330	F F	330 F	(increased Heinz bodies)	Bucci et al. 1997
	Neurological							
46	Mink (Ranch Wild)	13 mo ad lib (F)		330	F			Bucci et al. 1997
	Reproductive							
47	Mink (Ranch Wild)	1-11 mo ad lib (F)		330	F			Bucci et al. 1997

TABLE 2-1. Levels of Significant Exposure to Diisopropyl Methylphosphonate - Oral (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
	Immuno/Lymphor						
48	Mink (Ranch Wild)	13 mo ad lib (F)		330 F			Bucci et al. 1997

[a]The number corresponds to entries in Figure 2-1.

[b]An intermediate-duration Minimal Risk Level (MRL) of 0.8 mg/kg/day was derived based on this end point; dose of 75 mg/kg/day divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]A chronic-duration Minimal Risk Level (MRL) of 0.6 mg/kg/day based on this end point; dose of 57 mg/kg/day divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ad lib = ad libitum; Bd Wt = body weight; (C) = gelatin capsule; Cardio = cardivascular; d = day(s); F = female; (F) = feed; (G) = gavage (type unspecified); Gastro = gastrointestinal; Gd = gestation day(s); (GW) = gavage in water; Hemato = hematological; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; MCV = mean cell volume; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; RBC = red blood cells; Resp = respiratory; wk = week(s); x = times(s)

Figure 2-1. Levels of Significant Exposure to Diisopryl Methylphosphonate - Oral

Acute (≤14 days)

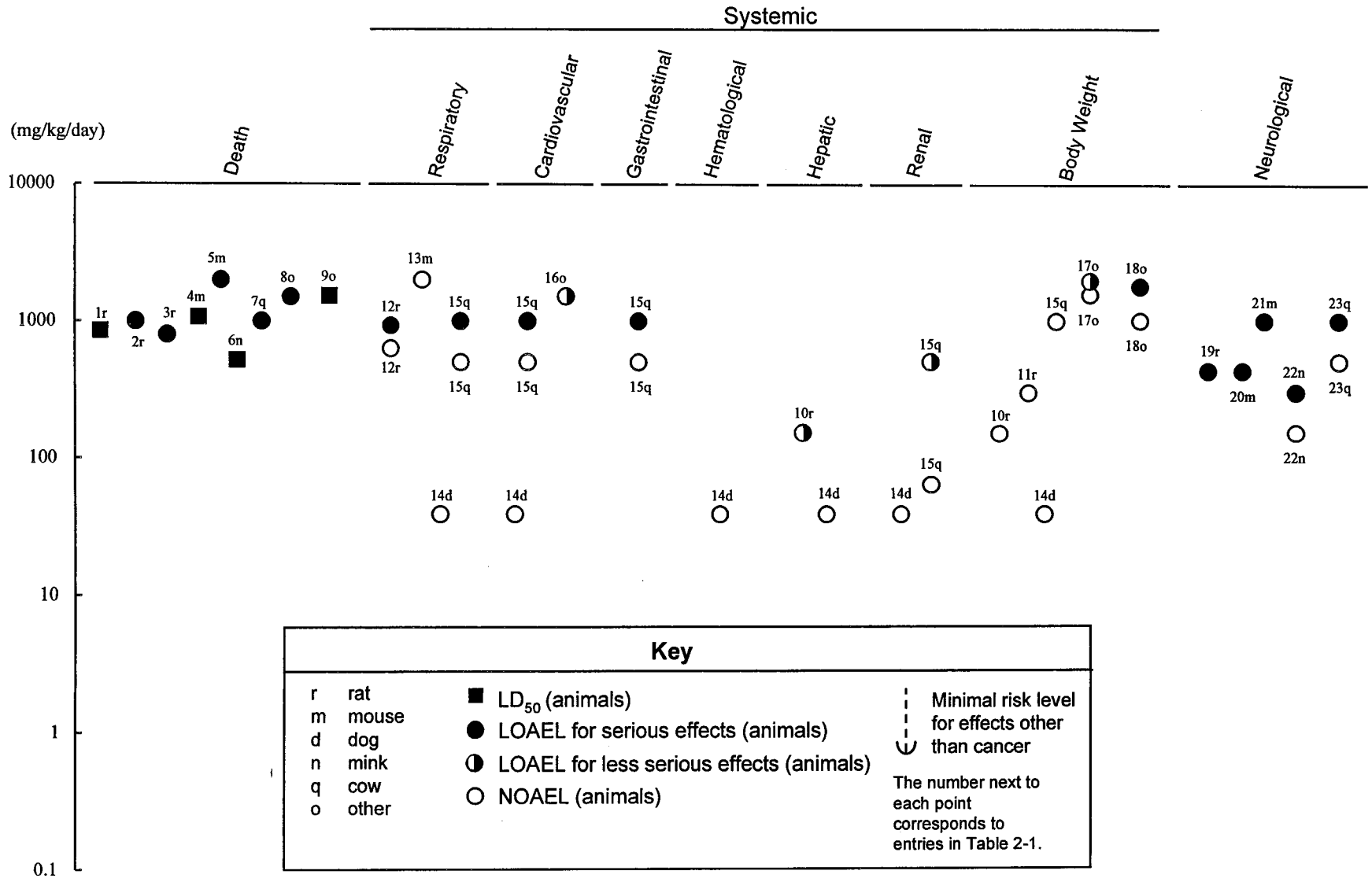


Figure 2-1. Levels of Significant Exposure to Diisopryl Methylphosphonate - Oral (cont.)

Intermediate (15-364 days)

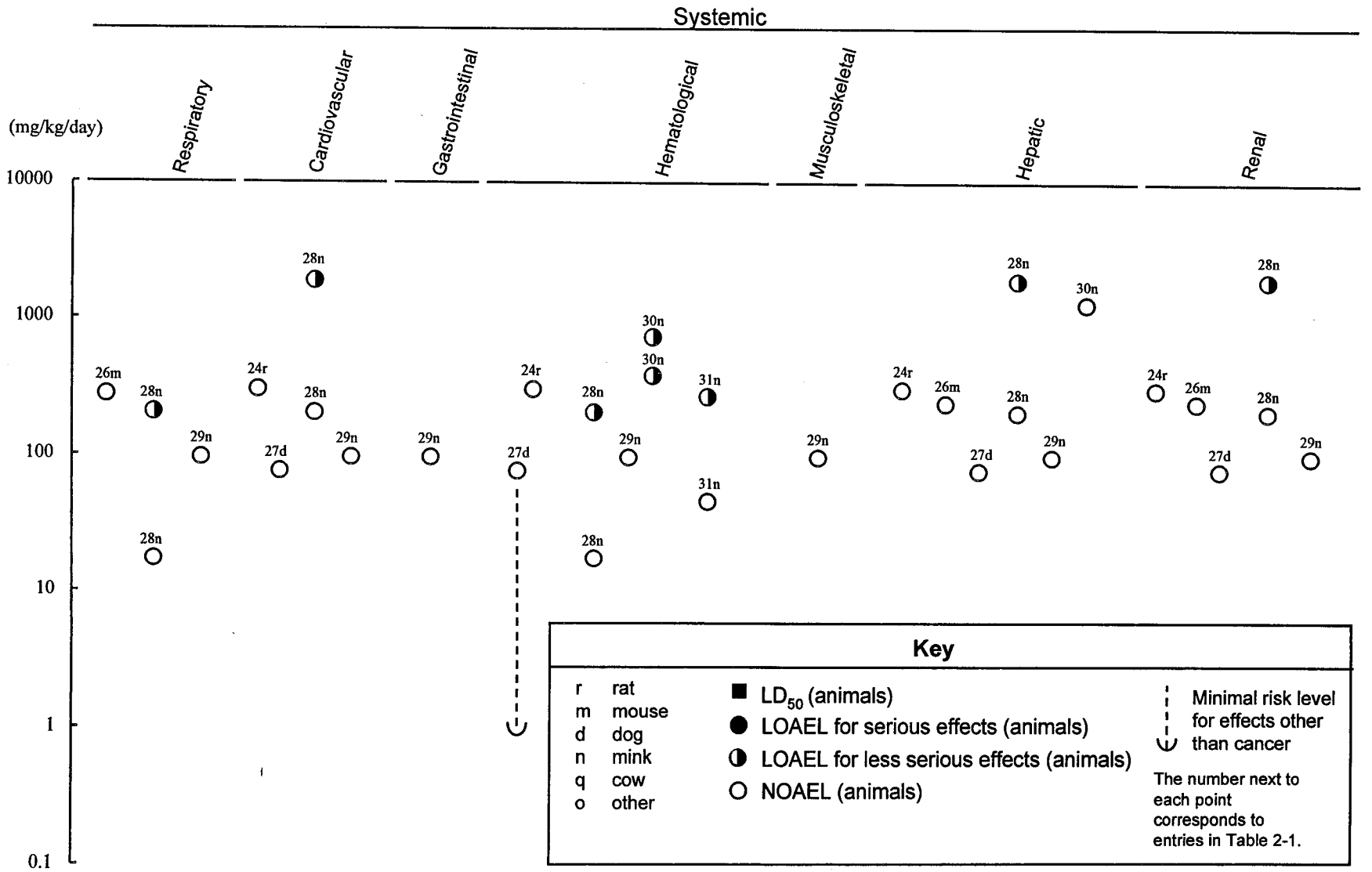


Figure 2-1. Levels of Significant Exposure to Diisopryl Methylphosphonate - Oral (cont.)

Intermediate (15-364 days)

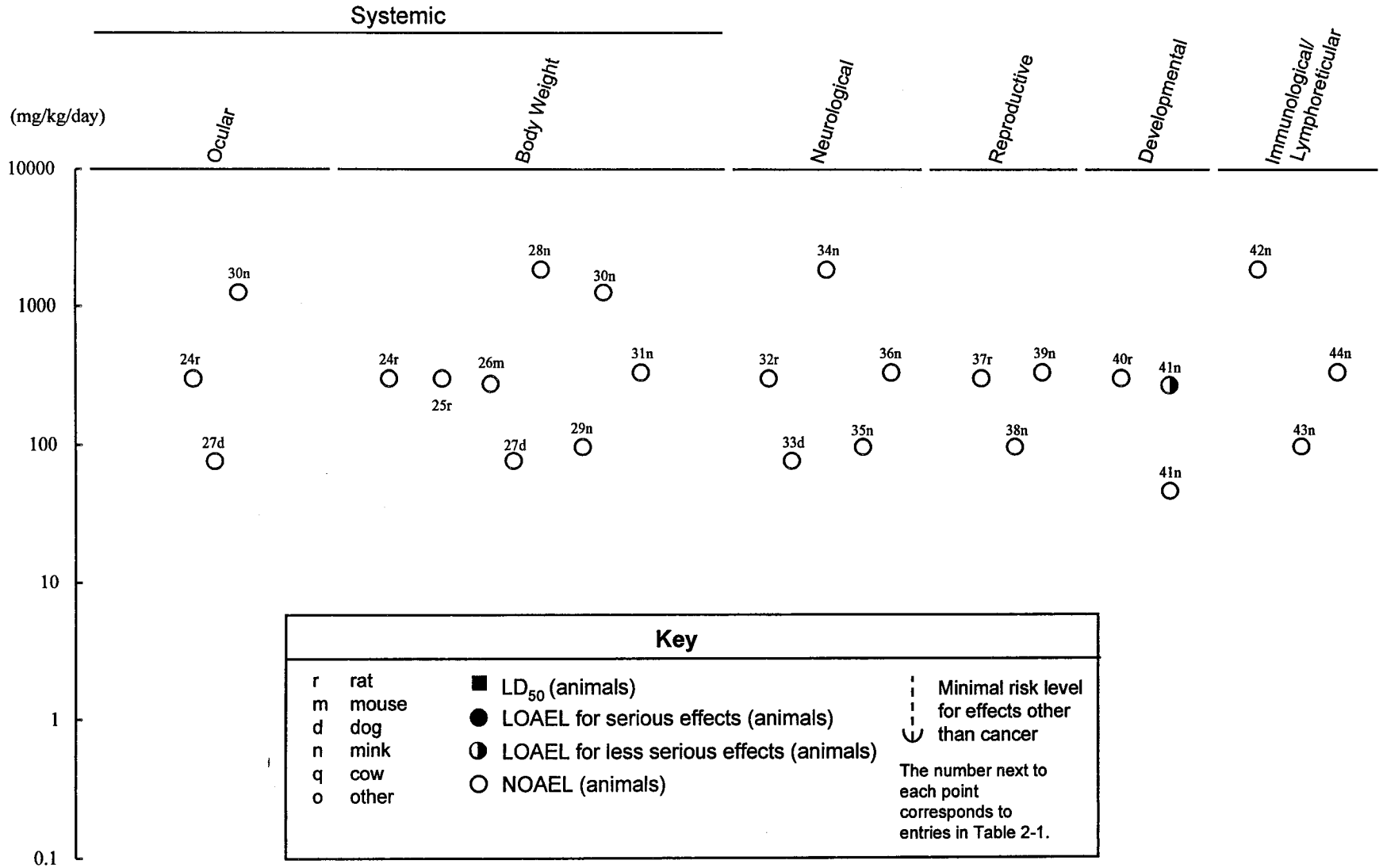
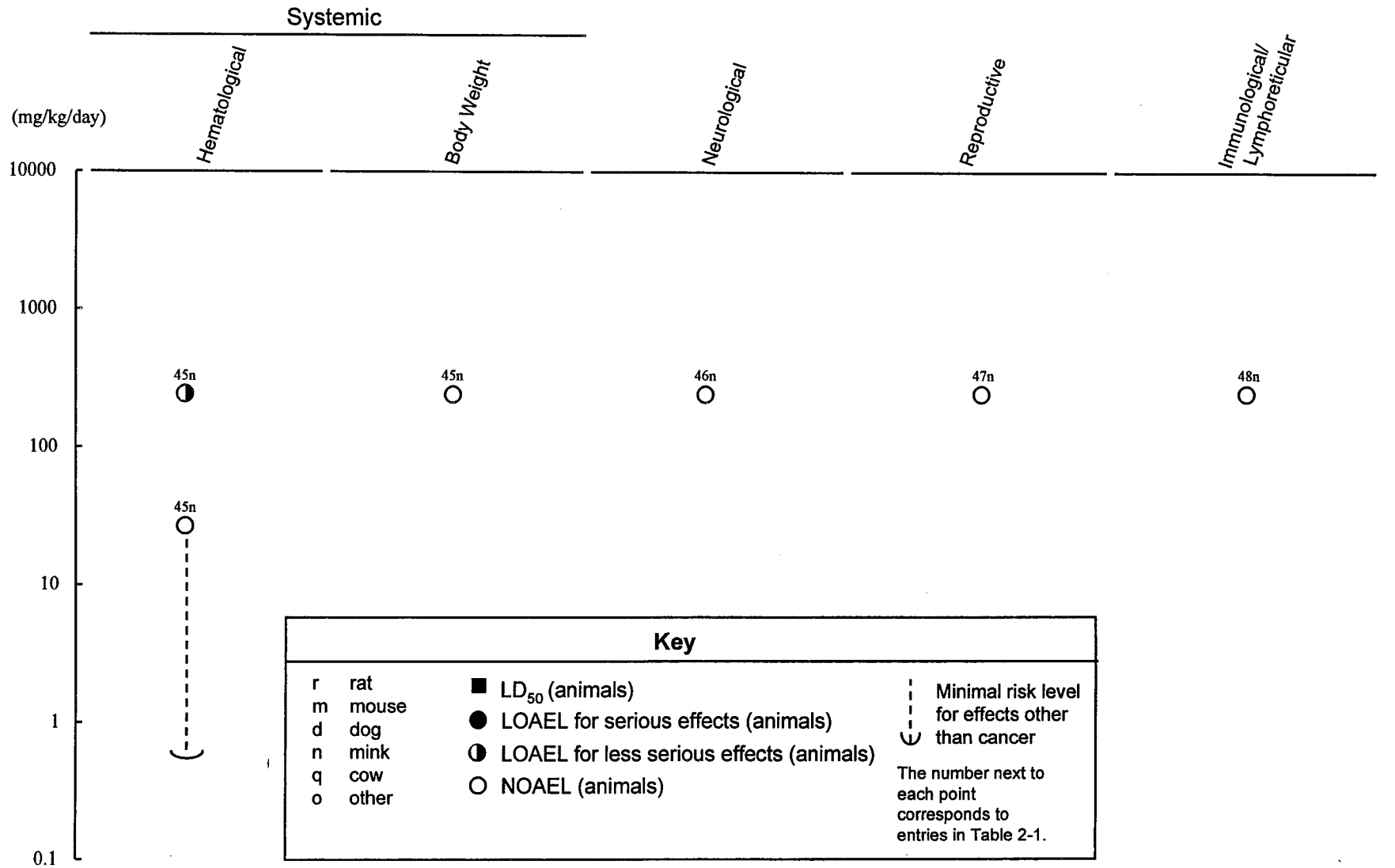


Figure 2-1. Levels of Significant Exposure to Diisopryl Methylphosphonate - Oral (cont.)

Chronic (≥365 days)



2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to diisopropyl methylphosphonate.

Three of five male Fisher-344 rats dosed once with 1,000 mg/kg diisopropyl methylphosphonate by gavage died (DOD 1991b). In a micronucleus assay using this species, 3 of 7 rats dosed on 3 successive days with 800 mg/kg died, but no deaths occurred at the next lower dose, 400 mg/kg (DOD 1991b). A repeat of this experiment in which rats were dosed with 400,600, or 800 mg/kg for 3 days resulted in the deaths of 2 of 7 high-dose rats (DOD 1991 b). Oral LD_{50}s of 1,125 and 826 mg/kg have been reported for male and female rats, respectively (Hart 1976). Single doses of diisopropyl methylphosphonate (430, 632, 928, 1,362, and 2,000 mg/kg) dissolved in polyethylene glycol 400 were administered by gastric intubation to 10 male and 10 female Sprague-Dawley rats at each dose level. The animals were examined for 14 days. By the day following the administration of the compound, all 10 males in the 1,362-mg/kg group and 9 of 10 males in the 2,000-mg/kg group had died (the remaining animal in the 2,000-mg/kg group died the following day). Similarly, all 10 females in the 1,362-mg/kg group and 9 of 10 females in the 2,000-mg/kg group had died (the remaining female died on day 2). Further, on the first 2 days subsequent to dosing 8 of 10 females but none of the 10 males in the 928-mg/kg group died. No deaths occurred in males or females administered 430 or 632 mg/kg. Signs of intoxication in both sexes included decreased activity, occasional ataxia, and prostration (Hart 1976).

Parallel experiments in Swiss Webster mice yielded LD_{50}s of 1,041 and 1,363 mg/kg in males and females, respectively (Hart 1976). Mortalities included 2 of 10 females in the 430-mg/kg group, 2 of 10 females and 3 of 10 males in the 928-mg/kg group, 3 of 10 females and 9 of 10 males in the 1,362-mg/kg group, and all 10 males and all 10 females in the 2,000-mg/kg group. As is the case with rats, nearly all deaths occurred on the 1st day following gavage treatment with diisopropyl methylphosphonate. Signs of intoxication included decreased activity and prostration (Hart 1976). In a range-finding study for a bone marrow micronucleus assay, 5 of 5 male mice receiving 2,000 mg/kg for 3 days by gavage died, but no deaths occurred in mice receiving 1,000 mg/kg (DOD 1991a).

An LD_{50} of 503 mg/kg was calculated from the results of a study in which adult female mink were administered single doses of diisopropyl methylphosphonate by gavage (doses of 75, 150, 300, 450, 500,

550, and 600 mg/kg) (Aulerich et al. 1979). Deaths occurred within a few hours after dosing. Animals that did not die recovered completely within several hours after administration of the compound.

In a 14-day lethality study of calves dosed with diisopropyl methylphosphonate at 62.5, 125, 250, 500, or 1,000 mg/kg via gelatin capsules gavaged using a balling gun, both of the two calves in the highest dose group died (Cysewski et al. 1981; Palmer et al. 1979). No animals in any of the other dose groups died. The study authors calculated the LD_{50} to be approximately 750 mg/kg.

An LD_{50} of 1,490 mg/kg was derived from a study using Mallard ducks in which groups of 10 of each sex were given a single dose of diisopropyl methylphosphonate by gavage at doses that ranged from 1,300 to 1,800 mg/kg and were observed for 14 days (Aulerich et al. 1979). The study authors attributed the deaths of many ducks to drowning on the copious amounts of saliva that were generated following exposure rather than true systemic toxicity. Necropsy showed no gross pathological changes.

No mortality occurred, nor were there judged to be any signs of toxicity, in beagle dogs receiving diisopropyl methylphosphonate in the diet for 2 weeks at 0, 4, 13, or 38 mg/kg/day (each dose group consisted of one male and one female) (Hart 1976). In a developmental toxicity study, no deaths occurred in female rats receiving diisopropyl methylphosphonate in the diet at 0, 10, 30, or 300 mg/kg/day on days 6-15 of gestation (Hart 1980).

No significant mortality related to diisopropyl methylphosphonate was noted in groups of 5 male and 5 female juvenile pastel mink receiving dietary doses of 0.2, 2, 17, 201, or 1,852 mg/kg/day for 21 days (Aulerich et al. 1979). A comparison of this study with the LD_{50} study in mink suggests that diisopropyl methylphosphonate is more toxic following gavage administration than following administration in the diet.

No mortality was noted in male or female rats that received diisopropyl methylphosphonate in drinking water at 0.0090 or 0.9 mg/kg/day for 13 weeks (males) or 19 weeks (females) (Hardisty et al. 1977). EPA (1989) indicated that actual doses could not be verified, considered the study inappropriate for human health risk assessment, and rejected it for use in the development of a hazard advisory. No mortality occurred and no toxic effects were noted in beagle dogs (4 per sex per dose group) that received diisopropyl methylphosphonate in the diet (0, 4, 38, or 75 mg/kg/day) for 13 weeks (Hart 1980).

No deaths or evidence of toxicity were attributable to diisopropyl methylphosphonate administered for 26 weeks in the drinking water of rats at concentrations of 0.6 ppb, 6.0 ppb, 10 ppm, and 1,000 ppm ($6.6x10^{-7}$, $6.6x10^{-5}$, 0.011, and 1.1 mg/kg/day, respectively) (Army 1978). It should be noted that there is some confusion concerning the concentration units used in this study (EPA 1989). EPA (1989) states that conversions between ppm and mg/L were incorrectly calculated using the air conversion factor. EPA (1989) also indicates that analysis of the diisopropyl methylphosphonate used in this study determined that it was only 65% pure. Therefore, results from the Army (1978) study are considered inappropriate for human health risk assessment. No deaths of adult rats were recorded in a three-generation study of reproductive effects in rats receiving diisopropyl methylphosphonate in the diet at 0, 30, or 300 mg/kg/day (Hart 1980).

In a 90-day toxicity study in which 179 mice received diisopropyl methylphosphonate in the diet at doses of 0,27,9 1, or 273 mg/kg/day, two deaths occurred in the 91 mg/kg/day male group. Since no deaths were observed in the 273-mg/kg/day group and no other signs of toxicity were observed, it was concluded that there was no evidence of toxicity in this 90-day study (Hart 1976). Four male rats died (1 control, 1 low-dose, and 2 high-dose animals) out of a total of 256 animals in a 90-day study, in which rats received diisopropyl methylphosphonate in the diet at doses of 0, 30, 100, or 300 mg/kg/day. The deaths were neither dose nor duration related and were not considered of toxicologic importance (Hart 1976).

In a reproductive study in which groups of 25 female and 6 male dark variety mink were fed diisopropyl methylphosphonate in the diet at 0, 11, 37, or 95 mg/kg/day for approximately 49 weeks, an increase in deaths occurred in females that was statistically significant at the high dose (Aulerich et al. 1979). No control females died, while 2 of 23, 3 of 24, and 5 of 24 died at the low, middle, and high doses, respectively. The first death that occurred was in the lowest dose group. No adverse symptoms were noted before the deaths occurred. Three of the five deaths at the high dose occurred between the time of mating and lactation. Among males, 1 of 6 controls and 1 of 6 treated at 11 mg/kg/day died. All male mink treated at the higher doses survived.

Although the increase in deaths in female mink was statistically significant at the high dose, it was not clear if the deaths were treatment related. In a concurrent study that was conducted to assess the toxicity of dicyclopentadiene, which used mink from the same lot, the mortality in the untreated female mink was 4 of 24, with 2 mink dying between the time of mating and lactation. It should also be noted that mink have a relatively high natural mortality (EPA 1989). The natural mortality for 1st-year mink in a commercial fur ranch operation approaches 6% annually, and up to 15% of lactating females may die in the late gestation

period from "nursing sickness" (Schneider and Hunter 1993). Although it is not clear when the mink in the Aulerich et al. (1979) study died in relation to lactation, some female mink did die during the time period that included lactation. Further evidence that the deaths of mink may not have been related to diisopropyl methylphosphonate treatment comes from a second study in mink (Bucci et al. 1992, 1994). In this 90-day study, no mortality was observed in male or female mink receiving diisopropyl methylphosphonate in the diet at doses of 0, 7, 63, 345, 749, or 1,009 mg/kg/day for males and 0, 9, 82, 455, 908, or 1,264 mg/kg/day for females. Although the Bucci et al. (1992, 1994) study lacks a breeding period, it was a better controlled study than the Aulerich et al. (1979) study. In the Bucci et al. (1992, 1994) study, diisopropyl methylphosphonate was measured in the diet, food intake was measured weekly, and the mink were housed in a laboratory. In the Aulerich et al. (1979) study, diisopropyl methylphosphonate dietary levels were not measured, food intake was estimated only once every 2 weeks, and the animals were housed outside in pens.

Recently, a two-generation reproductive study in mink was performed by Bucci et al. (1997). In the parental (F_0) generation, groups of 7 male and 35 female brown Ranch Wild mink were fed diisopropyl methylphosphonate in the diet at 0, 15,47, or 285 mg/kg/day (males) or 0, 26, 85, or 460 mg/kg/day (females) for 4 weeks (males) or 4 months (females). Two groups of control animals were used. However, the study authors noted that the DlMP consumption calculated for the F_1 generation should be used for extrapolation to humans, instead of the values obtained for F_0 animals, because body weight and food consumption were not monitored for F_0animals until they were brought inside from the farm at 8 months of age. Furthermore, the F_1 data offers a more conservative estimate, because the exposure was over a longer period of time. Although 3.5% (6/175) of the F_0 females (1 control, 2 low dose, 2 mid dose, and 1 high dose) died before the scheduled sacrifice, all of the animals except 1 mid dose animal showed signs of a stress syndrome characterized by stopping eating, lethargy, and weight loss that are associated with mink.

In the F_1 generation, 13 males and 35 females were used per group. Concentrations of diisopropyl methylphosphonate in feed corresponded to 0, 16,45, or 262 mg/kg/day (males) or 0, 20, 57, or 330 mg/kg/day (females) for either 8 months (males) or 13 months (females). Again, two groups of control animals were used. In the F_1 generation, 4.6% (8/175) of the females died before the scheduled sacrifice. Six of these (1 in each control group, 1 each in the low- and mid-dose groups, and 2 in the high-dose group) died of a stress syndrome common in minks that was induced by a slight anesthesia overdose. One mid-dose male also died of the same syndrome. A 7th female from the low-dose group, which was prone to seizures after being stressed during procedures such as cage cleaning, died unexpectedly after such a seizure. The 8th animal, a high-dose female who had shown signs of stress syndrome, was found to have a ruptured uterus.

Because the deaths either occurred early in the study, were randomly distributed among dose groups, or were associated with a stress syndrome common among mink, they are not believed to result from DIMP exposure. Thus, it appears that the deaths observed in the previous study by Aulerich et al. (1979) were not treatment related.

2.2.2.2 Systemic Effects

The highest NOAEL and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 2-1 and plotted .in Figure 2-1.

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

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to diisopropyl methylphosphonate.

The respiratory system does not appear to be a target of diisopropyl methylphosphonate. Exposure has only occasionally resulted in respiratory effects, and the effects did not appear to be dose or treatment related. Necropsy of both male and female rats that died as the result of a single dose (928, 1,362, or 2,000 mg/kg) of diisopropyl methylphosphonate, administered by gastric intubation, revealed some hyperemia of the lungs; however, most animals displayed no abnormalities (Hart 4976). Changes in the lungs were not observed in rats that survived treatment at 928 mg/kg or at lower doses (430, 632 mg/kg). No abnormal necropsy findings were noted in Swiss Webster mice dosed similarlly (430, 632, 928, 1,362, 2,000 mg/kg) in a companion study (Hart 1976). No important abnormalities were noted in the necropsy of rats receiving diisopropyl methylphosphonate in the diet (0, 30, 100, or 300 mg/kg/day) for 90 days (Hart 1976). No noteworthy deviations were noted in the necropsy or histopathology examination of male or female beagles receiving dietary doses of 0, 4, 13, or 38 mg/kg/day for 14 days (Hart 1976). No gross lesions were noted in rats subsequent to receiving drinking water containing diisopropyl methylphosphonate at doses of $6.6x10^{-7}$, $6.6x10^{-5}$, 0.011, or 1.1 mg/kg/day (Army 1978). However, as discussed in Section 2.2.2.1, there is some confusion concerning the concentration units and purity of the diisopropyl methylphosphonate used in the Army (1978) study (EPA 1989). Therefore, results from the Army (1978) study are considered inappropriate for human health risk assessment.

In a study of calves dosed with diisopropyl methylphosphonate at 62.5, 125, 250, 500, or 1,000 mg/kg via gelatin capsules placed with a balling gun, calves at the highest dose level displayed pulmonary emphysema upon autopsy (Palmer et al. 1979). No respiratory effects were observed at sublethal dose levels.

Male juvenile pastel mink receiving diisopropyl methylphosphonate in the diet at doses of 201 or 1,852 mg/kg/day for 21 days demonstrated significantly lower lung weights (toxicity is usually, but not always, associated with increased lung weight), although there were no significant changes observed during necropsy (Aulerich et al. 1979). The study autbors concluded that the compound was nontoxic in the 21-day test. Respiratory effects were not observed in mink treated with diisopropyl methylphosphonate in the diet at doses of 95 mg/kg/day for 49 weeks (Aulerich et al. 1979).

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

Mean blood pressure was decreased in male and female Mallard ducks given a single dose of 1,500 mg/kg of diisopropyl methylphosphonate via proventricular intubation (Iones et al. 1992). The proventriculus is a glandular stomach that precedes the gizzard in birds. However, pulse pressure and heart rate were not affected. Therefore, it is likely that cardiac output and/or resistance were decreased. The study authors speculated that diisopropyl methylphosphonate acts in a mechanism similar to that of meprobamate and glycerol guaiacolate (two psychotropic agents), i.e., by depressing or blocking nerve impulse transmission at the internuncial neuron level of the spinal cord, brain stem, and subcortical areas of the central nervous system.

There is no evidence from animal studies that diisopropyl methylphosphonate directly affects the cardiovascular system except at very high doses (Palmer et al. 1979). No anomalous necropsy or histopathological effects were noted in rats that received diisopropyl methylphosphonate in the diet at doses of 0, 30, 100, or 300 mg/kg/day for 90 days (Hart 1976). Similarly, no abnormalities were noted in mice that received the compound in the diet at doses of 0, 27, 91, or 273 mg/kg/day for 90 days (Hart 1976). In this study, beagle dogs were also treated with the compound in the diet at doses of 0, 4, 13, or 38 mg/kg/day for 14 days (Hart 1976). No deviation in heart weight or abnormal histopathological findings were reported in beagles that received diisopropyl methylphosphonate in the diet at doses of 0, 4, 38, or 75 mg/kg/day for 13 weeks (Hart 1980).

In a study of calves given a single dose of diisopropyl methylphosphonate at 62.5, 125, 250, 500, or 1,000 mg/kg via gelatin capsules placed with a balling gun, animals that died at the highest dose level displayed petechiae in the coronary grooves and ecchymotic hemorrhaging of the gastrointestinal tract upon autopsy (Palmer et al. 1979). No cardiovascular effects were observed at sublethal dose levels.

No significant changes in heart weight or histopathological findings were noted in mink receiving diisopropyl methylphosphonate in the diet at doses of 0.2, 2, 17,201, or 1,852 mg/kg/day for 21 days (Aulerich et al 1979). Gross and histopathological examination of mink that received 11, 37, or 95 mg/kg/day for 49 weeks revealed no consistent pathological changes (Aulerich et al. 1979).

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

In a study of calves dosed with diisopropyl methylphosphonate at 62.5, 125, 250, 500, or 1,000 mg/kg via gelatin capsules placed with a balling gun, calves that died at the highest dose level displayed acute gastroenteritis and ecchymotic hemorrhaging upon necropsy (Palmer et al. 1979). No gastrointestinal effects were observed at sublethal dose levels.

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

Hematological effects were noted in several animal studies. A few instances of significant differences were noted in the hematocytology (red blood cell [BBC] count, packed cell volume, hemoglobin, leukocyte count, and differential leukocyte count) in rats that had received diisopropyl methylphosphonate in the diet at doses of 0, 30, 100, or 300 mg/kg/day for 90 days. However, because the differences were so scattered and lacked clear dose response, they were considered of no toxicological importance (Hart 1976). Beagles that had been treated with the compound in the diet at doses of 4, 13, or 38 mg/kg/day for 14 days demonstrated values that were within normal limits for hemoglobin, hematocrit, BBC count, total leukocyte count, and differential leukocyte count (Hart 1976). In a 3-month study of beagles receiving diisopropyl methylphosphonate in the diet at concentrations that provided doses of 0, 4, 38, or 75 mg/kg/day, no clear dose-related adverse toxicological changes were noted in erythrocyte count, leukocyte count, differential leukocyte count, hemoglobin, packed cell volume, clotting time, blood glucose, or albumin (Hart 1980). Based on the NOAEL

of 75 mg/kg/day for hematological effects in dogs, an intermediate-duration oral MRL of 0.8 mg/kg/day was derived as described in the footnote in Table 2-l a

In a study of calves given a single dose of diisopropyl methylphosphonate at 62.5, 125, 250, 500, or 1,000 mg/kg via gelatin capsules placed with a balling gun, no hematological effects were observed at any dose level (Palmer et al. 1979).

Juvenile pastel mink that ingested 201 or 1,852 mg/kg/day diisopropyl methylphosphonate in food for 21 days showed a significantly depressed hematocrit (Aulerich et al. 1979). A significantly lower percentage of lymphocytes in peripheral blood was also noted in mink after ingestion of 2,201, or 1,852 mg/kg/day in the diet for 21 days. Mink that received food containing the compound at 17 mg/kg/day did not demonstrate a change in lymphocytes. The decreased hematocrit probably resulted from RBC damage and subsequent RBC clearance. This would explain the increase in reticulocytes and Heinz bodies noted in other studies. Increased hematocrit was noted in male dark variety mink treated with 37 or 95 mg/kg/day diisopropyl methylphosphonate in feed for 49 weeks, but not in males treated with 11 mg/kg/day or in females in any of the treatment groups. No differences in hemoglobin concentration or mean corpuscular hemoglobin were noted in either sex in any of the treatment groups (Aulerich et al. 1979).

Compared to controls, male dark brown Ranch Wild mink that were fed standard ranch diet containing diisopropyl methylphosphonate at doses of 1,009 mg/kg/day for 90 days had a significantly lower hematocrit at week 13, and lower hemoglobins at weeks 3, 7, and 13 (Bucci et al. 1994). Females that consumed 908 or 1,264 mg/kg/day had significantly lower hematocrit than the controls starting at week 3. There were no significant changes in mean erythrocyte cell volume, mean cell hemoglobin, or mean cell hemoglobin concentration. The mean number of reticulocytes in females treated at 1,264 mg/kg/day and in males at 747 mg/kg/day was significantly increased compared to the controls. The females that received 908 or 1,264 mg/kg/day had a significant increase in platelets (averaged across all time points) compared to the controls. Inconsistent changes in erythrocyte morphology were noted in the 3 highest dose groups (345, 747, 1,009 mg/kg/day for males; 455, 908, 1,264 mg/kg/day for females). All erythrocyte anomalies were normal by week 3 after treatment. Both males and females in the highest dose groups demonstrated a significant increase in Heinz bodies from week 3 through week 13, and at week 13 the females receiving 908 mg/kg/day showed a significant increase in Heinz bodies. Although not statistically significant, the number of Heinz bodies in males at 345 mg/kg/day and in females at 455 mg/kg/day was increased relative to the controls. Heinz bodies are inclusions in the RBCs resulting from the irreversible denaturation and precipitation of

hemoglobin. An increase in Heinz bodies causes the cells to be less deformable, have a reduced lifespan, and be vulnerable to splenic phagocytes and thus induces a predisposition for the RBCs to become trapped in the splenic sinuses and destroyed by phagocytes. The Heinz body data suggest that diisopropyl methylphosphonate may cause oxidative damage to the tertiary structure of hemoglobin, thus reducing the lifespan of RBCs, which in turn ultimately induces hematopoiesis. A marginal increase in the incidence and severity of splenic extramedullary hematopoiesis was observed in male mink exposed to 1,009 mg/kg/day of diisopropyl methylphosphonate in the diet for 90 days (Bucci et al. 1994). The RBC data and the reticulocyte data are consistent with a hematopoietic response to increased RBC destruction. The study authors indicated that the observed hematological changes suggested that diisopropyl methylphosphonate or its metabolites had oxidant properties that resulted in the denaturation of hemoglobin and shortened RBC lifespan (Bucci et al. 1992).

Similar results were observed in a two-generation study of Ranch Wild brown mink. Males were fed 0, 16, 45, or 262 mg/kg/day diisopropyl methylphosphonate for 8 months, and females were fed 0, 20, 57, or 330 mg/kg/day diisopropyf methylphosphonate for 13 months (Bucci et al. 1997). In the F_0 generation, no changes in hematologicaf parameters were observed in males. However, in F_0-generation females fed 330 mg/kg/day, RBC counts were significantly decreased, and reticulocyte counts, Heinz body counts, and mean cell volume were significantly increased. In the F_1-generation females fed 330 mg/kg/day diisopropyl methylphosphonate, Heinz body counts were increased at 6 and 13 months. Males fed this dose also had increased Heinz body counts. High-dose F_1 male kits bad significantly decreased RBC counts at 6 weeks of age, but this effect was not observed at the age of 4.1 weeks. The increased Heinz body counts observed in this study correlated with a decrease in RBC survival, which was shown by the decreased RBC count. These effects also correlate with the histopathological findings in the spleen of these animals, in which evidence of RBC replacement was observed. Based on the NOAEL of 57 mg/kg/day for hematological effects in mink in this study, a chronic-duration oral MRL, of 0.5 mg/kg/day was derived as described in the footnote in Table 2- 1.

Mallard ducks fed up to 10,000 ppm diisopropyl methylphosphonate in feed for up to 24 weeks showed no significant effects on blood hemoglobin levels, leukocyte count, and hematocrit (Aulerich et al. 1979). Doses could not be calculated in mg/kg/day because food intake and body weights were not measured in this study.

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

There is no evidence that diisopropyl methylphosphonate causes toxic effects in the liver or biliary systems. No changes of toxicologic significance were noted in serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase, liver weight, or liver histopathology in rats treated with diisopropyl methylphosphonate in the diet at doses of 0, 30, 100, or 300 mg/kg/day for 90 days (Hart 1976). No hepatic histopathological abnormalities or significant changes in liver weight were found in mice that received doses of 0, 27, 91, or 273 mg/kg/day diisopropyl methylphosphonate in food for 90 days (Hart 1976). No abnormal hepatic lesions or changes in SGPT, SGOT, or alkaline phosphatase were found in beagles treated with diisopropyl methylphosphonate in the diet at doses of 0,4,13, or 38 mg/kg/day for 14 days (Hart 1976). Rats pretreated with diisopropyl methylphosphonate in the diet (450 mg/kg/day for 4 days) showed a marked decrease in sleep time subsequent to the administration of hexobarbital, which is consistent with the induction of the P-450 mixed-function oxidase system. Liver weights, however, were not increased (Hart 1976). No changes in SGOT, SGPT, alkaline phosphatase, liver histopathology, or liver weight were reported in male or female beagles that received 0, 4, 38, or 75 mg/kg/day diisopropyl methylphosphonate in food for 90 days (Hart 1980).

Juvenile male pastel mink that ingested 1,852 mg/kg/day diisopropyl methylphosphonate for 21 days showed a significant decrease in liver weight; however, no associated lesions were noted (Aulerich et al. 1979). The study authors indicated that the decreases in organ weights noted in this high-dose group may be related to significantly reduced food consumption and were not necessarily a toxic response. Since a pair-fed control was not maintained for this study, the causes of the organ weight differences were not easily identifiable. Finally, the authors considered the compound nontoxic in this 21-day study (Aulerich et al. 1979). No gross or histopathological hepatic lesions or decreases in liver weight were noted in male and female dark variety mink that received diisopropyl methylphosphonate in feed at doses of 0, 11, 37, or 95 mg/kg/day for 49 weeks (Aulerich et al. 1979). No lesions were noted in the livers of Ranch Wild mink in a 90-day dietary study in which males received doses of 0, 7, 63, 345, 747, or 1,009 mg/kg/day and females received doses of 0, 9, 82, 455, 908, or 1,264 mg/kg/day (Bucci et al. 1992, 1994). The absence of hepatic lesions and the healthy appearance of the animals throughout the 90-day study are in agreement with the results reported by Aulerich et al. (1979).

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to diisopropyl methylphosphonate.

In a study of calves given a single dose of diisopropyl methylphosphonate at 62.5, 125, 250, 500, or 1,000 mg/kg via gelatin capsules placed with a balling gun, animals at the 500-mg/kg and higher dose levels displayed mild congestion of the renal cortex upon necropsy (Palmer et al. 1979). All calves at 1,000 mg/kg died.

Diisopropyl methylphosphonate does not appear to cause renal effects in laboratory animals. In a study of mink that were fed 1,852 mg/kg/day for 21 days, significant kidney weight loss but no renal pathology occurred (Aulerich et al. 1979). The study authors indicated that the decreases in organ weights noted in this high-dose group may be related to significantly reduced food consumption and were not necessarily a function of toxicity. In a 90-day study, small but significant increases in blood urea nitrogen were observed in Ranch Wild mink fed diisopropyl methylphosphonate in the diet at 747 and 1,009 mg/kg/day for males and 908 and 1,264 mg/kg/day for females, with no increase at 345 mg/kg/day in males and 455 mg/kg/day in females (Bucci et al. 1992). Microscopic examination of the kidneys of the mink did not reveal any treatment-related lesions (Bucci et al. 1992, 1994).

No deviations from normal were noted in the urinalyses (color, specific gravity, pH, sugar albumin, ketones, and microscopic examination of sediment), weights, or histopathology of kidneys in rats given diisopropyl methylphosphonate in the diet at doses of 0, 30, 100, or 300 mg/kg/day for 90 days (Hart 1976). No abnormal renal lesions (gross or histopathological) or changes in kidney weight were noted in mice fed chow with 0, 27, 91, or 273 mg/kg/day diisopropyl methylphosphonate for 90 days (Hart 1976). In beagle dogs given 0, 4, 13, or 38 mg/kg/day diisopropyl methylphosphonate in the diet for 14 days, urinalyses (pH, specific gravity, glucose, ketones, and total protein) resulted in values within normal limits, and no gross or histopathological renal lesions were noted (Hart 1976). No toxicologically significant anomalies in kidney weight, kidney-body weight ratios, or pathology of the kidneys were noted in dogs that received dietary diisopropyl methylphosphonate for 90 days at doses up to 75 mg/kg/day (Hart 1980). No gross diisopropyl methylphosphonate-related renal lesions were noted in rats that had received the compound in drinking water ($6.6x10^{-7}$, $6.6x10^{-5}$, 0.011, 1.1 mg/kg/day) for 26 weeks (Army 1978). However, as discussed in Section 2.2.2.1, there is some confusion concerning the concentration units and purity of the diisopropyl methylphosphonate used in the Army (1978) study (EPA 1989), and therefore the results are considered inappropriate for human health risk assessment.

Ocular Effects. No studies were located regarding ocular effects in humans after oral exposure to diisopropyl methylphosphonate.

An ophthalmic examination was performed during week 12 of a 13-week study of rats that received diisopropyl methylphosphonate in food (0, 30, 100, or 300 mg/kg/day); 12 of 64 animals displayed some opacity of the lens (Hart 1976). Although the finding was considered by the pathologist to be normal for the rat, the study author suggested that lens opacity may deserve attention in longer studies. No changes were noted in the eyes of beagle dogs that received diisopropyl methylphosphonate in their food (0, 4, 38, or 75 mg/kg/day) for 90 days (Hart 1980). Bucci (1997) reported no clinical observations related to treatment in mink exposed to up to 262 mg/kg/day (males) or 330 mg/kg/day (females) diisopropyl methylphosphonate in feed. The eyes were specifically examined at necropsy.

Body Weight Effects. No studies were located regarding body weight effects in humans after oral exposure to diisopropyl methylphosphonate.

Decreased body weight was observed in several animal studies; however, the decreases appeared related to the unpalatability of the food and to decreased food consumption rather than to a direct toxic effect. Rats that received diisopropyl methylphosphonate (0, 30, 100, or 300 mg/kg/day) in their diet for 90 days showed occasional significant changes in weight; however, the changes were inconsistent and judged not to be of toxicological significance (Hart 1976). Although individual weights of mice were not recorded, the study author indicated that it seemed “clear that growth was alike in all groups” of mice that had ingested diisopropyl methylphosphonate in the diet (0, 27, 91, or 273 mg/kg/day) for 90 days (Hart 1976). Calves given a single dose of 500 mg/kg diisopropyl methylphosphonate by gelatin capsule showed no significant body weight effects during the 14-day observation period (Palmer et al. 1979). Body weights of beagles fed diisopropyl methylphosphonate (0, 4, 13, or 38 mg/kg/day) for 14 days fluctuated within the confines of what was considered to be normal for adult dogs (Hart 1976). Rats receiving diisopropyl methylphosphonate in drinking water (0, 0.009, or 0.9 mg/kg/day) showed no apparent changes in weight after 10 weeks (Hardisty et al. 1977). However, as discussed in Section 2.2.2.1, there is some confusion regarding the actual doses used in this study. Therefore, results from Hardisty et al. (1977) are considered inappropriate for human health risk assessment. No changes in growth rate as measured by weight were noted in rats that received diisopropyl methylphosphonate in drinking water for 26 weeks (Army 1978), but as previously noted in Section 2.2.2.1, there is some confusion concerning the concentration units and purity of the diisopropyl

methylphosphonate used in the Army (1978) study (EPA 1989). Therefore, results from this study are considered inappropriate for human health risk assessment. Pregnant female rats that were administered diisopropyl methylphosphonate in the diet (0, 10, 30, 100, or 300 mg/kg/day) during days 6-15 of gestation showed no difference in mean body weight compared to controls (Hart 1980). No differences were noted in the body weight of rats in the first generation of parents in a three-generation study, after the rats received diisopropyl methylphosphonate in the diet (0, 30, or 300 mg/kg/day) for a reproductive cycle and the 12 weeks preceding the cycle. However, second-generation parents that received 300 mg/kg/day diisopropyl methylphosphonate had a significant decrease in body weight lasting from week 4 to week 9 (Hart 1980). No significant differences were reported among beagle dogs that were fed chow containing diisopropyl methylphosphonate (0,4,38, or 75 mg/kg/day) for 90 days (Hart 1980).

Significant weight loss was noted in male and female pastel mink that received diisopropyl methylphosphonate in their feed at a dose of 1,851 mg/kg/day for 21 days (Aulerich et al. 1979). A significant reduction in food consumption was also noted in this high-dose treatment group. Subsequent to treatment (during the recovery period), the group displayed increased food consumption suggesting that the weight loss may have been due to decreased food consumption resulting from a palatability problem and was not a function of toxicity. To determine if palatability of the feed was the cause of the weight loss, an attempt was made to "pair feed" a control group to match voluntary food consumption (Bucci et al. 1994). Similar to the findings of Aulerich and coworkers (1979), male and female mink receiving feed containing diisopropyl methylphosphonate at doses of 1,009 mg/kg/day for males and 1,264 mg/kg/day for females for 21 days consumed significantly less food than did the controls and demonstrated decreased body weight throughout the study. Although only partially successful, the pair-feed data for the high-dose male group (1,009 mg/kg/day) provided substantial evidence that the weight loss was the result of decreased food consumption rather than diisopropyl methylphosphonate toxicity (Bucci et al. 1994). No differences in body weight or in the percentage change in body weight were found in mink receiving diisopropyl methylphosphonate in feed at doses of 0, 11, 37, or 95 mg/kg/day for 49 weeks (Aulerich et al. 1979). Similarly, no significant changes in food consumption or mean body weight were observed in any generation of mink fed 0, 16, 45, or 262 mg/kg/day (males) or 0, 20, 57, or 330 mg/kg/day (females) diisopropyl methylphosphonate in the diet compared to controls for up to 8 or 13 months, respectively, in a two-generation reproductive study (Bucci et al. 1997). However, it should be noted that F_0 females consumed almost 50% more feed than F_1 females in this study. The study authors suggested that there may have been food wastage in the F_0 generation or that because the F_0 generation was 8 months old when they arrived in the laboratory from the ranch they may

have retained more of their ranch leanness, whereas because the F_1 animals were housed in the laboratory throughout their lives they may have become heavier on less food (Bucci et al. 1997).

Aulerich et al. (1979) reported a 14.8% weight loss in Mallard ducks given a single gavage dose of 1,800 mg/kg of diisopropyl methylphosphonate. Twelve-day-old Mallard ducklings dosed with 1,796 or 2,062 mg/kg/day of diisopropyl methylphosphonate in their feed for 5 days, followed by a 3-day normal feed period, showed significant decrease in body weight gain and food consumption (Aulerich et al. 1979). However, the study authors speculated that this decrease was due to the animals refusing to eat the feed that contained very high percentages of the chemical instead of from a loss of appetite from toxic effects. Following the dosing period, animals consumed an amount inversely proportional to the decreases in food consumption observed during the dosing period, indicating a lack of residual effects on appetite. Female Mallard ducks that received diisopropyl methylphosphonate (beginning 10 weeks prior to egg production) in their feed at a concentration of 1,000 ppm or more lost significantly less weight than the controls. Food intake and body weights were not measured in this study, and therefore, mg/kg/day doses could not be calculated.

2.2.2.3 Immunological and Lymphoreticular Effects

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

Histological changes in the spleen related to diisopropyl methylphosphonate intake were not observed in male or female rats exposed to 1 mg/kg/day of diisopropyl methylphosphonate in their drinking water for 26 weeks (Army 1978). As discussed in Section 2.2.2.1, there is some confusion concerning the concentration units and purity of the diisopropyl methylphosphonate used in the Army (1978) study (EPA 1989), and therefore results from the Army (1978) study are considered inappropriate for human health risk assessment. No changes in spleen weight were noted in male or female mink exposed to 1,851 mg/kg/day of diisopropyl methylphosphonate in the diet for 21 days (Aulerich et al. 1979). Similarly, spleen weight measurements and gross and histopathological examinations did not reveal any significant immunological or lymphoreticular effects in male or female mink exposed to 95 mg/kg/day of diisopropyl methylphosphonate in the diet for 12 months (Aulerich et al. 1979). A marginal increase in the incidence and severity of splenic extramedullary hematopoiesis was observed in male mink exposed to 1,009 mg/kg/day of diisopropyl methylphosphonate in the diet for 90 days (Bucci et al. 1994). No adverse effects were observed in mink exposed to 747

mg/kg/day. Similarly, female mink fed 330 mg/kg/day, but not 57 mg/kg/day, diisopropyl methylphosphonate in the diet for 4 months exhibited significant increases in absolute and relative spleen weight and the ratio of spleen-to-brain weight (Bucci et al. 1997). This was accompanied by splenic hematopoietic cell proliferation. The splenic extramedullary hematopoiesis in both studies is probably due to erythrocyte damage and shortened survival of erythrocytes rather than a direct lymphoreticular effect.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to diisopropyl methylphosphonate.

Single-dose oral toxicity studies in both rats and mice indicated that signs of diisopropyl methylphosphonate intoxication included decreased activity, occasional ataxia, and prostration within 1-4 hours after dosing (Hart 1976). Acute toxicity studies on Mallard duclcs (Aulerich et al. 1979) treated orally with doses of up to 1,800 mg/kg diisopropyl methylphosphonate demonstrated that the most notable clinical sign was salivation. Acute studies in female minks at doses up to 600 mg/kg (Aulerich et al. 1979) indicated clinical signs included salivation, lethargy, myasthenia, immobilization, vomiting, and death. Male $B6C3F_1$ mice treated with 1,000 mg/kg diisopropyl methylphosphonate for 3 days were lethargic or comatose after each treatment, but recovered within an hour (DOD 1991a). Calves that received a single dose of 1,000 mg/kg diisopropyl methylphosphonate in a gelatin capsule suffered neurological effects including depression, ataxia, tympanitis, engorgement of the meningeal vessels, excess fluid in cerebral ventricles, and prostration followed by death within 2 hours after treatment (Palmer et al. 1979). Mink that received 1,852 mg/kg/day diisopropyl methylphosphonate in the feed for 21 days displayed aggressive behavior (Aulerich et al. 1979). However, the study authors concluded that this behavior was probably due to hunger resulting from the unpalatability of the feed.

Inconsistent fluctuations were observed in RBC or plasma cholinesterase activity in rats that received diisopropyl methylphosphonate in the diet (0, 30, 100, or 300 mg/kg/day) for 90 days (Hart 1976). The most significant difference was a decrease in plasma cholinesterase activity in the high-dose female group at 13 weeks. The study author pointed out, however, that the control group value was exceptionally high compared to that of other time intervals (Hart 1976). In a 90-day study of dogs using diisopropyl methylphosphonate in the diet (0, 4, 38, or 75 mg/kg/day), a slight increase in plasma cholinesterase was observed; however, problems with the experimental protocol (failure to measure cholinesterase at 4 and 8

weeks) make any interpretations difficult (Hart 1980). Dark brown Ranch Wild mink that ingested 375, 742, or 1,009 mg/kg/day (males) or 455, 908, or 1,264 mg/kg/day (females) demonstrated decreased plasma cholinesterase compared to the controls, although the change was rapidly reversible. No effect was noted in erythrocyte cholinesterase activity. The study authors indicated that plasma cholinesterase is labile and sufficiently unrelated to the nervous system and that, therefore, the observed changes in the low-dose groups were toxicologically unimportant. Further, they noted that, even in the high-dose groups with large decreases (61%) in plasma cholinesterase, there was no reduction in erythrocyte cholinesterase or signs of acetylcholinesterase inhibition (Bucci et al. 1992, 1994). Similar results were also observed in a two-generation reproductive study using brown Ranch Wild mink that ingested 0, 16, 45, or 262 mg/kg/day (males) or 0, 20, 57, or 330 mg/kg/day (females) (Bucci et al. 1997). No changes in brain acetylcholinesterase were observed in either the F_1 or F_2 offspring. Decreases (42%) in plasma cholinesterase were observed in F_0 females at 4 months, and F_1 females at 4 (22%), 8 (21 %), and 13 (31%) months of age at high doses. These effects were not observed in males or F_2 kits. No significant changes or biological significance (>20%) were observed in RBC or whole blood cholinesterase in any group or generation. There were no clinical signs of acetylcholinesterase inhibition observed in any generation or treatment group in this study. Based on the studies discussed, it appears that diisopropyl methylphosphonate may inhibit plasma cholinesterase but not RBC cholinesterase.

Consistent decreases in plasma cholinesterase may not have been observed in rats and dogs because they were treated with lower doses of diisopropyl methylphosphonate. In general, depression of plasma cholinesterase, also known as pseudocholinesterase or butyrylcholinesterase, is considered a marker of exposure rather than an adverse effect. Depression of cholinesterase activity in red blood cells (acetylcholinesterase) is a neurological effect thought to parallel the inhibition of brain acetylcholinesterase activity. It is considered an adverse effect. Acetylcholinesterase is found mainly in nervous tissue and erythrocytes. Diisopropyl methylphosphonate was not found to inhibit RBC cholinesterase at doses at which plasma cholinesterase was significantly inhibited. Thus, although the 330-mg/kg/day dose in females appears to be a lowest-observable-effect level (LOAEL) for plasma cholinesterase inhibition and 57 mg/kg/day females or 262 mg/kg/day in males appears to be a no-observable-effect level (NOAEL) for this end point based on the study by Bucci et al. (1997), it is not a biologically significant change.

2.2.2.5 Reproductive Effects

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

In a single-generation reproductive study, male and female rats received diisopropyl methylphosphonate in the drinking water for 10 weeks at doses of 0, 0.009, or 0.9 mg/kg/day (Hardisty et al. 1977). Dosing continued during gestation and lactation in females. There were no differences in male fertility (calculated by the number of females that became pregnant) among the treatment groups and controls. No differences were noted in the litter sizes among those treated and the controls. No differences were noted in the number of stillborn pups or in pup weights. The study authors concluded that there was no evidence of adverse diisopropyl methylphosphonate-induced reproductive effects. However, as discussed in Section 2.2.2.1, there is some confusion regarding the actual doses to which the animals were exposed in the Hardisty et al. (1977) study. Therefore, results from this study are considered inappropriate for human health risk assessment.

In a three-generation reproductive study, male and female rats received diisopropyl methylphosphonate in their feed (0, 30, or 300 mg/kg/day) for 11 weeks before being mated with animals of the same dose group in the 12th week (Hart 1980). Dosing continued during gestation and lactation. A week after lactation of the F_{1A} pups, the F_0 females were remated with a different male. A week after lactation of the F_{1B} pups, the F_0 parents were sacrificed and necropsied. Male and female F_{1B} animals were selected and mated as above. Similarly F_{2B} offspring were mated, yielding third-generation (F_{3A} and F_{3B}) offspring. No differences in male virility or female fertility were noted in the F_0 and F_1 parents, and no differences in newborn viability or pup weights were noted in the F_1 and F_2 offspring. A significant number of pup losses were noted in the F_{3A} offspring from the 300-mg/kg/day group; however, since the Posses were not observed in the second mating (F_{3B} offspring), the losses were probably not related to treatment. Further, pup appearance and gross examination at necropsy did not reveal any evidence of diisopropyl methylphosphonate-related effects in the F_{3A} or F_{3B} pups, although the histopathological changes were apparently not evaluated. No significant differences in body weight and food consumption among the F_0 (parent), F_{1B} and F_{2B} generations were observed. Necropsy observations did not indicate any dose-dependent relationships of diisopropyl methylphosphonate in the feed at doses of 30 or 300 mg/kg/day in the rat in 3 successive generations with 2 matings per generation (Hart 1980).

Reproductive toxicity in mink was assayed in a 49-week feed study (Aulerich et al. 1979). Male and female dark variety mink received feed containing diisopropyl methylphosphonate at doses of 0, 11, 37, or 95 mg/kg/day. Male fertility, estimated by the presence of sperm in post-coital vaginal aspirations, was not adversely affected. Further, no significant differences were noted in whelping dam and kit performance, kit mortality, kit weight, or the body weight of lactating females at 4 weeks post-par-turn (Aulerich et al. 1979). h-r the study, an increase in deaths occurred in females that was statistically significant at the high dose. No control females died, while 2 of 23, 3 of 24, and 5 of 24 died at the low, middle, and high doses, respectively. However, the deaths may not be treatment related. In a concurrent study conducted to assess the toxicity of dicyclopentadiene which used mink from the same lot, the mortality in the untreated female mink was 4 of 24, with 2 mink dying between the time of mating and lactation. The conclusion that female deaths in the Aulerich et al. (1979) study were probably not DIMP treatment-related was supported by a two-generation reproductive study performed using Ranch Wild mink fed 0, 16, 45, or 262 mg/kg/day (males) or 0, 20, 57, or 330 mg/kg/day (females) diisopropyl methylphosphonate in the diet (Bucci et al. 1997). The F_0 males and females were exposed for 1 and 4 months, respectively, and the F_1 males and females for 8 and 13 months, respectively. No treatment-related effects were observed in kits/litter, live kits/litter, litter weights at birth or at 28 days, or kit development. No dose-related deaths was observed in females in either the F_0 and F_1 generations. Ovarian follicles were counted in control and high-dose F_1 females to examine possible ovarian toxicity. There was a significant ($p<0.01$) increase in the mean follicle count of high-dose females (645 ± 157) compared to controls (329 ± 153 or 460 ± 148). Only the control and high-dose animals' ovaries were examined. However, it is not clear whether this end point represents an adverse effect because the treated dams of both the F_0 and F_1 generations produced as many offspring as controls. The study authors noted that the effect could be representative of disrupted follicle maturation with retention of ova. Semen quality in F_0 and F_1 males, as measured by sperm motility, epididymal sperm count, and incidence of head/tail abnormalities, was unaffected by treatment.

Another study was performed in which both male and female Mallard ducks received feed containing 1,000, 3,200, or 10,000 ppm diisopropyl methylphosphonate from 40 weeks prior to the beginning of egg production (Aulerich et al. 1979). The dosing continued through the egg production period and for an additional 10 weeks after egg production levels reached 50%. No significant changes in maternal death or in gonad weight in either males or females were reported at any dose level. Food intake and body weights were not measured in this study, and therefore mg/kg/day doses could not be calculated.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to diisopropyl methylphosphonate.

Diisopropyl methylphosphonate was administered to pregnant female rats in the diet at doses of 0, 10, 30, 100, or 300 mg/kg/day on day 6 through day 15 of gestation (Hart 1980). No deaths occurred among the rats. No differences were noted in mean body weight or food consumption. Observations of the uterine contents indicated no compound-related effects" Gross examination revealed no abnormalities of the internal organs. There was a slight increase in the abnormal-normal fetus ratio in the 300-mg/kg/day group; however, the effect was not statistically significant. Further, the changes noted did not suggest a specific area of involvement. The study author concluded that the administration of diisopropyl methylphosphonate in the diet at these concentrations produced no effect on dams and no compound-induced terata, embryo toxicity, or inhibition of fetal growth and development. In a single-generation study, male and female rats received diisopropyf methylphosphonate in the drinking water (0, 0.009, 0.9 mg/kg/day) for 10 weeks (Hardisty et al. 1977). Dosing continued through gestation and lactation in females. No skeletal or visceral anomalies were found among the rats that were examined. However, as discussed in Section 2.2.2.1, there is some confusion regarding the actual dosages that the animals were exposed to in this study. Therefore, results from the Hardisty et al. (1977) study are considered inappropriate for human health risk assessment.

Decreased red blood cell counts were observed in F_2 male, but not female, kits at 6 weeks of age in a two-generation reproductive study in which Ranch Wild mink were fed 0, 16,45, or 262 mg/kg/day (males) or 0, 20, 57, or 330 mg/kg/day (females) diisopropyl methylphosphonate (Bucci et al. 1997). This effect was not observed in F_1 kits at 11 weeks.

Both male and female Mallard ducks received feed containing 1,000, 3,200, or 10,000 ppm diisopropyl methylphosphonate beginning 10 weeks prior to the beginning of egg production (Aulerich et al. 1979). The dosing continued through the egg production period and for an additional 10 weeks after egg production levels reached 50%. No significant difference was reported between dosed animals and controls in number of cracked shells, shell thickness, egg incubation parameters (viability, fertile versus nonfertile eggs, survival in shell), incidence of developmental abnormalities, or 14-day survival in chicks. Food intake and body weights were not measured in this study, and therefore mg/kg/day doses could not be calculated.

2.2.2.7 Genotoxic Effects

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

Genotoxicity studies in animals are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding carcinogenic effects in humans or animals after oral exposure to diisopropyl methylphosphonate.

2.2.3 Dermal Exposure

Limited data are available concerning health effects in humans and animals following dermal exposure to diisopropyl methylphosphonate. Results of these studies are discussed below and presented in Table 2-2.

2.2.3.1 Death

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

Twelve New Zealand White rabbits were divided into three dose groups, each consisting of four animals (Hart 1976). Diisopropyl methylphosphonate was applied neat, in single doses (200, 630, or 2,000 mg/kg) to areas of the back that had been closely clipped. Three rabbits that received 2,000 mg/kg were found dead the morning after application, and a fourth animal that had received 630 mg/kg was found dead on the third morning. Based on these results, a dermal LD_{50} of 1,100 mg/kg was calculated. The study author noted that there was “no antemortem indication of systemic intoxication based on the general appearance and behavior” of the animals. In fact, the body weights of the surviving animals increased during the 2-week observation period. Further, no edema or eschar formation of the skin was observed, and the hair growth appeared normal in the shaved areas. No treatment-related abnormalities were noted during necropsy (Hart 1976).

TABLE 2-2. Levels of Significant Exposure to Diisopropyl Methylphosphonate - Dermal

Species (strain)	Exposure duration/ frequency/ (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect)			Reference
				Less serious (mg/kg/day)		Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
Rabbit (New Zealand)	once					1100 M (LD_{50})	Hart 1976
Systemic							
Rabbit (New Zealand)	once	Ocular		0.1	(diffuse opacity of the corneal surface)		Hart 1976
Rabbit (New Zealand)	once	Derm	2000				Hart 1976

Derm = dermal; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, or renal effects in humans or animals after dermal exposure to diisopropyl methylphosphonate.

Dermal Effects. Skin irritation was noted in wildlife officers at the RMA after they handled sick or dead ducks without gloves (NIOSH 1981). Although the investigators concluded that diisopropyl methylphosphonate contributed to the local effects, a number of other compounds were present. Analysis of the pond water indicated the presence of a number of organic and inorganic contaminants, including diisopropyl methylphosphonate (11.3 ppm); aldrin (0.368 ppm); dieldrin (0.0744 ppm); dicyclopentadiene, bicycloheptadiene, diethyl benzene, dimethyl disulfide, methyl acetate, methyl isobutyl ketone, toluene, and sodium (49,500 ppm); chloride (52,000 ppm); arsenic (1,470 ppm); potassium (180 ppm); fluoride (63 ppm); copper (2.4 ppm); and chromium (0.27 ppm). Because of the presence of numerous compounds, it is unclear whether diisopropyl methylphosphonate was related to the irritation.

Only minimal indications of skin irritation were noted in New Zealand White rabbits that received a single dose of diisopropyl methylphosphonate (200, 630, or 2,000 mg/kg) on intact or abraded skin (Hart 1976). In the same series of studies, the sensitization potential for diisopropyl methylphosphonate was evaluated in guinea pigs. Eight albino guinea pigs received 10 intracutaneous injections (3 times per week to a total of 10) of diisopropyl methylphosphonate in corn oil on one side and of the corn oil vehicle on the other side. Four positive control guinea pigs received intracutaneous injections of a known sensitizer (2,4-dinitro-1-chlorobenzene. Following a 2-week period, the animals received one or more challenges, applied in the same manner as the initial material, and were graded for sensitization. Diisopropyl methylphosphonate was not judged by the study author to be a strong sensitizer in guinea pigs; however, this was considered a preliminary study (Hart 1976).

Ocular Effects. No studies were located regarding ocular effects in humans after dermal exposure to diisopropyl methylphosphonate.

The potential of diisopropyl methylphosphonate to cause eye irritation was evaluated with the Draize Test (Hart 1976). The compound was directly applied to the conjunctival sac of one eye in each of nine New Zealand White rabbits. Significant irritation of the conjunctivae was observed in all rabbits, and the cornea1 surface was characterized by a diffuse opacity. The opacity was temporary and cleared within 8 days. Irrigation with lukewarm water following application of diisopropyl methylphosphonate reduced but did not prevent irritation (Hart 1976).

No studies were located regarding the following health effects in humans or animals after dermal exposure to diisopropyl methylphosphonate.

2.2.3.3 Immunological and Lymphoreticuiar Effects

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to diisopropyl methylphosphonate.

2.3 TOXICOKINETICS

Diisopropyl methylphosphonate is rapidly absorbed following oral administration in animals and is widely distributed throughout the body. Two metabolites of diisopropyl methylphosphonate are isopropyl methylphosphonic acid (IMPA) and methylphosphonic acid (MPA). Excretion of these metabolites in animals occurs primarily through the urine.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

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

2.3.1.2 Oral Exposure

No studies were located regarding absorption in humans after oral exposure to diisopropyl methylphosphonate.

Diisopropyl methylphosphonate was absorbed from the gastrointestinal tract of mink, rats, mice, dogs, and cattle after oral administration of the [^{14}C]-radiolabeled compound as indicated by the appearance of the radiolabel in the blood after exposure (Bucci et al. 1992; Hart 1976; Ivie 1980). Quantitative measurements of absorption can only be approximated (Hart 1976; Ivie 1980).

Single doses (225 mg/kg) of [^{14}C]-radiolabeled diisopropyl methylphosphonate dissolved in polyethylene glycol were administered by gavage to fasted male mice, rats, and dogs (Hart 1976). Plasma concentrations of the labeled diisopropyl methylphosphonate were monitored after 5, 15, and 30 minutes and after 1, 2, 4, 6, and 24 hours in the mice, rats, and dogs as well as at 48 and 72 hours in the mice and rats. The plasma data indicated that there were species differences in absorption In mice, the highest level of radioactivity (172 μg/mL) was noted in the blood at 15 minutes. In rats, plasma concentrations peaked at 151 μg/mL about 2 hours after compound administration, suggesting slower movement through the gastrointestinal tract and uptake by the mucosa. In dogs, peak plasma concentrations also occurred at 2 hours post-administration at a concentration of 276 μg/mL. This value suggests that movement through the gastrointestinal tract in dogs was comparable to that in rats. However, for each measurement taken in the first 2 hours, the plasma levels of diisopropyl methylphosphonate in dogs were higher than those in rats. This suggests that the total amount of intestinal uptake in the dogs exceeded that for the rats.

Studies in mink (Bucci et al. 1992) and rats (Weiss et al. 1994) indicate that absorption following oral exposure to a single dose of [^{14}C]-radiolabeled diisopropyl methylphosphonate is rapid. Male and female rats

were treated with a gavage dose of 66 or 660 mg/kg, and mink were treated with a gavage dose of 27 or 270 mg/kg. Blood levels of radioactivity peaked in 2-3 hours in rats and at 2 hours in mink, except in high-dose male mink, in which the peak was observed at 4 hours. In male rats, 86-97% of the radioactivity was recovered in the urine. Recovery in the urine of female rats (67-72%) and mink (70-91%) was lower, but this was thought to be a result of difficulties in urine collection during blood sampling rather than a difference in the absorption or disposition of diisopropyl methylphosphonate.

Plasma levels of diisopropyl methylphosphonate were measured in a single lactating Jersey cow after the sixth day of diisopropyl methylphosphonate oral administration (10 mg/kg/day) by gelatin capsule (Ivie 1980). For the first 5 days the cow was given unlabeled compound and fed hay *ad libitum.* The diisopropyl methylphosphonate administered on the 6th day was labeled with carbon 14. Based on measurements of label in the plasma, absorption in the cow paralleled that in rats and dogs, with the highest concentration detected in the plasma at 2 hours after administration.

After doses of 10 mg/kg/day in a cow and 225 mg/kg in male mice, rats, and dogs (fasted for 18 hours prior to administration of diisopropyl methylphosphonate), approximately 90% of the diisopropyl methylphosphonate was absorbed from the gastrointestinal tract. This estimate is based on the small percentage of the label found in the feces in the 2-3-day period after dosing and the 84-97% excreted in the urine (Hart 1976; Ivie 1980).

2.3.1.3 Dermal Exposure

No studies were located regarding absorption in humans after dermal exposure to diisopropyl methylphosphonate.

Some dermal uptake of diisopropyl methylphosphonate through the skin of rabbits can be inferred based on the death of 3 of 4 animals treated with 2,000 mg/kg neat applied to shaved abraded or unabraded skin for a 24-hour period (Hart 1976).

Snodgrass and Metker (1992) reported that male pigs dermally treated with [^{14}C]-radiolabeled diisopropyl methylphosphonate dissolved in 95% ethanol (1-mL dose volume over a 100-cm^2 area) absorbed less than 7% of the percutaneously-applied dose regardless of dose level. The remaining dermally-applied diisopropyl methylphosphonate is assumed to have volatilized or been lost along with exfoliated skin.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

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

2.3.2.2 Oral Exposure

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

In animals, diisopropyl methylphosphonate absorbed from the gastrointestinal tract is rapidly distributed to the tissues as indicated by the decay in peak plasma levels after absorption (Hart 1976; Ivie 1980). In mice, plasma radioactivity declined slowly from 15 minutes to 1 hour after exposure and then dropped rapidly during the next 2 hours. At the end of 24 hours, the label in the blood was 0.63 μg/mL or 0.3% of the 173-μg/mL peak concentration (Hart 1976). The radiolabel in the plasma of rats after 24 hours was nearly identical to that in mice (0.61 μg/L) and was 0.4% of the peak concentration. Clearance of label was slower in dogs with 1.3% of the 276-μg/mL peak concentration present after 24 hours.

Diisopropyl methylphosphonate is initially distributed to the liver by way of the portal circulation after absorption from the intestines, and then to the kidneys for excretion (Hart 1976). High concentrations of radiolabel were detected in the urinary bladder itself, exclusive of any urine of mice at 15 minutes and persisted for up to 6 hours. A similar pattern of distribution to the liver, kidney, and urinary bladder was seen in rats over the first 6 hours after oral administration of diisopropyl methylphosphonate.

Tissue levels of diisopropyl methylphosphonate radiolabel in dogs were determined at 4 and 24 hours on the day of compound administration (Hart 1976). When the liver, kidney, and bladder values for dogs at 4-hours are compared to those for mice and rats, it appears that clearance from the body is slower in canine species. At 24 hours, the organ concentrations of radiolabel in the dogs' tissues were generally 4-8 times higher than those in mice and rats, but tissue:blood concentration ratios were lower (Hart 1976).

Based on a single-dose study and tissue:blood concentration ratios, diisopropyl methylphosphonate is taken up by the lungs of mice, rats, and dogs (Hart 1976). The ltmgzblood concentration ratios are 4.5 for mice, 3.6 for rats, and 2.0 for dogs. There is also some uptake of the [^{14}C]-label by the testes where the testes:blood concentration ratios are 2.7 for mice, 2.3 for rats, but only 1.1 for dogs.

Fat tissues do not appear to highly concentrate diisopropyl methylphosphonate or its metabolites. Tissue:blood ratios for adipose deposits range from 1.3 to 3.6 in the species studied (Hart 1976). There was a surprisingly high concentration of radiolabeI in the skin for mice with a tissue:blood ratio of 14.6 (Hart 1976). It has been suggested, however, that the skin samples were contaminated with urine. Values for rats and dogs were much lower (Hart 1976).

In a single-dose oral study in male and female rats (Bucci et al. 1992), only 0.5% of the radioactivity from a dose of 660 mg/kg [^{14}C]-radiolabeled diisopropyl methylphosphonate was found in the tissues 120 hours after dosing. The investigators indicated that no important tissue depot for diisopropyl methylphosphonate or its metabolites could be identified from the data obtained.

The study of diisopropyl methylphosphonate distribution in a lactating Jersey cow was the only study that used multiple doses of diisopropyl methylphosphonate (Ivie 1980). In this single cow, radioactivity was detected in the blood 2 hours after dosing with [^{14}C]-radiolabeled compound but not in the tissues. The animal had received diisopropyl methylphosphonate in one gelatin capsule for 5 days before the radiolabeled dose was administered. If tissue uptake in the cow was similar to that in dogs, measurements made 2 hours after dosing may not have provided an opportunity to measure tissue uptake of label. After 24 hours, 0.1% of the administered label was found in the cow’s milk (Ivie 1980).

2.3.2.3 Dermal Exposure

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

2.3.3 Metabolism

Metabolism of diisopropyl methylphosphonate can be inferred based on the identification (Hart 1976; Ivie 1980) and quantification of its urinary metabolites in various animal species (Bucci et al. 1992; Hart 1976; Ivie 1980; Snodgrass and Metker 1992; Weiss et al. I994). Hydrolysis of one of the two phosphate ester bonds liberates isopropanol and converts diisopropyl metbylphosphonate to IMPA. The locations of the enzymes capable of catalyzing diisopropyl methylphosphonate phosphate ester hydrolysis have not been identified.

At low doses, the metabolism of diisopropyl methylphosphonate to IMPA in the body is rapid and nearly complete. After oral exposure to diisopropyl methylphosphonate, the principal metabolite isolated from both urine (93-99%) and feces (≥97%) in mink, mice, rats, dogs, and cattle is IMPA (Bucci et al. 1992; Hart 1976; Ivie 1980). Less than 0.5% of the radiolabel was detected in the exhaled air of rats and mice as carbon dioxide after diisopropyl methylphosphonate ingestion (Hart 1976). Thus, complete metabolism of diisopropyl methylphosphonate occurs only to a minor extent.

A sex difference in the rate of conversion of DIMP to its primary metabolite was observed after intravenous administration of ^{14}C-DIMP in rats (Bucci et al. 1992). The males appeared to convert DIMP to IMPA more actively than the females. The apparent plasma elimination half-life of DIMP was about 45 minutes in males and up to 250 minutes in females. Both the rate and total excretion of the administered dose in urine were also higher in male rats. However, this sex difference was not observed for orally-administered DIMP in minks (Bucci et al. 1992; Weiss et al. 1994).

In studies in rats and mink that used more than one dose, the area under the plasma-IMPA concentration time curves indicated that at high doses the principal pathway for the conversion of diisopropyl methylphosphonate to IMPA was saturated (Bucci et al. 1992). In rats, metabolism was saturated at an oral dose of 660 mg/kg, but not at 66 mg/kg; in mink, an oral dose of 270 mg/kg caused metabolic saturation which did not occur at 27 mg/kg.

The second ester bond in diisopropyl methylphosphonate is more stable to hydrolysis than the first, and IMPA undergoes relatively little decomposition to MPA. After intraperitoneal administration of 160 mg/kg [^{32}P]-labeled IMPA to a single male rat, the IMPA was excreted unmodified in the urine with no evidence of further metabolism (Hoskin 1956).

Support for the limited metabolism of IMPA to MPA with resultant retention of the MPA is provided by distribution studies of Sarin (GB). Twenty-four hours after intravenous injection of [^{3}H]-labeled Sarin, unextractable label was present in all tissues except the plasma and kidney. At least some unextractable label was presumed by the study authors to be protein-bound MPA (Little et al. 1986, 1988). The concentration of bound MPA was found to be 20-65% of bound IMPA in different mouse brain areas, with the highest concentration found in the hypothalamus.

2.3.4 Elimination and Excretion

2.3.4.1 Inhalation Exposure

No studies were located regarding excretion in humans or animals after inhalation exposure to diisopropyl methylphosphonate.

2.3.4.2 Oral Exposure

No studies were located regarding excretion in bumans after oral exposure to diisopropyl methylphosphonate.

Urine is the principal excretory route for elimination of diisopropyl methylphosphonate after oral administration to mice, rats, pigs, mink, or dogs (Hart 1976; Snodgrass and Metker 1992; Weiss et al. 1994). However, the rate of excretion differs among species. Peak urinary excretion of a single oral dose of 225 mg/kg [^{14}C]-radiolabeled diisopropyl methylphosphonate occurred at 6 hours in mice, 24 hours in rats, and 72 hours in dogs (Hart 1976). Over the 72-hour period after dosing, a total of 96% of the recovered label was found in the urine of mice, 86% of the recovered label was found in the urine of rats, and 97% was found in the urine of dogs. Fecal excretion was low in some species (3-30%). Only 0.06% of the label was found in the bile of dogs; biliary excretion in mice and rats was not determined. Minimal label (<0.5%) was removed from the body with exhaled air. Dose administration and recovery in the Hart (1976) study may not have been very accurate as total recovery of radioactivity was 126% in mice, 108% in rats, and 90% in dogs.

In rats given 66 or 660 mg/kg diisopropyl methylphosphonate, peak radioactivity in the blood was at 2-3 hours in both sexes at both doses; however, radioactivity was still detectable in the blood 24 hours post-administration in the 66-mg/kg group (Weiss et al. 1994). After intravenous administration of 66 mg/kg, the elimination half-life of diisopropyl methylphosphonate was estimated at 45 minutes in males and 250 minutes

in females; the rate of excretion was greater in males than in females. Urine and feces together accounted for 86-97% of administered radioactivity in males and 57-62% in females.

Mink fasted overnight and given 27 or 270 mg/kg diisopropyl methylphosphonate by gavage exhibited peak radioactivity in the blood at 2-4 hours in both sexes (Weiss et al. 1994). In male and female mink given 270 mg/kg, urinary excretion accounted for 83.3% and 83.6%, respectively, of the total radioactivity administered. In male and female mink given 27 mg/kg, urine accounted for 86.9% and 91.5%, respectively, of the administered radioactivity. In male and female mink given 270 mg/kg, feces accounted for only 1.7% and 2.5%, respectively, of the total radioactivity administered. In male and female mink given 27 mg/kg, feces accounted for only 3.1% and 3.7%, respectively, of the total radioactivity administered. The study authors indicated that total recoveries may have been low because of difficulties in urine collection, especially while blood samples were being taken.

In a single lactating Jersey cow, 30% of a [^{14}C]-radiolabeled dose of 10 mg/kg/day was excreted in the urine 4 hours after dosing, and 84% was excreted in a 96-hour period (Ivie 1980). The amount of label after 96 hours was 7% in the feces and less than 1% in the cow's milk. Before being given the radiolabeled material, the cow had been administered unlabelled diisopropyl methylphosphonate in a gelatin capsule for 5 consecutive days.

2.3.4.3 Dermal Exposure

No studies were located regarding excretion in humans after dermal exposure to diisopropyl methylphosphonate.

Yorkshire cross pigs were treated with diisopropyl methylphosphonate percutaneously and subcutaneously (Snodgrass and Metker 1992). In animals treated subcutaneously with 40 mg labelled diisopropyl methylphosphonate, 9 1% of the injected dose was recovered in the urine within 24 hours, with a total of 94% recovered in urine and 6% recovered in feces by 7 days. In animals exposed percutaneously to 0.4, 4, or 40 mg diisopropyl methylphosphonate (dissolved in ethanol and applied to a loo-cm2 clipped area on the back), percentages of the total radioactivity administered (including nonabsorbed diisopropyl methylphosphonate) in urine were 7%, 3%, and 4%, respectively. Radioactivity in feces was very low, ranging from 0.31% to 0.45%. As the total percentage of administered radioactivity absorbed was only 7%, 3%, and 4% for the three dose levels, almost all radioactivity absorbed was accounted for in urine or feces.

2.3.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simnllation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A

simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically-sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 2-2 shows a conceptualized representation of a PBPK model.

If PBPK models for diisopropyl methylphosphonate exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

No data were located regarding PBPK/PD modeling to explain the biological basis for the dose-response relationship in humans or animals after exposure to diisopropyl methylphosphonate.

2.4 MECHANISMS OF ACTION

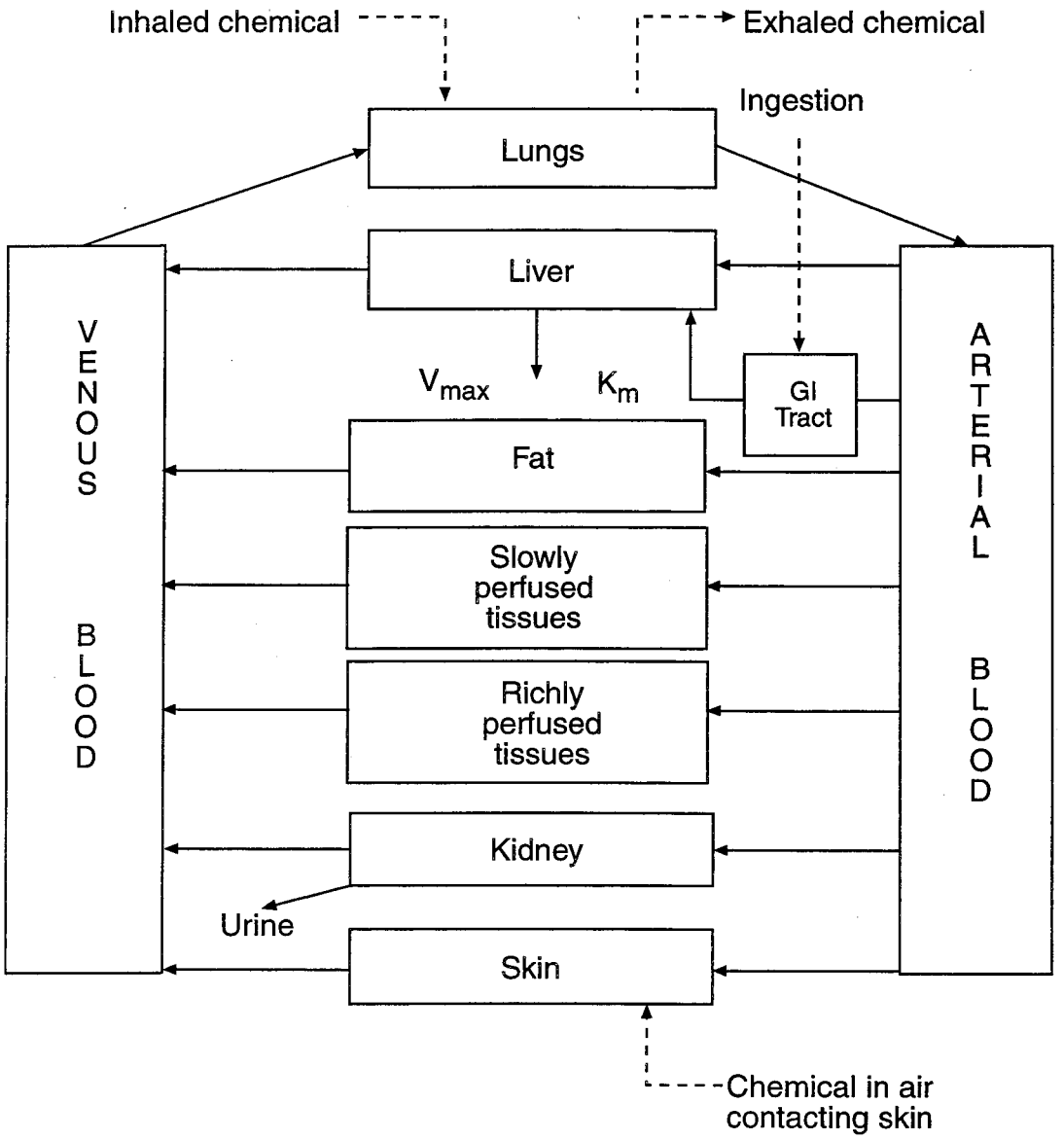
2.4.1 Pharmacokinetic Mechanisms

Absorption. No studies were located regarding the mechanism of absorption in humans or animals after inhalation, oral, or dermal exposure to diisopropyl methylphosphonate. Both facilitated transport and diffusion through the lipophilic portions of the membrane could be involved in absorption processes. No data were found regarding lipid solubility or partition coefficients.

Distribution. No studies were located regarding the mechanism of distribution in humans after inhalation, oral, or dermal exposure to diisopropyl methylphosphonate.

In animals, radiolabel distribution studies demonstrate rapid movement of the label to the liver. Average plasma concentrations of label were greater than average blood concentrations in mice, rats, and dogs after oral administration of a single dose of 225 mg/kg diisopropyl methylphosphonate (Hart 1976). This suggests

Figure 2-2. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

greater solubility of the radiolabeled compound in the plasma or protein adsorption rather than adsorption to the cellular components of the blood.

There may be some hydrolysis of IMPA to MPA in the liver or in other tissues, but the MPA that is liberated is apparently bound to other molecules and is not immediately excreted. Most of the IMPA is rapidly removed from the blood by the kidneys and excreted by way of the urinary bladder. In mice, the concentrations of radiolabel in the empty urinary bladder from a single 225-mg/kg dose were greater than 1,000 μg/mL in all samples taken between 30 minutes and 4 hours after compound administration (Hart 1976). In rats, concentrations of the label were 1,000 μg/mL or greater for all measurements taken between 2 and 6 hours after dosing (Hart 1976).

Storage. No studies were located regarding storage of the parent compound or its metabolites in humans after inhalation, oral, or dermal exposure to diisopropyl methylphosphonate.

Animal data suggest that there is no storage of diisopropyl methylphosphonate, IMPA, or MPA in the body, although portions of the [^{3}H]-label may become incorporated in biomolecules leading to some retention of label in the form of an unextractable labeled compound (Little et al. 1986, 1988).

The concentrations of [^{14}C]-radiolabel were very low in the adipose deposits of mice, rats, and dogs at 1,2, and 3 days after administration of a single dose of diisopropyl methylphosphonate (Hart 1976). This demonstrates lack of storage in body lipids.

Excretion. No studies were located regarding the mechanism of excretion in humans after inhalation, oral, or dermal exposure to diisopropyl methylphosphonate.

Based on the data from animal studies, diisopropyl methylphosphonate is principally excreted in the urine as the metabolite IMPA (Hart 1976; Ivie 1980). Chromatographic behavior of urinary metabolites does not change after the urine is treated with glucuronidase and sulfatase, so there is no conjugation of diisopropyl methylphosphonate or IMPA by microsomal enzymes (Hart 1976). There was minimal excretion of diisopropyl methylphosphonate metabolites in bile (Hart 1976) or in the milk of a lactating cow (<1 %) (Palmer et al. 1979).

2.4.2 Mechanisms of Toxicity

Effects of Metabolism on Toxicity. Whether the toxic effects seen after exposure to diisopropyl methylphosphonate are caused by the parent compound or its metabolites is unknown. Studies of IMPA show that acute-duration exposure to IMPA results in reduced motor activity, prostration, and ataxia-effects also seen after exposure to diisopropyl methylphosphonate (EPA 1992). Other studies (Little et al. 1986, 1988) show that IMPA, the major metabolite of diisopropyl methylphosphonate, has an affinity for both lung and brain tissues and will bind to proteins in these tissues-effects that were not seen after exposure to diisopropyl methylphosphonate (EPA 1992; Little et al. 1988). These data and other data on the toxicity of IMPA neither support nor contradict the data found in the diisopropyl methylphosphonate studies, so it is not possible to attribute the effects after exposure to diisopropyl methylphosphonate to IMPA. Metabolites of IMPA other than MPA have not been identified.

Target Organ Toxicity. The mechanism of toxicity for diisopropyl methylphosphonate on the red blood cells is not understood. However, it has been suggested that its toxicity in general may be due to the ability of the chemical to inhibit the enzyme cholinesterase (NIOSH 1981). However, no quantitative data exist to support this hypothesis, and Bucci et al. (1992, 1994) indicated that there were no signs of acetylcholinesterase inhibition in mink that received diisopropyl methylphosphonate in the diet at concentrations of 2,700, 5,400, or 8,000 ppm (400, 827, or 1,136 mg/kg/day) for 90 days. The effects of diisopropyl methylphosphonate exposure in acute animal toxicity studies range from ataxia and decreased activity to coma and death at higher doses (Aulerich et al. 1979; Hart 1976). These effects suggest that diisopropyl methylphosphonate, like a number of other organophosphorous compounds, affects the nervous system. This mechanism, however, is speculative, and biologically significant changes in brain cholinesterase activity have not been measured. Neurotoxicity data for diisopropyl methylphosphonate are limited; therefore, the mechanism of nervous system toxicity is not known.

Carcinogenesis. There are no human or animal data concerning the carcinogenic effects of diisopropyl methylphosphonate. Diisopropyl methylphosphonate has been classified by the EPA as a Group D substance, not classifiable as to human carcinogenicity (EPA 1994).

2.4.3 Animal-to-Human Extrapolations

No studies were located regarding toxicokinetic data in humans. Limited information is available regarding the toxicokinetic differences among animal species. Rats, mice, mink, and dogs showed rapid absorption, wide distribution, and over 90% urinary excretion of diisopropyl methylphosphonate or its metabolites. However, the rates of absorption and patterns of distribution varied (Hart 1976; Weiss et al. 1994). The mechanism of toxicity is also undetermined. From the limited data available, it is not possible to determine the degree of correlation between humans and animals

The use of data derived from mink for human bealth assessment, and in particular the mink diisopropyl methylphosphonate data, has been recommended by Calabrese (1990). His recommendation is primarily based on the documentation of "the biology of the mink" available in the scientific literature (including information concerning "nutrition, reproduction and breeding, physiology and biochemistry, disease and parasitism, anatomy and embryology, genetics, behavior, ecology, paleontology and taxonomy") and on the development of a historical database and quantitative assessment of natural background mortality. Calabrese indicated that mink have been used for toxicological research into a number of compounds (e.g., lead, mercury, polychlorinated biphenyls, selenium, and dioxin). It should be noted, however, while mink can be used as a predictive animal model for animal-to-human extrapolations, the database of mink studies being developed has far fewer data than are available for rodents, rabbits, and dogs, species that have traditionally been used for toxicity studies.

2.5 RELEVANCE TO PUBLIC HEALTH

No animal or human data were available for inhalation exposure. There are no data regarding effects in humans after oral exposure. Information is available in animals regarding health effects following acute, intermediate, and chronic oral ingestion of diisopropyl methylphosphonate. The animal data obtained after oral exposure indicate that diisopropyl methylphosphonate is moderately toxic after acute bolus exposure but has a lower order of toxicity after intermediate and chronic exposures in food. No data were found on the toxicity of diisopropyl methylphosphonate after exposure in drinking water. Further, diisopropyl methylphophonate is rapidly metabolized and excreted and does not accumulate. It does not appear to have reproductive or developmental effects. At the doses tested, it does not appear to be an acetylcholinesterase inhibitor, although this issue has not been resolved yet. Limited data are available for dermal exposure in humans and animals. Diisopropyl methylphosphonate does not appear to be a skin irritant; dermal

absorption data on pigs (Snodgrass and Metker 1992) indicated skin absorption in humans is expected to be less than 10%. It does appear to be an eye irritant. Diisopropyl methylphosphonate is not a strong sensitizer in animals, although this conclusion is based on a preliminary study. Animals have shown hematological effects to diisopropyl methylphosphonate after oral exposure.

Minimal Risk Levels for Diisopropyl Methylphosphonate

Inhalation MRLS

Minimal risk level (MRL) values were not derived for acute, intermediate, or chronic inhalation exposures to diisopropyl methylphosphonate because no toxicity data were identified concerning the inhalation of the compound.

Oral MRLs

- An intermediate-duration MRL of 0.8 mg/kg/day was derived from a NOAEL of 75 mg/kg/day (3,000-ppm concentration in the diet) at which no hematological or other effects were noted in beagle dogs (Hart 1980). The NOAEL was divided by an uncertainty factor of 100 (10 each for interspecies and intraspecies variability).

  Purebred beagles, 4 males and 4 females, received diisopropyl methylphosphonate in the diet at doses of 4, 38, or 75 mg/kg/day for 90 days (Hart 1980). A control group of four males and four females was also maintained. At the outset of the study and at 4, 8, and 13 weeks, hemograms and clinical chemistry parameters were determined for all of the dogs. The dogs appeared in good condition throughout the study. No hematological effects were ascribed to diisopropyl methylphosphonate. At the termination of the study, a gross necropsy was performed on all of the dogs and no meaningful changes were observed. In addition, the liver, brain, thyroid, kidneys, adrenal glands, testes, ovaries, heart, and spleen were removed and weighed. No significant weight changes were noted. Microscopic examination of major organs did not reveal any adverse effects.

  Although this study (Hart 1980) did not identify an effect level, the NOAEL is below the LOAEL found in all studies examining the toxicity of diisopropyl methylphosphonate. The LOAEL for diisopropyl methylphosphonate is 262 mg/kg/day for male mink and 330 mg/kg/day for female mink

(Bucci et al. 1997), doses at which statistically significant decreases in plasma cholinesterase (butyrylcholinesterase) but not RBC cholinesterase (acetylcholinesterase) activity were observed (Bucci et al. 1997). In general, a decrease in plasma cbolinesterase activity is considered to be a marker of exposure rather than a marker of adverse effect, while a decrease in RBC acetyl-cholinesterase activity is a neurological effect thought to parallel the inhibition of brain acetyl-cholinesterase activity and is thus considered an adverse effect. Diisopropyl methylphosphonate was not found to inhibit red blood cell cholinesterase at doses at which plasma cholinesterase was significantly inhibited. No effects were observed in males at 45 mg/kg/day (Bucci et al. 1997) or at 63 mg/kg/day (Bucci et al. 1994), and no effects were observed in females at 82 mg/kg/day (Bucci et al. 1994), or at 57 mg/kg/day (Bucci et al. 1997).

Adverse effects (shortened RBC survival, increased Heinz body formation, increased number of reticulocytes, and reduced blood cell counts) were observed at 747 mg/kg/day in males and 907 mg/kg/day in females (average 827 mg/kg/day) (Bucci et al. 1994). Although not statistically significant, the number of Heinz bodies was increased relative to the controls and to the rats treated at 400 mg/kg/day. The observed effects are consistent with a direct effect on RBC and a decrease in their survival.

EPA derived a reference dose (RfD) of 0.08 mg/kg/day based on the same NOAEL (75 mg/kg/day) from the Hart (1980) study. The RfD, however, utilized an additional uncertainty factor of 10 to extrapolate to chronic exposure.

• A chronic-duration MRL of 0.6 mg/kg/day was derived from a NOAEL of 57 mg/kg/day (490-ppm concentration in the diet) at which no hematological or other effects were noted in female mink (Bucci et al. 1997). The NOAEL was divided by an uncertainty factor of 100 (10 for extrapolations from animals to humans and 10 for human variability).

In a two-generation reproductive study, Ranch Wild mink received diisopropyl methylphosphonate in the diet at doses of 0, 16, 45, or 262 mg/kg/day (males) or 0, 20, 57, or 330 mg/kg/day (females) (Bucci et al. 1997). F_1 -generation females were treated for up to 13 months, while other generations and F_1 males were treated for 8 months or less. Two groups of control animals were used. In addition to standard examinations (body and organ weights, hematology, clinical chemistry, gross and

histopathological parameters), plasma and whole blood cholinesterase, and brain acetylcholinesterase were measured. Ovarian follicles were also counted in high-dose females.

No effects were observed in F_1 females at 57 mg/kg/day that were attributable to diisopropyl methylphosphonate after 13 months of exposure. However, at 330 mg/kg/day, Heinz body counts were increased in F_1 females. There was a 31% decrease in plasma cholinesterase in animals fed 330 mg/kg/day for 13 months, but this is not considered to be biologically significant. There was also a significant increase in ovarian follicles among animals at this concentration (the only level examined). However, because treated dams of both generations produced as many offspring as the control animals, the biological significance of these findings is unclear.

This study is supported by intermediate-duration NOAELs for hematological effects of 75 mg/kg/day in a dog study (Hart 1980) and 45 mg/kg/day (males) and 57 mg/kg/day (females) in a mink study (Bucci et al. 1997). In the Bucci et al. (1997) mink study, the next highest level, 262 or 330 mg/kg/day in males and females, respectively, produced hematological changes that included increased Heinz body counts, reticulocytes, mean cell volume, and decreased RBC counts.

No MRL was derived for acute oral exposure to diisopropyl methyl phosphonate because the data were considered too limited to determine an appropriate threshold and derive an MRL.

Death

No studies were located regarding death in humans after inhalation, oral, or dermal exposure to diisopropyl methylphosphonate.

Oral LD_{50}s of 1,125 and 826 mg/kg have been reported for male and female rats, respectively (Hart 1976). Swiss Webster mice yielded oral LD_{50}s of 1,041 and 1,363 mg/kg for males and females, respectively (Hart 1976). No deaths occurred in female rats receiving diisopropyl methylphosphonate for 10 days in the diet at doses of 10, 30, or 300 mg/kg/day on days 6-15 of gestation (Hart 1980). An oral LD_{50} of approximately 750 mg/kg for calves was estimated in a study of pairs of calves given 62.5, 125, 250, 500, or 1,000 mg/kg diisopropyl methylphosphonate in a gelatin capsule (Palmer et al. 1979). All animals at the highest dose died. No deaths of adult rats were recorded in a three-generation study of reproductive effects in rats receiving diisopropyl methylphosphonate in the diet at 0, 30, or 300 mg/kg/day (Hart 1980). Four male rats

died (1 control, 1 low dose, and 2 high dose) out of a total of 256 animals in a 90-day study in which rats received diisopropyl methylphosphonate in the diet at doses of 30, 100, or 3,000 mg/kg/day. The study author concluded that the deaths were not treatment related and that there was no evidence of toxicity (Hart 1976). Similarly, the 3 deaths in the 91-mg/kg/day group were not considered evidence of toxicity since no deaths occurred in the low- or high-dose groups among 180 mice receiving diisopropyl methylphosphonate in the diet at doses of 27, 91, or 273 mg/kg/day (Hart 1976). No mortality occurred, nor were there judged to be any signs of toxicity in beagle dogs receiving dietary doses of 4, 13, or 38 mg/kg/day for 2 weeks (Hart 1976). No deaths or evidence of toxicity were attributable to diisopropyl methylphosphonate administered for 26 weeks in the drinking water of rats at doses of $6.6x10^{-7}$, $6.6x10^{-5}$, 0.011, or 1.1 mg/kg/day (Army 1978). However, as discussed in Section 2.2.2.1, there is some confusion concerning the concentration units and purity of the diisopropyl methylphosphonate used in the Army (1978) study (EPA 1989), and therefore results from the Army (1978) study are considered inappropriate for human health risk assessment.

Adult female mink dosed by gavage with single doses of diisopropyl methylphosphonate (75, 150, 300, 450, 500, 550, and 600 mg/kg) yielded an LD_{50} of 503 mg/kg (Aulerich et al. 1979). No mortality related to diisopropyl methylphosphonate was noted in juvenile pastel mink receiving dietary doses of 0.2, 2, 17, 201, or 1,852 mg/kg/day in a 21-day feed study. The calculated doses of diisopropyl methylphosphonate suggest that it is not a cumulative toxin since the amounts ingested with food exceed the LD_{50} found in a companion study. The effects may also have been lessened because it was mixed with food (Aulerich et al. 1979). During a 90-day study, no mortality occurred in male or female mink receiving diisopropyl methylphosphonate in the diet at doses of 7, 63, 345, 747, or 1,009 mg/kg/day for males and 9, 82, 455, 908, or 1,264 mg/kg/day for females (Bucci et al. 1992, 1994). No treatment-related mortality was observed in parental or F_1 animals in a two-generation reproductive study in mink in which animals consumed 16, 45, or 262 mg/kg/day (males) or 20, 57, or 330 mg/kg/day (females) of diisopropyl methylphosphonate in the diet for up to 13 months (Bucci et al. 1997). A slight though significant increase in mortality (5/24) was noted among female dark variety mink in the high-dose group in a reproductive study approximately 49 weeks in duration in which the animals received doses of 11, 37, or 95 mg/kg/day diisopropyl methylphosphonate in the diet (Aulerich et al. (1979). It is not clear if the deaths in the Aulerich et al. (1979) study were actually a result of diisopropyl methylphosphonate treatment. No symptoms of toxicity were noted before the mink died. In a concurrent study conducted to assess the toxicity of dicyclopentadiene, which used mink from the same lot, the mortality in the untreated female mink was 4 of 24; 2 died between the time of mating and lactation. It should also be noted that mink have a relatively high natural mortality (EPA 1989). The natural mortality for 1st year mink in a commercial fur ranch operation approaches 6% annually, and up to 15% of

lactating females may die in the late gestation period from "nursing sickness" (Schneider and Hunter 1993). Although it is not clear when in relation to lactation the mink in the Aulerich et al. (1979) study died, some female mink did die during the period that included lactation. The Bucci et al. (1994, 1997) studies, which did not observe deaths in mink that were fed higher doses, provide further evidence that the deaths of mink in the Aulerich et al. (1979) study probably were not related to diisopropyl methylphosphonate treatment.

Four out of 12 rabbits died after a single dermal administration of diisopropyl methylphosphonate (dose range, 200 to 2,000 mg/kg), yielding a dermal LD_{50} of 1,100 mg/kg. The author notes that there was "no antemortem indication of systemic toxicity based on general appearance and behavior." In fact, the body weights of the surviving animals increased during the 2-week observation period. Further, no edema or eschar formation of the skin was observed, and the hair growth appeared normal in the shaved areas. No treatment-related abnormalities were noted during necropsy (Hart 1976). Based on the available animal data, it is highly improbable that people living near the RMA will be exposed to lethal exposures of diisopropyl methylphosphonate in their drinking water.

Systemic Effects

Respiratory Effects. No studies were located regarding respiratory effects in humans after inhalation, oral, or dermal exposure or in animals after dermal or inhalation exposure to diisopropyl methylphosphonate.

Oral ingestion of diisopropyl methylphosphonate does not appear to induce respiratory effects. Necropsy of both male and female rats that died as the result of a single dose (928, 1362, or 2,000 mg/kg) of diisopropyl methylphosphonate administered by gastric intubation revealed some hyperemia of the lungs; however, most animals displayed no abnormalities (Hart 1976). No abnormal necropsy findings were noted in Swiss Webster mice dosed similarly in a companion study (Hart 1976). No important abnormalities were noted in the necropsy of rats receiving diisopropyl methylphosphonate in the diet (0, 100, or 300 mg/kg/day) for 90 days (Hart 1976). A study in pairs of calves given 62.5, 125, 250, 500, or 1,000 mg/kg diisopropyl methylphosphonate in a gelatin capsule (Palmer et al. 1979) showed that animals at the highest dose displayed pulmonary emphysema; however, no effects were seen at non-lethal doses. No noteworthy deviations were noted in the necropsy or histopathology examination of male or female beagles receiving dietary doses of 38 mg/kg/day (Hart 1976). No gross lesions were noted in rats subsequent to receiving drinking water containing diisopropyl methylphosphonate at doses of $6.6x10^{-7}$, $6.6x10^{-5}$, 0.011, or 1.1 mg/kg/day (Army 1978). However, as described in Section 2.2.2.1, there is some confusion concerning

the concentration units and purity of the diisopropyl methylphosphonate used in the Army (1978) study (EPA 1989), and therefore results from Army (1978) are considered inappropriate for human health risk assessment. Available animal data indicates that no respiratory effects will be expected in the population living near the RMA.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation, oral, or dermal exposure or in animals after dermal or inhalation exposure to diisopropyl methylphosphonate.

Ingestion of diisopropyl methylphosphonate does not appear to induce cardiovascular effects. No anomalous necropsy or histopathological effects were noted in rats receiving diisopropyl methylphosphonate in the diet at doses of 30, 100, or 300 mg/kg/day for 90 days (Hart 1976). Similarly, no abnormalities were noted in mice receiving the compound in the diet at doses of 27, 91, or 273 mg/kg/day for 90 days (Hart 1976). A study in pairs of calves given 62.5, 125, 250, 500, or 1,000 mg/kg diisopropyl methylphosphonate in a gelatin capsule (Palmer et al. 1979) showed that animals at the highest dose displayed petechiae in the coronary grooves and ecchymosis in the gastrointestinal tract. However, no effects were seen at non-lethal doses. Gross necropsy and histopathological examination revealed hemorrhage and erythrophagocytosis in the mesenteric lymph node, although the incidences in the treated and control groups did not indicate a relation with the treatment. In no instance was the tissue or organ examined different from corresponding tissue or organ in the same or other treatment groups or in the controls in beagle dogs receiving the compound in the diet at doses of 4, 13, or 38 mg/kg/day for 14 days (Hart 1976). No deviation in heart weight or abnormal histopathological findings were reported in beagles that received diisopropyl methylphosphonate in the diet at doses of 4, 38, or 75 mg/kg/day for 90 days (Hart 1980).

No significant changes in heart weight or histopathological findings were noted in mink receiving diisopropyl methylphosphonate in the diet at doses of 0.2, 2, 17, 201, or 1,852 mg/kg/day (Aulerich et al 1979). Gross and histopathological examination of mink that received 11, 37, or 95 mg/kg/day revealed no consistent pathological changes (Aulerich et al. 1979). Persons living near the RMA who are exposed to low levels of diisopropyl methylphosphonate are not expected to experience cardiovascular effects.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after inhalation, oral, or dermal exposure or in animals after dermal or inhalation exposure to diisopropyl methylphosphonate.

A study in pairs of calves given 62.5, 125, 250, 500, or 1,000 mg/kg diisopropyl methylphosphonate in a gelatin capsule (Palmer et al. 1979) showed that animals at the highest dose displayed acute gastroenteritis and ecchymotic hemorrhaging. However, no effects were seen at non-lethal doses. It is not expected that persons living near the RMA and exposed to low levels of diisopropyl methylphosphonate would experience gastrointestinal effects.

Hematological Effects. No studies were located regarding hematological effects in humans after inhalation, oral, or dermal exposure or in animals after dermal or inhalation exposure to diisopropyl methylphosphonate.

No significant differences were noted in the hemocytology of ducks, rats, calves, or dogs subsequent to ingestion of diisopropyl methylphosphonate in acute or intermediate protocols (Aulerich et al. 1979; Hart 1976, 1980; Palmer et al. 1979). However, increase in activated partial thromboplastin time (APTT) was observed in calves dosed orally with DIMP in an acute study (Palmer et al. 1979). Alternatively, mink ingesting diisopropyl methylphosphonate for 90 days demonstrated significant changes in hemocytology. Such changes included depressed hematocrits, increased numbers of reticulocytes, and an increase in Heinz bodies (Bucci et al. 1994, 1997). The increase in Heinz bodies suggests that diisopropyl methylphosphonate may cause oxidative damage to the tertiary structure of hemoglobin, thus reducing the lifespan of RBCs, which in turn ultimately induces hematopoiesis. The RBC data and the reticulocyte data are consistent with a mechanism for this hematopoietic response. A marginal increase in the incidence and severity of splenic extramedullary hematopoiesis was observed in male mink exposed to diisopropyl methylphosphonate in the diet at 1,009 mg/kg/day for 90 days (Bucci et al. 1994) and in female mink exposed to 330 mg/kg/day in the diet for 4 months (Bucci et al. 1997). This effect was not observed in the male mink exposed to 747 mg/kg/day in the Bucci et al. (1994) study, or in the female mink exposed to 57 mg/kg/day in the Bucci et al. (1997) study. Results of groundwater monitoring near the RMA in 1997 indicated the maximum detected concentration of DIMP is 1,500 µg/L; and DIMP is not detected in surface soil. The exposure dose associated with the maximum concentration of DIMP in the groundwater is below the chronic MRL of 0.6 mg/kg/day for hematological effect. Therefore, no hematological effects are expected to occur in persons exposed to diisopropyl methylphosphonate while living near the RMA.

Hepatic Effects. No studies were located regarding hepatic effects in humans after inhalation, oral, or dermal exposure or in animals after dermal or inhalation exposure to diisopropyl methylphosphonate.

No changes of toxicological significance were noted in SGOT, SGPT, alkaline phosphatase, liver weight, or liver histopathology in rats, mice, or dogs receiving diisopropyl methylphosphonate in their feed in acute or intermediate protocols (Hart 1976). However, rats pretreated with diisopropyl methylphosphonate in the diet (450 mg/kg/day for 4 days) showed a marked decrease in sleep time subsequent to the administration of hexobarbital, which is consistent with the induction of the P-450 mixed-function oxidase system. Liver weights, however, were not increased (Hart 1976).

Juvenile male pastel mink that ingested 1,852 mg/kg/day diisopropyl methylphosphonate for 21 days showed a significant decrease in liver weight. However, no associated hepatic lesions were noted. The study authors indicated that the decreases in organ weights noted in this high-dose group may be related to significantly reduced food consumption and were not necessarily a function of toxicity. It was noted that, since a pair-fed control was not maintained for this study, the causes of the organ weight differences were not easily identifiable. Finally, the study authors considered the compound nontoxic in this 21-day study (Aulerich et al. 1979).

The available animal data suggest that diisopropyl methylphosphonate is not a hepatotoxin, at least in the doses and time courses studied. Therefore, exposure of persons living near the RMA is not expected to cause hepatic effects.

Renal Effects. No studies were located regarding renal effects in humans after inhalation, oral, or dermal exposure or in animals after dermal or inhalation exposure to diisopropyl methylphosphonate.

Renal toxicity data are limited. In rats, dogs, and mice, no deviations from normal were noted in urinalyses (color, specific gravity, pH, sugar albumin, ketones, and microscopic examination of sediment), weights, or histopathology of kidneys after administration of diisopropyl methylphosphonate in the diet (Hart 1976) or drinking water (Army 1978). As discussed in Section 2.2.2.1, however, there is some confusion concerning the concentration units and purity of the diisopropyl methylphosphonate used in the Army (1978) study (EPA 1989). Therefore, results from the Army (1978) study are considered inappropriate for human health risk assessment. A study in pairs of calves given 62.5, 125, 250, 500, or 1,000 mg/kg diisopropyl methylphosphonate in a gelatin capsule (Palmer et al. 1979) showed that animals at the 500-mg/kg dose and above displayed mild congestion of the renal cortex; however, no effects were seen at the lower doses.

Significant kidney weight loss, but no renal pathology, was found in mink that ingested 1,852 mg/kg/day for 21 days. The study authors indicated that the decreases in organ weights noted in this high-dose group may be related to significantly reduced food consumption and were not necessarily a function of toxicity (Aulerich et al. 1979). No renal pathology was noted in a similar study (Bucci et al. 1992, 1994). The absence of renal lesions and the healthy appearance of the animals throughout the 90-day study are in agreement with Aulerich et al. (1979). Further, the decrease in kidney weight that was attributed to decreased food intake suggests that the renal toxicity of diisopropyl methylphosphonate may be limited. However, the available data are inadequate to draw firm conclusions.

Dermal Effects. No studies were located regarding dermal effects in humans after inhalation or oral exposure or in animals after oral or inhalation exposure to diisopropyl methylphosphonate.

Skin irritation was noted in wildlife officers at the IWA who handled dead or sick ducks without gloves. Analysis of the pond water indicated the presence of a number of organic and inorganic contaminants, including diisopropyl methylphosphonate. Although the investigators concluded that diisopropyl methylphosphonate contributed to the effects, a number of other compounds were identified in the pond, the presence of which makes it unclear whether diisopropyl methylphosphonate was related to the irritation (NIOSH 1981).

Only minimal indications of skin irritation were noted in New Zealand White rabbits that received a single dose of diisopropyl methylphosphonate (200, 630, or 2,000 mg/kg) applied to intact or abraded skin (Hart 1976). Diisopropyl methylphosphonate was not considered a strong sensitizer in guinea pigs (Hart 1976). These studies, however, do not conclusively predict the human risk of skin irritation or sensitivity, but it is not believed that levels found in water near the IWA would cause dermal effects.

Ocular Effects. No studies were located regarding ocular effects in humans after inhalation, oral, or dermal exposure or in animals after inhalation exposure to diisopropyl methylphosphonate.

An ophthalmic examination was performed during week 12 of a 13-week study of rats that received diisopropyl methylphosphonate in their feed (30, 100, or 300 mg/kg/day); 12 of 64 animals displayed some opacity of the lens (Hart 1976). Although the finding was considered normal, the study author suggested that lens opacity may deserve attention in longer studies. No changes were noted in the eyes of beagles that received diisopropyl methylphosphonate in their food (at doses up to 75 mg/kg/day) for 90 days (Hart 1980)

or in mink that received dietary concentrations up to 330 mg/kg/day in a two-generation reproductive study (Bucci et al. 1997).

The potential of diisopropyl methylphosphonate to cause eye irritation was evaluated by direct application of the compound to the conjunctival sac of one eye in each of nine New Zealand White rabbits. Significant irritation of the conjunctivae was observed in all rabbits, and the cornea1 surface was characterized by diffuse opacity. The opacity was temporary and cleared within 8 days. Irrigation with lukewarm water following application of diisopropyl methylphosphonate reduced but did not prevent irritation (Hart 1976). These data indicate that diisopropyl methylphosphonate is probably an eye irritant in humans at sufficiently high concentrations. However, eye irritation is not expected to occur as a result of groundwater contamination in persons living near the RMA.

Body Weight Effects. No studies were located regarding body weight effects in humans after inhalation, oral, or dermal exposure or in animals after inhalation or dermal exposure to diisopropyl methylphosphonate.

No toxicologically important compound-related weight loss in rats (Army 1978; Hardisty et al. 1977; Hart 1976), pregnant rats (Hart 1980), calves (Palmer et al. 1979), or dogs (Hart 1980) was noted subsequent to acute (Hart 1980) or intermediate (Army 1978; Hardisty et al. 1977; Hart 1976, 1980) oral administration of diisopropyl methylphosphonate. However, as discussed in Section 2.2.2.1, there is some confusion concerning the concentration units of the diisopropyl methylphosphonate used in the Army (1978) and Hardisty et al. (1977) studies (EPA 1989), and the actual doses received may be lower. Therefore, results from the Army (1978) and Hardisty et al. (1977) studies are considered inappropriate for human health risk assessment. In addition, the purity of the compound was only 65% in the Army (1978) study. Significant weight loss and decreased food consumption were noted in male and female pastel mink that received diisopropyl methylphosphonate in their feed at a dose of 1,852 mg/kg/day. However, subsequent to treatment (during the recovery period), the group displayed increased food consumption, suggesting that the weight loss may have been due to decreased food consumption resulting from a palatability problem and not a function of toxicity (Aulerich et al. 1979). To determine if unpalatability of the feed was the cause of the weight loss, an attempt was made to “pair feed’” a control group to match voluntary food consumption. Similar to the findings of Aulerich and coworkers (1979), mink that received feed containing diisopropyl methylphosphonate at doses of 1,009 mg/kg/day for males and 1,264 mg/kg/day for females consumed significantly less feed than did the controls and demonstrated decreased body weight throughout the study. Although only partially successful, the pair-feed data for the male high-dose group provided substantial

evidence that the weight loss was the result of decreased food consumption rather than diisopropyl methylphosphonate toxicity (Bucci et al. 1994). No weight loss was noted in mink subsequent to oral administration of diisopropyl methylphosphonate for 49 weeks (Aulerich et al. 1979) or up to 13 months in a two-generation reproductive study (Bucci et al. 1997). Mallard ducks exposed to a single dose of 1,800 mg/kg by gavage or fed 1,796 mg/kg/day for 5 days exhibited decreased weight gain, although this may have been because of refusal to eat feed containing a very high concentration of the chemical (Aulerich et al. 1979). As a whole, these data suggest that intermediate-duration oral administration of diisopropyl methylphosphonate does not cause serious toxicity.

Immunological and Lymphoreticular Effects. No studies were located regarding immunological and lymphoreticular effects in humans after inhalation, oral, or dermal exposure or in animals after inhalation or dermal exposure to diisopropyl methylphosphonate.

Histological changes in the spleen related to diisopropyl methylphosphonate intake were not observed in male or female rats exposed to 1.1 mg/kg/day of diisopropyl methylphosphonate in their drinking water for 26 weeks (Army 1978). However, as stated in Section 2.2.2.1, there is some confusion concerning the concentration units and purity of the diisopropyl methylphosphonate used in the Army (1978) study (EPA 1989). Therefore, results from the Army (1978) study are considered inappropriate for human health risk assessment. No changes in spleen weight were noted in male or female mink exposed to 1,852 mg/kg/day of diisopropyl methylphosphonate in the diet for 21 days (Aulerich et al. 1979). Similarly, spleen weight measurements and gross and histopathological examinations did not reveal any significant immunological or lymphoreticular effects in male or female mink exposed to 95 mg/kg/day of diisopropyl methylphosphonate in the diet for 49 weeks (Aulerich et al. 1979). A marginal increase in the incidence and severity of hematopoiesis in the spleen was observed in male mink exposed to 1,009 mg/kg/day of diisopropyl methylphosphonate in the diet for 90 days (Bucci et al. 1994). An increase in hematopoiesis was not observed in male mink exposed to 747 mg/kg/day. Splenic hematopoiesis accompanied by increased spleen weights also were observed in female mink fed 330 mg/kg/day diisopropyl methylphosphonate in the diet for 4 months (Bucci et al. 1997). However, in both of these studies the splenic extramedullary hematopoiesis was probably a compensatory effect due to damage to erythrocytes and not a direct lymphoreticular effect. Data are insufficient to determine if diisopropyl methylphosphonate affects the function of the immune system.

Neurological Effects. No studies were located regarding neurological effects in humans after inhalation, oral, or dermal exposure or in animals after inhalation or dermal exposure to diisopropyl methylphosphonate.

Single-dose oral toxicity studies in rats, mice, and cattle indicate that signs of diisopropyl methylphosphonate intoxication include decreased activity, ataxia, tympanitis, and prostration within l-4 hours after dosing (Hart 1976; Palmer et al. 1979). Mink that received 1,852 mg/kg/day diisopropyl methylphosphonate in their feed displayed aggressive behavior. However, it was concluded that this behavior was probably due to hunger resulting from the unpalatability of the feed (Aulerich et al. 1979).

Inconsistent fluctuations were observed in RBC and plasma cholinesterase activity in rats that received diisopropyl methylphosphonate in the diet (30, 100, or 300 mg/kg/day) for 90 days. The most significant difference was a decrease in plasma cholinesterase activity in the 300-mg/kg/day female group at 13 weeks (Hart 1976). The study author pointed out, however, that the control group value was exceptionally high compared to those at other time intervals (Hart 1976). In a go-day study of diisopropyl methylphosphonate in the diet (4, 38,75 mg/kg/day) of dogs, a slight increase in plasma cholinesterase was observed; however, problems with the experimental protocol (failure to measure cholinesterase at 4 and 8 weeks) make any interpretations difficult (Hart 1980). Dark brown Ranch Wild mink that ingested 345, 747, or 1,009 mg/kg/day for males and 455, 908, or 1,284 mg/kg/day for females demonstrated decreased plasma cholinesterase compared to the controls, although the change was rapidly reversible. No effect was noted in erythrocyte acetylcholinesterase activity. Similarly, in a two-generation reproductive study using the same species fed 16, 45, or 262 mg/kg/day (males) or 20, 57, or 330 mg/kg/day (females) of diisopropyl methylphosphonate in the diet for up to 13 months, high-dose females of the parental and F_1 generations showed decreased plasma cholinesterase compared to controls (Bucci et al. 1997). The study authors indicated that plasma cholinesterase is labile and sufficiently unrelated to the nervous system, and that therefore any changes in the low-dose groups were toxicologically unimportant. Further, they noted that even in the high-dose groups with large decreases (61%) in plasma cholinesterase, there was no reduction in erythrocyte or brain cholinesterase and no signs of acetylcholinesterase inhibition (Bucci et al. 1992, 1994, 1997). Based on the studies discussed, it appears that diisopropyl methylphosphonate may inhibit plasma cholinesterase but not RBC cholinesterase. Consistent decreases in plasma cholinesterase may not have been observed in rats and dogs because they were treated with lower doses of diisopropyl methylphosphonate. These data do suggest that at the doses tested, the main effect of diisopropyl methylphosphonate appears to be on the red blood cells, and not on acetylcholinesterase inhibition. In the absence of other systemic effects, it may be speculated that the neurotoxic effects of diisopropyl methylphosphonate may be the cause of death

in those animals receiving sufficient amounts of the compound. However, it is not expected that persons living near the RMA would experience these effects.

Reproductive Effects. No studies were located regarding reproductive effects in humans after inhalation, oral, or dermal exposure or in animals after inhalation or den-oaf exposure to diisopropyl methylphosphonate.

No toxicologically important dose-related reproductive responses were noted in a single-generation reproductive study in which male and female rats received diisopropyl methylphosphonate in the drinking water (0.009 or 0.9 mg/kg/day) for 10 weeks (Hardisty et al. 1977), in a three-generation reproductive study (with 2 matings per generation) in which male and female rats received diisopropyl methylphosphonate in feed at doses of 30 or 300 mg/kg/day (Hart 1980), or in reproductive studies in which mink received feed containing diisopropyl methylphosphonate at doses of 11, 37, or 95 mg/kg/day (Aulerich et al. 1979) or up to 262 mg/kg/day (males) or 330 mg/kg/day (females) (Bucci et al. 1997). However, in the Bucci et al. (1997) study, there was a significant increase in ovarian follicles in high-dose F_1 females, the only treated animals examined, compared to controls. Because there was no difference in the actual breeding outcome (number of offspring) in F_0 or F_1 females, the study authors concluded that there was insufficient evidence to suggest that the increase in ovarian follicles represented an adverse effect. However, the authors also reported that it is possible that these data represent a disrupted follicular maturation process. Because only the high-dose animals in this study were examined for this end point, a NOAEL could not be established for it.

As discussed in Section 2.2.2.1, there is some confusion regarding the actual doses the animals were exposed to in the Hardisty et al. (1977) study. Therefore, results from Hardisty et al. (1977) are considered inappropriate for human health risk assessment. A study in Mallard ducks revealed no changes in gonad weight or maternal death in animals given up to 10,000 ppm diisopropyl methylphosphonate in their feed beginning 10 weeks prior to egg production and terminating 10 weeks after egg production reached 50% (Aulerich et al. 1979). Doses (mg/kg/day) could not be calculated for this study. Reproductive effects are not expected to occur in persons living near the RMA who are exposed to diisopropyl methylphosphonate.

Developmental Effects. No studies were located regarding developmental effects in humans after inhalation, oral, or dermal exposure or in animals after inhalation or dermal exposure to diisopropyl methylphosphonate.

Diisopropyl methylphosphonate was administered in the diet at doses of 10, 30, and 300 mg/kg/day to pregnant female rats on day 6 through day 15 of gestation Observations of the uterine contents indicated no compound-related effects. Gross examination revealed no abnormalities of the internal organs. There was a slight increase in the abnormal-normal fetus ratio in the 300-mg/kg/day group; however, the effect was not statistically significant. Further, the changes noted did not suggest a specific area of involvement. The study author concluded that the administration of diisopropyl methylphosphonate in the diet at these concentrations produced no effect on development (Hart 1980). Up to 10,000 ppm diisopropyl methylphosphonate, given in the feed of Mallard ducks for 10 weeks prior to egg production through 10 weeks after egg production reached 50%, produced no increases in incubation parameters, shell thickness, number of cracked shells, developmental abnormalities, or chick 14-day survival (Aulerich et al. 1979). Doses (mg/kg/day) could not be calculated for this study. Developmental effects are not expected to occur after exposure to low levels of diisopropyl methylphosphonate near the RMA.

Genotoxic Effects. No studies were located regarding genotoxic effects in humans after inhalation, oral, or dermal exposure or in animals after inhalation or dermal exposure to diisopropyl methylphosphonate.

In a micronucleus assay using male $B6C3F_1$ mice dosed with 0, 250, 500, or 1,000 mg/kg diisopropyl methylphosphonate, a small but significant increase in micronuclei were observed at mid- and high-dose levels (DOD 1991a). However, the maximum response was found to be within the laboratory historical control limits. The assay was repeated and the increase in micronuclei was not observed, therefore, it is believed that diisopropyl methylphosphonate did not cause micronuclei induction in this experiment. Diisopropyl methylphosphonate was also negative for the induction of micronuclei in the rat bone marrow after administration of up to 800 mg/kg (DOD 1991b).

Treatment of male $B6C3F_1$ mice with 1,000 mg/kg diisopropyl methylphosphonate for 13 days did not result in a significant increase in DNA damage to either liver parenchymal cells or leukocytes in a single cell assay (DOD 1991 f). However, the same assay performed using Fischer-344 rats showed DNA damage to leukocytes but not liver parenchymal cells (DOD 1991g). However, since no increase in damage in the positive controls was found, the liver cell results are questionable.

The mutagenic potential of diisopropyl methylphosphonate was investigated using the Ames assay. The compound was obtained from two different sources and tested on *Salmonella typhimurium* strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100, both with and without S-9 activation. The compound did not demonstrate mutagenic activity in any of the assays (Hart 1980). Diisopropyl methylphosphonate was also negative for gene mutation in *Saccharomyces cerevisiae* (Hart 1980).

No DNA damage or increases in sister chromatid exchange were evident using Chinese hamster ovary cells treated with diisopropyl methylphosphonate either with or without activation (DOD 1991c, 1991e). Exposure of Chinese hamster ovary cells to diisopropyl methylphosphonate did increase clastogenic damage at levels of 5 and 15 μL/mL in the absence and presence of S-9 activation, respectively (DOD 1991d). Diisopropyl methylphosphonate tested negative in the L5178Y TK+/- mouse lymphoma mutagenesis assay with S-9 activation, but without activation equivocal results were observed (DOD 1991h).

The results of these genotoxicity studies are summarized in Table 2-3 and Table 2-4.

Cancer. No quantitative data for carcinogenic effects in humans or animals were located involving inhalation, oral, or dermal exposure to diisopropyl methylphosphonate.

2.6 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very Limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of

TABLE 2-3. Genotoxicity of Diisopropyl Methylphosphonate *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian cells:			
Mouse (bone marrow)	Micronucleus induction	–	DOD 1991a
Rat (bone marrow)	Micronucleus induction	–	DOD 1991b
Mouse (liver, leukocytes)	DNA damage	–	DOD 1991f
Rat (liver)	DNA damage	–	DOD 1991g
Rat (leukocytes)	DNA damage	+	DOD 1991g

– = negative result; + = positive result

TABLE 2-4. Genotoxicity of Diisopropyl Methylphosphonate *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium	Gene mutation	–	–	Hart 1980
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae	Gene mutation	–	–	Hart 1980
Mammalian cells:				
Chinese hamster ovary	DNA damage	–	–	DOD 1991c
Chinese hamster ovary	Chromosome aberration	+	+	DOD 1991d
Chinese hamster ovary	Sister chromatid exchange	–	–	DOD 1991e
Mouse lymphoma (L5178Y TK+/-)	Gene mutation	–	±	DOD 1991h

– = negative result; + = positive result; ± = equivocal result

exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to diisopropyl methylphosphonate are discussed in Section 2.6.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by diisopropyl methylphosphonate are discussed in Section 2.6.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.8, Populations That Are Unusually Susceptible.

2.6.1 Biomarkers Used to Identify or Quantify Exposure to Diisopropyl Methylphosphonate

No studies were located regarding the parent compound as a biomarker of exposure in humans. Animal exposure to diisopropyl methylphosphonate can be determined by measuring the parent compound in urine. However, since less than 3% of the parent compound was excreted in the urine of mice and rats, concentrations of the parent compound may be below detection levels (Hart 1976). No accumulation of the parent compound in tissues was identified. Elimination of diisopropyl methylphosphonate from the body occurs rapidly. Excretion is primarily in the urine as the metabolite IMPA.

IMPA, the major metabolite of diisopropyl methylphosphonate, has been suggested as a possible biomarker of exposure for diisopropyl methylphosphonate. The excretion of IMPA is not unique to diisopropyl methylphosphonate exposure; IMPA is also a major metabolite of GB (Sarin) (Little et al. 1986). Thus,

IMPA in the urine may be an accurate biomarker of recent diisopropyl methylphosphonate exposure. IMPA can be detected in the blood as well as in the urine. However, since IMPA is cleared from the blood rapidly, its detection may only be useful for monitoring recent exposure.

2.6.2 Biomarkers Used to Characterize Effects Caused by Diisopropyl Methylphosphonate

No biomarkers have been identified to characterize the effects associated with exposure to diisopropyl methylphosphonate. At high doses in animals, diisopropyl methylphosphonate affects RBC and the nervous system. However, because the effects are not unique to diisopropyl methylphosphonate, they would not serve as useful biomarkers of effects. No clinical signs or symptoms in humans have been positively linked to diisopropyl methylphosphonate exposure.

2.7 INTERACTIONS WITH OTHER SUBSTANCES

No studies were located regarding interactions with other substances in humans or animals after exposure to diisopropyl methylphosphonate. However, the potential for multiple chemical interactions does exist. Diisopropyl methylphosphonate has been identified in the RMA in the presence of many other chemicals (such as endrin, dieldrin, dicyclopentadiene, bicycloheptdiene, diethyl benzene, and diethyl disulfide). The nervous system is a target of many of these compounds found at the RMA, including diisopropyl methylphosphonate. Therefore, there is potential for interaction, and studies examining multiple exposures would be useful in predicting risk to humans. Workers at the RMA reported skin irritation after dermal exposure to diisopropyl methylphosphonate at concentrations around 11.3 ppm in water. However, several other chemicals were also in the area (NIOSH 1981). Therefore, it is not clear if diisopropyl methylphosphonate contributed to the effects.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to diisopropyl methylphosphonate than will most persons exposed to the same level of diisopropyl methylphosphonate in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters may result in reduced detoxification or excretion of diisopropyl methylphosphonate, or compromised function of organs affected by diisopropyl methylphosphonate.

Populations who are at greater risk due to their unusually high exposure to diisopropyl methylphosphonate are discussed in Section 5.6, Populations With Potentially High Exposures.

No populations that are unusually susceptible to the toxic effects of diisopropyl methylphosphonate have been identified. At high doses, the RBC is an important target of diisopropyl methylphosphonate in animals. Therefore, persons with more fragile blood cells, possibly those at high altitudes, or individuals with sickle cell anemia may be at greater risk for the hematological effects of diisopropyl methylphosphonate following exposure at high doses. Persons with neuromuscular diseases such as myasthenia gravis or other degenerative conditions may also be at greater risk based on the neurological effects observed in animals.

2.9 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to diisopropyl methylphosphonate. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to diisopropyl methylphosphonate. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to chemical substances. Bronstein and Currance 1988; Ellenhorn and Barceloux 1988; Ellenhorn and Barceloux 1997; Stutz and Ulin 1992. However, exposure to diisopropylmethyl phosphonate is rare; therefore, these texts do not provide information regarding overexposure that is specific to this chemical, but do provide generalized information on procedures to be followed after various types of exposure.

2.9.1 Reducing Peak Absorption FolIlowing Exposure

No studies were located for reducing absorption in humans or animals exposed to diisopropyl methylphosphonate. Standard methods such as cathartics or activated carbon could be used. However, exposure would have to be identified within 4-6 hours since diisopropyl methylphosphonate is rapidly absorbed from the gastrointestinal tract (Ellenhorn and Barceloux 1988).

Common methods for reducing dermal absorption of diisopropyl methylphosphonate include removing contaminated clothes and washing contacted skin with soap and water (Ellenhorn and Barceloux 1988). Following eye contact with diisopropyl methylphosphonate, eyes should be flushed with copious amounts of

water. In rabbits, rinsing the eyes immediately after direct exposure to diisopropyl methylphosphonate has been shown to reduce effects (Hart 1976).

2.9.2 Reducing Body Burden

In studies of mice, rats, and dogs, diisopropyl metbylphosphonate was rapidly absorbed into plasma (Hart 1976). The plasma data indicate that all three species rapidly absorbed diisopropyl methylphosphonate, although the exact rate was species specific. Although no studies were located regarding human absorption, diisopropyl methylphosphonate is also likely to be absorbed rapidly into the plasma of humans. The ability of porous polymeric sorbents, activated carbon, and dialysis to remove diisopropyl methylphosphonate from human plasma has been studied (McPhillips 1983). The grafted butyl-XAD-4 was found to be the most efficient sorbent for the removal of diisopropyl methylphosphonate from human plasma. Hemoperfusion of plasma over synthetic XAD-4 or butyl-XAD-4 sorbent resin was more efficient than dialysis/ultrafiltration for the removal of diisopropyl methylphosphonate from human plasma; the smaller surface of the packed resins provided less area to minimize damage to molecular constituents of the plasma. These methods are useful in reducing diisopropyl methylphosphonate concentrations in the plasma. However, since diisopropyl methylphosphonate and its metabolites are not retained by the body, the need for methods to reduce body burden is uncertain.

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism of the toxic action of diisopropyl metbylphosphonate is unclear. Therefore, therapies that could interfere with its mechanism are unknown.

2.10 ADEQUACY OF THE DATABASE

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

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

2.10.1 Existing Information on Health Effects of Diisopropyl Methylphosphonate

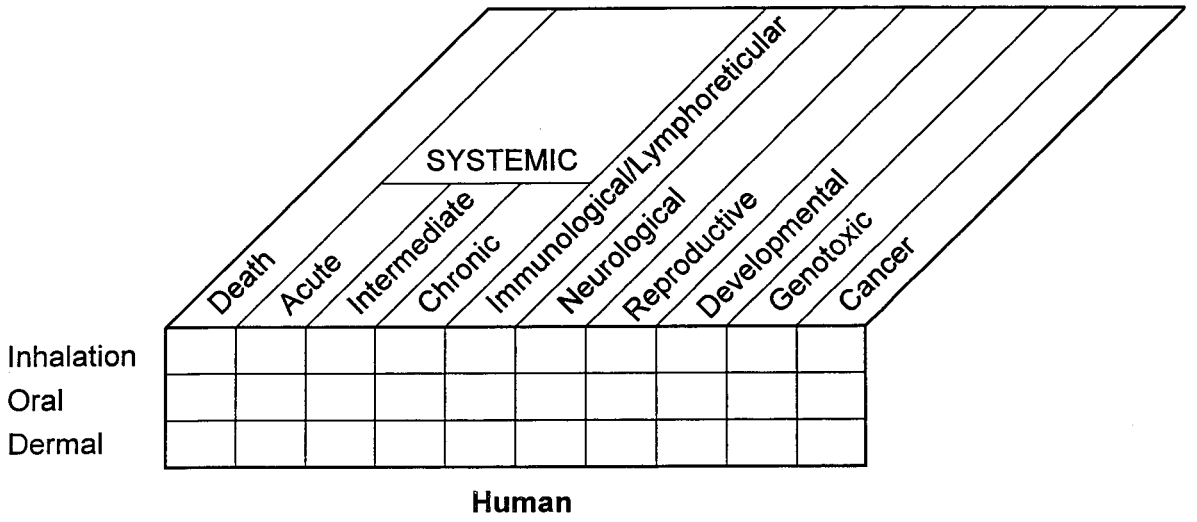
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to diisopropyl methylphosphonate are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of diisopropyl methylphosphonate. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Zdentifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

No studies were found on the health effects of diisopropyl methylphosphonate in humans following inhalation or oral exposure. The results of the one study that was located in which humans were exposed dermally to diisopropyl methylphosphonate was confounded by concurrent exposure to other chemicals. Limited animal data are available on the health effects of diisopropyl methylphosphonate following oral and dermal exposures.

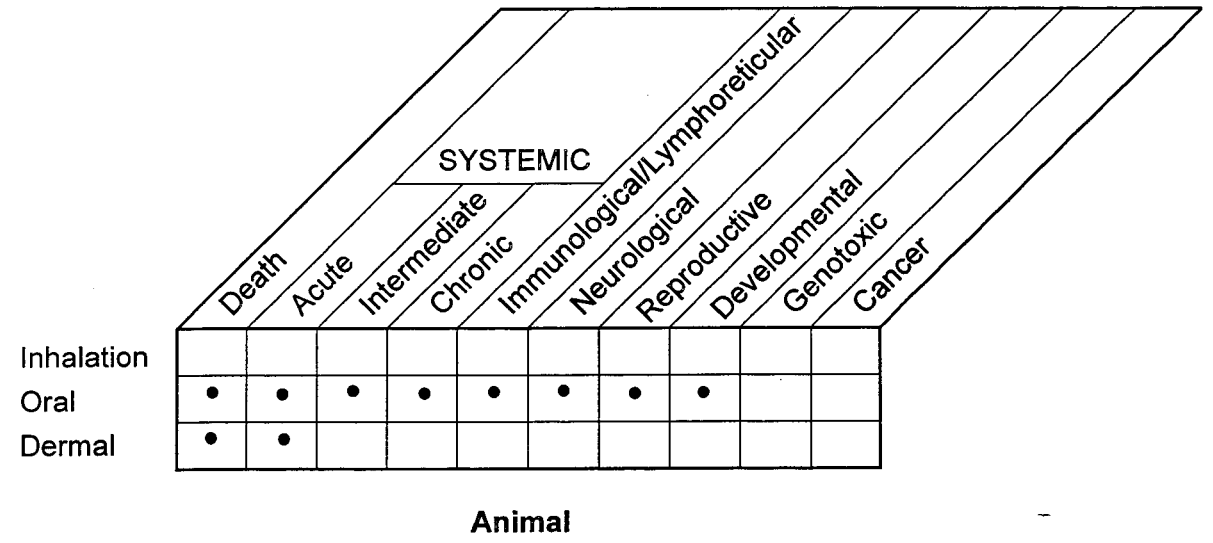
2.10.2 Identification of Data Needs

The following are topical sections that identify gaps in the present state of knowledge concerning the toxicology of diisopropyl methylphosphonate. Each of the sections identifies specific areas in which additional data may be helpful to gain a greater understanding of the toxicity of diisopropyl methylphosphonate as well as of the biochemical mechanisms of toxicity.

FIGURE 2-3. Existing Information on Health Effects of Diisopropyl Methylphosphonate



Human



Animal

• Existing Studies

Acute-Duration Exposure. Acute oral LD_{50} data are available for mice and rats (Hart 1976) and for ducks (Aulerich et al. 1979). Acute oral toxicity studies, including histopathological observations, are available for ducks, mice, rats, dogs, and mink (Aulerich et al. 1979; Hardisty et al. 1977; Hart 1976, 1980). Limited acute dermal toxicity are available for rats (Hart 1976). These data suggest a relatively low toxicity. However, a clear relationship between dose and effect has not been elucidated. Inhalation data of any kind were not identified, and dermal data were very limited.

Intermediate-Duration Exposure. No human or annimal data were located concerning the toxicity of diisopropyl methylphosphonate after exposure via inhalation. Animal data are available for intermediate exposures by ingestion. Aulerich et al. (1979) reported deaths in mink treated with diisopropyl methylphosphonate. However, it is not clear if the deaths were truly treatment related. A more recent study by Bucci et al. (1997) did not find any treatment-related death in mink due to diisopropyl methylphosphonate after intermediate-duration exposure. Bucci et al (1992, 1994) found effects consistent with shortened RBC survival in mink treated with diisopropyl methylphosphonate in the diet at 747 mg/kg/day for males and 908 mg/kg/day for females, with no significant effects at lower doses. Plasma cholinesterase activity was significantly decreased at 345 mg/kg/day for males and 455 mg/kg/day for females, but not at lower doses. No change in RBC cholinesterase activity was observed. A change in plasma but not RBC cholinesterase is not considered adverse. Decreased weight gain was observed in ducks fed diisopropyl methylphosphonate at high doses. Additional intermediate-duration studies in rats and dogs did not find evidence of toxicity (Hardisty et al. 1977; Hart 1976, 1980), and a NOAEL in a dog study was used to derive an intermediateduration oral MRL (Hart 1980).

Chronic-Duration Exposure and Cancer. No studies were located regarding the health effects of chronic-duration exposure to diisopropyl methylphosphonate by inhalation or dermal route of exposure. There was no treatment-related lethality, changes in body weight, or other effects observed in mink fed 57 mg/kg/day diisopropyl methylphosphonate compared to controls for up to 13 months in a two-generation reproductive study (Bucci et al. 1997) although effects were observed at higher doses. However, F_0 females consumed almost 50% more feed than F_1 females, which was not completely explained by the study authors. This NOAEL was used to derive an chronic-duration oral MRL. Additional data are needed regarding all routes of exposures in various species to determine what, if any, toxicity exists. Future studies should focus on the understanding of the toxicity and the mechanism of toxicity of diisopropyl methylphosphonate. The design and protocol should be to study DIMP toxicity in an integrated manner, not to focus on a single

endpoint. In addition, better understanding of the metabolism of diisopropyl methylphosphonate in longterm studies will also be helpful in elucidating its mechanism of action.

Genotoxicity. Several *in vitro* (DOD 1991c, 1991d, 1991e, 1991h; Hart 1980) and *in vivo* (DOD 1991a, 1991b, 1991f, 1991g) studies addressed the genotoxicity of diisopropyl methylphosphonate. Further *in vivo* studies may be useful to clarify any questions concerning the genotoxicity of the compound since equivocal results were sometimes observed.

Reproductive Toxicity. Significant amounts of work have been conducted in the reproductive toxicity of diisopropyl methylphosphonate (Aulerich et al. 1979; Bucci et al. 1997; Hart 1980). It seems fairly clear that diisopropyl methylphosphonate does not have reproductive effects, at least in the range of the doses tested. In addition, as discussed in Section 2.2.2.1, there is some confusion regarding the actual doses that the animals were exposed to in Hardisty et al. (1977). In addition, in the two-generation reproductive study by Bucci et al. (1997), an increase in ovarian follicles was found in high-dose F_1 animals compared to controls. The biological significance of these data are not clear, since there was no effect observed on breeding outcome. Nonetheless, further study is needed to determine whether this effect represents disrupted follicle maturation and to identify a NOAEL/LOAEL for this end point.

Developmental Toxicity. A single rat study, which found no adverse effects, addressed the developmental effects of diisopropyl methylphosphonate (Hart 1980). Additional data that utilize a greater range of doses in a few different species may be heIpfu1.

Immunotoxicity. Limited data that addressed the immunological and lymphoreticular effects were available. No adverse responses were noted in the spleen of male or female rats (histopathological examination) (Army 1978) or in the spleen of mink (spleen weight, gross and histopathological examination) (Aulerich et al. 1979; Bucci et al. 1994, 1997). Additional data concerning various exposure pathways and a range of doses may be useful to corroborate the negative findings.

Neurotoxicity. The primary neurological concern with organophosphorous compounds is the potential for acetylcholinesterase inhibition. Plasma and erythrocyte cholinesterase measurements have been made in rats and mink (Bucci et al. 1992, 1994, 1997; Hart 1976,1980). Even when large decreases (61%) in plasma cholinesterase were demonstrated, there were no decreases in erythrocyte cholinesterase nor were there any clinical signs of acetylcholinesterase inhibition. The data strongly suggest that diisopropyl methylphos-

phonate is not an inhibitor of acetylcholinesterase. Additional chronic studies in which higher doses are used may be useful in determining the toxicological importance of long-term plasma cholinesterase depression. Measurements of acetylcholinesterase at lethal doses of diisopropyl methylphosphonate may also illuminate its mechanism of action In addition, quantitation of the distribution of DIMP and its metabolites (IMPA and MPA) among different brain areas and its correaation with the degree of cholinesterase inhibition found in a given brain area may help to understand its mechanism of action.

Epidemiological and Human Dosimetry Studies. No epidemiological studies were located. Elowever, prior to conducting any large-scale studies, animal studies are needed to determine what, if any, chronic health effects occur after exposure to diisopropyl methylphosphonate. In addition, appropriate cohorts would be very difficult to identify.

Biomarkers of Exposure and Effect

Exposure. Few studies were found regarding the measurement of diisopropyl methylphosphonate or its metabolites as indicators of exposure. IMPA in urine or plasma has been suggested as a biomarker of acute exposure. It would be useful to more fully explore urinary excretion of IMPA to determine dose relationships and its utility as a bioindicator of diisopropyl methylphosphonate exposure.

Effect. No biomarkers have been identified to characterize the effects associated with exposure to diisopropyl methylphosphonate. Toxicity data for diisopropyl methylphosphonate are limited. No specific clinical signs or symptoms in humans have been positively linked to diisopropyl methylphosphonate exposure. Further research is needed to identify the mechanism of neurological effects and the clinical symptoms of diisopropyl methylphosphonate exposure.

Absorption, Distribution, Metabolism, and Excretion. There are no data available on the absorption, distribution, metabolism, or excretion of diisopropyl methylphosphonate in humans. Limited animal data suggest that diisopropyl methylphosphonate is absorbed following oral and dermal exposure. Fat tissues do not appear to concentrate diisopropyl methylphosphonate or its metabolites to any significant extent. Nearly complete metabolism of diisopropyl metbylphosphonate can be inferred based on the identification and quantification of its urinary metabolites; however, at high doses the metabolism of diisopropyl methylphosphonate appears to be saturated. Animal studies have indicated that the urine is the principal excretory route for removal of diisopropyl methylphosphonate after oral and dermal administration.

Because in most of the animal toxicity studies administration of diisopropyl methylphosphonate is in food, a pharmacokinetic study with the compound in food would be especially useful. It could help determine if the metabolism of diisopropyl methylphosphonate becomes saturated when given in the diet and if the levels of saturation are similar to those that result in significant adverse effects.

Comparative Toxicokinetics. There are no data on the kinetics of diisopropyl methylphosphonate in humans. Studies in animals suggest that metabolism and urinary metabolite profiles are qualitatively similar among species. Additional studies would be useful in understanding the differences in metabolic rates in species and in determining which animal species is the most appropriate model for human exposure.

Interactions With Other Substances. Little information is available regarding the interactions of diisopropyl methylphosphonate with other substances. As diisopropyl methylphosphonate has been identified at the RMA in the presence of many other chemicals, further studies of mixed chemical interactions should be done using chemicals found at the RMA to determine if the response to diisopropyl methylphosphonate is altered after multichemical exposure. Although the synergistic action of diisopropyl methylphosphonate and multiple pesticides has been suggested by one study (NIOSH 1981), the potential interaction of this substance with commonly-used antidotes for organophosphate poisoning (atropine and pralidoxime) might be explored. Subtle differences in the structure of organophosphates can result in different effects as well as different interactions with antidotes.

Methods for Reducing Toxic Effects. Little information is available regarding reducing the toxic effects of diisopropyl methylphosphonate following exposure. Recommended treatments include general hygienic procedures for rapid decontamination. The ability of porous polymeric sorbents, activated carbon, and dialysis to remove diisopropyl methylphospbonate from human plasma has been studied. However, since diisopropyl methylphosphonate and its metabolites are not retained by the body, the need for methods to reduce body burden is uncertain.

2.10.3 On-going Studies

No on-going studies on diisopropyl methylphosphonate were located.