CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 2,4-D. 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.

Most of the information available regarding exposure to 2,4-D and health endpoints in humans comes from studies of individuals occupationally exposed either through farming activities or manufacture, formulation, or packaging of herbicide products containing 2,4-D. In these activities, exposure is likely to be predominantly by dermal contact with products containing 2,4-D, with inhalation exposure playing a lesser role. However, the reader should keep in mind that the health outcomes described are the result of exposure through multiple routes, usually a combination of inhalation, oral, and dermal. It is important to keep in mind that although most human exposures are to chemical mixtures containing 2,4-D, exposure to 2,4-D is the common factor between the studies.

This profile discusses 2,4-D and simple salts (e.g., sodium, ammonium) as representatives of the various forms present in commercial formulations.

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

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to 2,4-D, but may not be inclusive of the entire body of literature.

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

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of 2,4-D are indicated in Table 2-2 and Figure 2-3.

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

Although several studies evaluated the effects of 2,4-D exposure in dogs, the results are not included in LSE tables or figures because dogs appear to be more sensitive than other species (including humans) to 2,4-D toxicity due to a significantly lower capacity to eliminate 2,4-D via the kidneys (Timchalk 2004). For this reason, dogs are not considered to represent a relevant species for evaluation of human health risk assessment. However, results from dog studies are summarized in appropriate health effects sections of Chapter 2.

The health effects of 2,4-D have been evaluated in a number of human studies and a variety of animal studies. As illustrated in Figure 2-1, the oral exposure route was employed in the majority of animal

studies. The most examined endpoints in animal studies were body weight (53% of the animal studies), hepatic effects (32% of the animal studies), renal toxicity (36% of the animal studies), endocrine effects (31% of the animal studies), neurotoxicity (32% of the animal studies), and reproductive effects (35% of the animal studies). Cancer was the most examined endpoint in epidemiological studies (57% of the human studies).

Animal studies suggest that relative sensitive noncancer targets of 2,4-D include the hematological system, renal system, and endocrine system.

- **Hematological endpoints:** Limited human data are available. Decreases in selected hematology parameters (platelets, erythrocytes, and hematocrit) were observed in 2,4-D-treated animals.

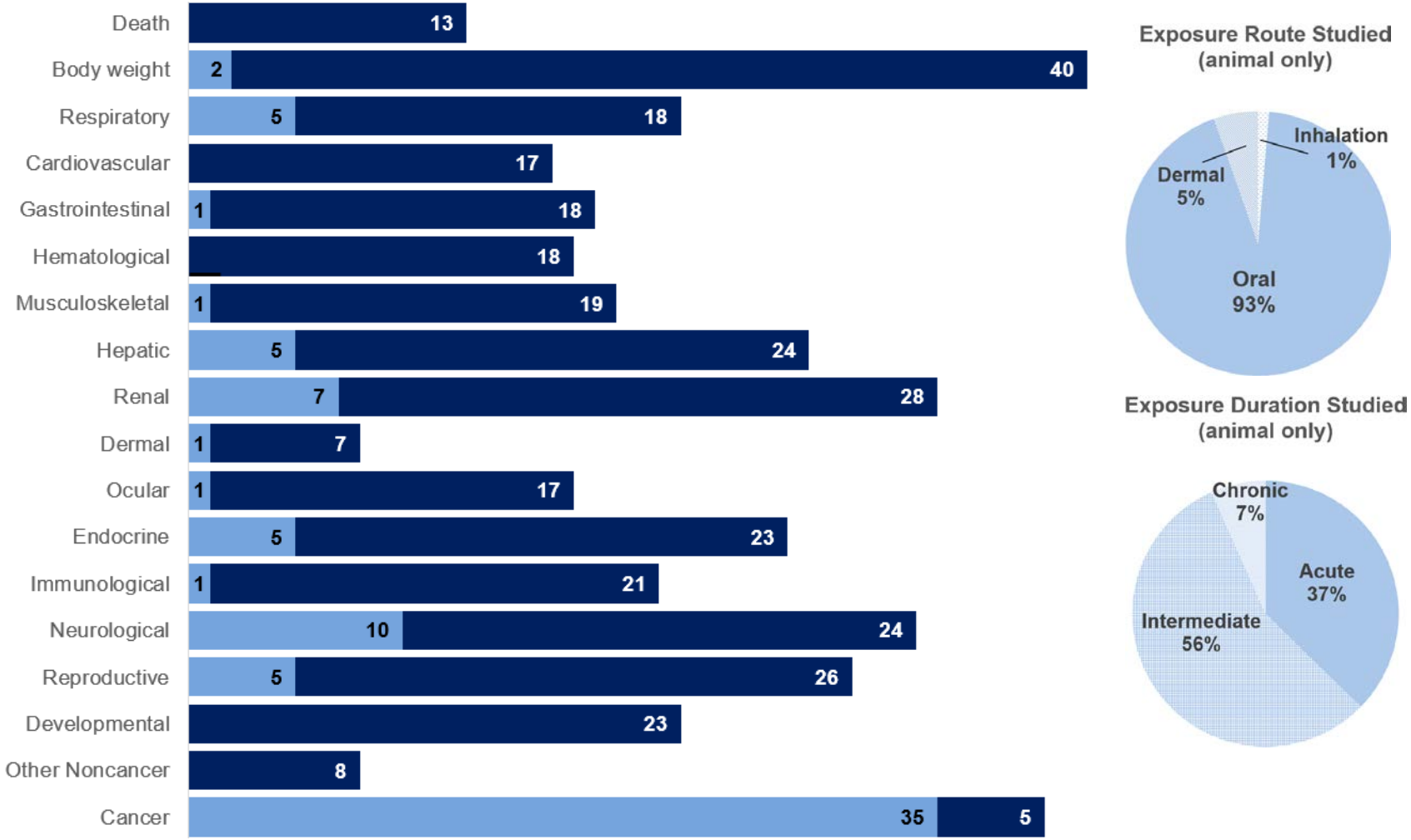
- **Renal endpoints:** Available human data are restricted to a few case reports of kidney damage following intentional ingestion of 2,4-D products. Kidney damage has been reported in a variety of animal studies that employed oral exposure to 2,4-D.

- **Endocrine system:** Limited human data are available. Evidence of 2,4-D-related adverse thyroid effects (decreased serum triiodothyronine [T3] and thyroxine [T4]) has been reported in animal studies that employed oral exposure to 2,4-D.

Figure 2-1. Overview of the Number of Studies Examining 2,4-D Health Effects

Most studies examined the potential body weight and renal effects of 2,4-D

Fewer studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 110 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to 2,4-D – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
INTERMEDIATE EXPOSURE									
1	Rat (Sprague-Dawley) 10 M, 10 F	28 days 5 days/week 6 hours/day	0, 50, 100, 300, 1,000	BI, BW, CS, FI, GN, HE, HP, LE, OW	Bd wt	1000 M 300 F	1,000 F		11–13% reduced body weight during recovery
					Resp		50	1,000	50: hyperplasia, metaplasia in larynx 1,000: labored breathing
					Cardio	1,000			
					Gastro	1,000			
					Hemato	100	300		20–26% decrease in reticulocytes
					Musc/skel	1,000			
					Hepatic	1000 M 100 F	300 F		24% increased serum AP
					Renal	1,000			
					Dermal	1,000			
					Ocular	1,000			
					Endocr	1,000			
					Immuno	1,000			
					Neuro	1,000			
					Repro	1,000			
					Other noncancer	1,000			

EPA 2008

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

2,4-D = 2,4-dichlorophenoxyacetic acid; AP = alkaline phosphatase; Bd wt or BW = body weight; BI = biochemical changes; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); FI = food intake; Gastro = gastrointestinal; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; OW = organ weight; Repro = reproductive; Resp = respiratory

Figure 2-2. Levels of Significant Exposure to 2,4-D – Inhalation

Intermediate (15-364 days)

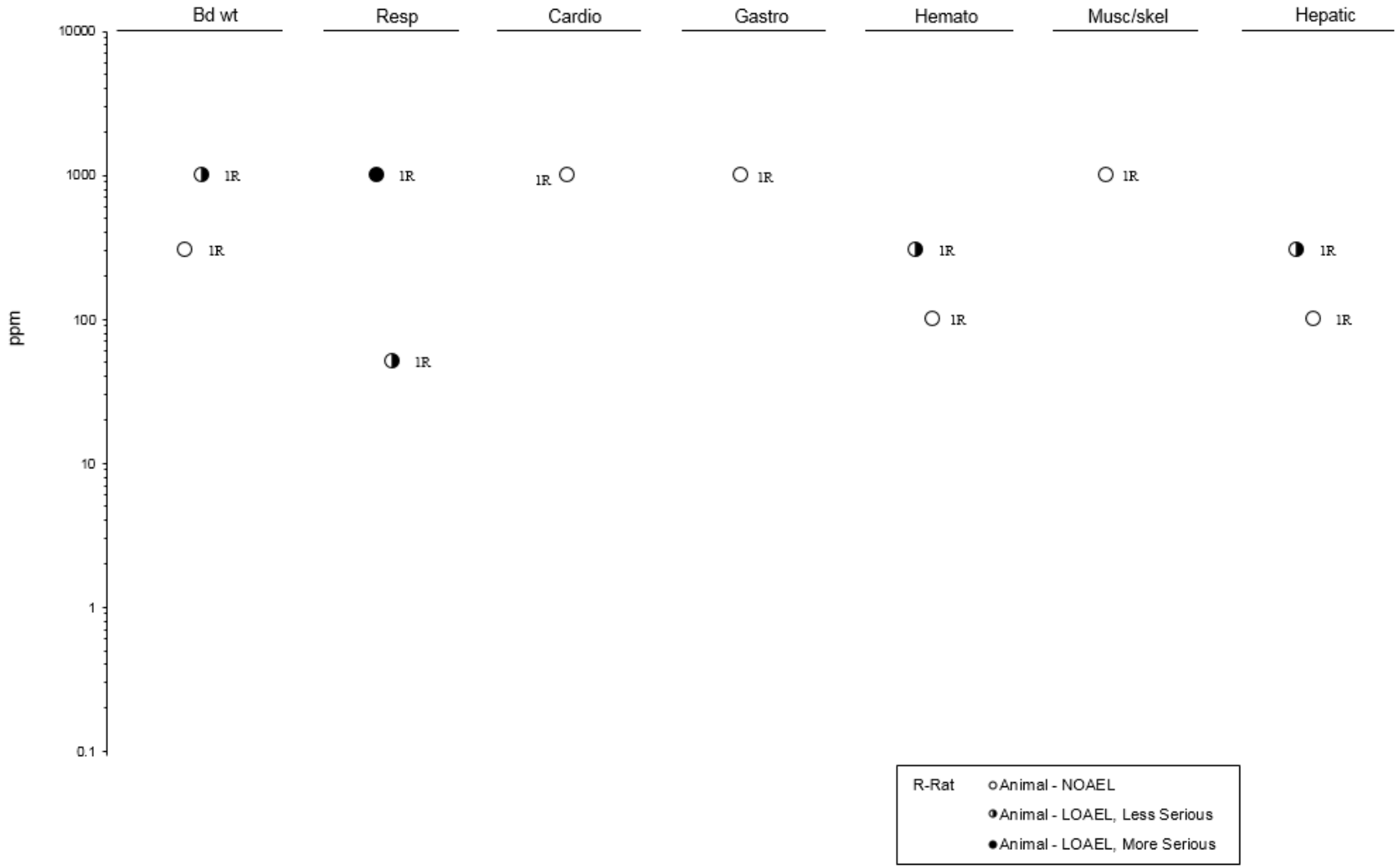


Figure 2-2. Levels of Significant Exposure to 2,4-D – Inhalation

Intermediate (15-364 days)

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
1	Rat (Fischer 344) 35 F	GDs 6–15 1 time/day (GW)	0, 8, 25, 75	BW, CS, DX, FX, LE, MX, TG	Bd wt	75			
					Develop	75			
Charles et al. 2001 – 2,4-D									
2	Rat (Sprague-Dawley) 25 F	GDs 6–15 1 time/day (GO)	0, 115	BW, DX, MX, TG	Death			115	4/25 maternal rats died
					Bd wt			115	Up to 41% decreased maternal weight gain during treatment
					Develop		115		Increased incidence of supernumerary ribs
Chernoff et al. 1990 – 2,4-D									
3	Rat (Sprague-Dawley) 7 M	Once (G)	375, 583, 844	LE	Death			600	LD_{50}
Elo et al. 1988 – 2,4-D									
4	Rat (Sprague-Dawley) 4 or 8 M	Once (G)	150, 300, 600	HP	Neuro	150		300	Vascular damage in the CNS
Elo et al. 1988 – 2,4-D									
5	Rat (Wistar) 5 F	GDs 6–15 1 time/day (GW)	0, 50, 70, 110, 150	BW, CS, DX, GN, MX, TG	Bd wt		50		Maternal body weight loss
					Develop	50		70	Increased resorptions, renal malformations
Fofana et al. 2000 – 2,4-D									
6	Rat (Wistar) 3 F	GDs 6–15 1 time/day (GW)	0, 70, 110	DX, LE	Develop			70	50% pup mortality during 4 weeks postpartum
Fofana et al. 2002 – 2,4-D									

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
7	Rat (Wistar) 3 F	GDs 6–10 1 time/day (GW)	0, 70, 110	DX, LE	Develop			70	25% pup mortality during 4 weeks postpartum
Fofana et al. 2002 – 2,4-D									
8	Rat (Wistar) 3 F	GDs 11–15 1 time/day (GW)	0, 150	DX, LE	Develop			150	27% pup mortality during 4 weeks postpartum
Fofana et al. 2002 – 2,4-D									
9	Rat (Fischer 344) 5 M, 5 F	Once (GO)	NS	LE	Death			639[b] M 764 F	LD_{50}
Gorzinski et al. 1987 – 2,4-D									
10	Rat (White) 6 NS	Once (GW)	0, 333, 666, 1,000	LE	Death			666	3/6 rats died
Hill and Carlisle 1947 –Sodium salt of 2,4-D									
11	Rat (White) 4 NS	Once (GW)	0, 333, 666, 1,000	LE	Death			666	2/4 rats died
Hill and Carlisle 1947 – Purified sodium salt of 2,4-D									
12	Rat (Fischer 344) 3 M, 3 F	Once (GO)	0, 50, 100, 150, 200, 250, 500, 750, 1,000	LE	Death			500	Deaths at ≥500 mg/kg
Mattsson et al. 1997 – 2,4-D									
13	Rat (Fischer 344) 10 M, 10 F	Once (GO)	0, 15, 75, 250	BH, BW, CS, GN, HP	Bd wt	250			
					Musc/skel	250			
					Ocular	250			
					Endocr	250			
					Neuro	75	250		Altered gait, decreased motor activity on treatment day
Mattsson et al. 1997 – 2,4-D									

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
14	Rat (Sprague-Dawley) 17–19 F	GDs 6–15 1 time/day (GO)	0, 12.5, 25, 50, 75, 87.5	BW, DX, MX, TG	Bd wt	87.5			
					Develop	25	50		6% depressed fetal weight, increased incidence of soft-tissue and skeletal anomalies
Schwetz et al. 1971 – 2,4-D									
15	Mouse (ICR) 11–13 F	GDs 0–9 (W)	0, 0.01, 0.1, 100	BI, BW, DX, MX	Bd wt	100			
					Repro	100			
Dinamarca et al. 2007 – 2,4-D									
16	Mouse (White) NS	Once (GW)	NS	LE	Death			375	50% mortality
Hill and Carlisle 1947 – Sodium salt of 2,4-D									
17	Mouse (CD-1) 30 F	GDs 8–12 1 time/day (GO)	0, 87.5	DX, TG	Develop		87.5		7% depressed PPD 1 pup weight
Kavlock et al. 1987 – 2,4-D									
18	Guinea pig (NS) NS	Once (GW)	NS	LE	Death			1000	50% mortality
Hill and Carlisle 1947 – Sodium (2,4-dichlorophenoxy) acetate									
19	Hamster (Golden Syrian) 10–11 F	GDs 6–10 1 time/day (GO)	0, 20, 40, 60, 100	DX, LE, MX, TG	Develop	100			
Collins and Williams 1971 – 2,4-D									
20	Rabbit (New Zealand) 20 F	GDs 6–18 1 time/day (GW)	0, 10, 30, 90	BW, CS, DX, FX, LE, MX, TG	Bd wt	90			
					Develop	90			
Charles et al. 2001 – 2,4-D									

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
21	Rabbit (NS) 4 NS	Once (GW)	NS	LE	Death			800	50% mortality
Hill and Carlisle 1947 – Sodium (2,4-dichlorophenoxy) acetate									
INTERMEDIATE EXPOSURE									
22	Rat (Wistar) 40 F	28 days GD 16 to PPD 23 (F)	0, 70	BH, BW, CS, DX, MX	Bd wt	70			
					Develop		70		Preweaning: 12–15% depressed pup weight; neurobehavioral alterations
Bortolozzi et al. 1999 – 2,4-D									
23	Rat (Wistar) 20 litters of M and F	PPDs 23–90 after 28 days of maternal exposure (F)	0, 70	BH, BW, CS, DX, MX	Bd wt	70 F	70 M		11–12% depressed body weight at PPDs 75 and 90
					Develop		70		Neurobehavioral alterations
Bortolozzi et al. 1999 – 2,4-D									
24	Rat (Fischer 344) 10 M, 10 F	13 weeks (F)	0, 1, 15, 100, 300	BI, BW, CS, GN, HE, HP, LE, OP, OW	Bd wt	100		300	Depressed weight gain (38% in males, 57% in females)
					Resp	300			
					Cardio	300			
					Gastro	300			
					Hemato	15	100		Decreased platelets
					Musc/skel	300			
					Hepatic	100	300		Hepatocellular hypertrophy
					Renal	15	100		Increased kidney weight
					Ocular	300 M 100 F	300 F		Cataracts
					Endocr	15	100		M: decreased serum T4; increased thyroid weight F: decreased serum T3 and T4; adrenal cortex hypertrophy
					Immuno	300			

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Neuro	300			
					Repro	300			
					Other noncancer	300			
Charles et al. 1996b – 2,4-D									
25	Rat (Fischer 344) 10 M, 10 F	52 weeks (F)	0, 5, 75, 150	BH, BW, CS, GN, HP	Bd wt	75 M 5 F	150 M 75 F	150 F	M: 18% depressed body weight gain F: 10 and 27% depressed body weight gain at 75 and 150 mg/kg/day, respectively
					Resp	75 M 5 F	150 M 75 F		Multifocal alveolar histiocytosis
					Cardio	150			
					Gastro	150			
					Hemato	75 M 5 F	150 M 75 F		M: decreased platelets F: decreases in erythrocytes, platelets, hematocrit
					Musc/skel	150			
					Hepatic	150			
					Renal	5	75		Degeneration in descending proximal convoluted tubules
					Ocular	75 F		150 F	Retinal degeneration, cataracts
					Endocr	5	75		13 and 65% decreased serum T4 in males and females, respectively; increased thyroid weight in females
					Immuno	150			
					Neuro	150			

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Repro	75 M 150 F		150 M	Testicular atrophy
					Other noncancer	5 F	75 F		Atrophy of adipose tissue
Charles et al. 1996a; EPA 1996a – 2,4-D									
26	Rat (Fischer 344) 20 M, 20 F	13 weeks (F)	0, 1, 5, 15, 45	BI, BW, CS, GN, HE, HP, LE, OW	Bd wt	45			
					Resp	45			
					Cardio	45			
					Gastro	45			
					Hemato	45			
					Musc/skel	45			
					Hepatic	45			
					Ocular	45			
					Endocr	45			
					Immuno	45			
					Neuro	45			
					Repro	45			
					Other noncancer	45			
EPA 1984 – 2,4-D									
27	Rat (Fischer 344) 10 M, 10 F 52-week interim sacrifice in a 2-year study	52 weeks (F)	0, 1, 5, 15, 45	BI, BW, CS, FI, GN, HE, HP, LE, OW, UR	Bd wt	45			
					Resp	45			
					Cardio	45			
					Gastro	45			
					Hemato	45			
					Musc/skel	45			
					Hepatic	45			
					Dermal	45			
					Ocular	45			
					Endocr	45			

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Immuno	45			
					Neuro	45			
					Repro	45			
					Other noncancer	45			
EPA 1985 – 2,4-D									
28	Rat (Fischer 344) 30 M, 30 F	40 weeks (F)	F0: 0, 5, 20, 80 (target doses) TWA doses to F0 dams on GD 0–LD 14 for F1b pups: 0, 9, 32, 110	BW, CS, DX, GN, HP, MX, OW	Bd wt	80			
					Hepatic	80			
					Repro	80			
					Develop	32		110	F1b pups: 24% depressed pup body weight on PPD 21 (TWA dose to F0 dams during GD 0–LD 14)
EPA 1986, 1987b – 2,4-D									
29	Rat (Fischer 344) 15 M, 15 F	13 weeks (F)	0, 15, 60, 100, 150	BI, BW, CS, GN, HE, HP, LE, OW, UR	Bd wt	150 M 100 F	150 F		21% depressed weight gain
					Resp	150			
					Cardio	150			
					Gastro	150			
					Hemato	150			
					Musc/skel	150			
					Hepatic	100	150		Slight swelling and increased cytoplasmic homogeneity of hepatocytes
					Renal	15	60		Slight multifocal degeneration of descending proximal tubules
					Ocular	150			
					Endocr	150 M 60 F	100 F		Decreased serum T4
					Immuno	150			

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Neuro	150			
					Repro	150			
Gorzinski et al. 1987 – 2,4-D									
30	Rat (Osborne Mendel) 10 M, 20 F	3-gen (F)	0, 7.4, 37, 111	DX, MX, OF	Repro	111			
					Develop	37		111	Depressed pup body weight, decreased pup viability
Hansen et al. 1971 – 2,4-D									
31	Rat (Albino) 6 M	30 days 1 time/day (GO)	0, 50, 100, 150	BI, BW, HP, OF, OW	Repro		50		Decreased sperm count and motility; testicular histopathology
Joshi et al. 2012 – 2,4-D									
32	Rat (Wistar) 14 M	30 days 1 time/day (GW)	0, 100, 200	BW, CS, FI, GN, HP, LE, OW, WI	Repro			100	Decreased weight of testis, seminal vesicles, prostate; decreased number of spermatozoa; decreased sperm quality; morphological changes in prostate and seminiferous tubules; decreased serum testosterone; increased serum LH and FSH
Marouani et al. 2017 – 2,4-D									

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
33	Rat (Sprague-Dawley) 27 M, 27 F (parental rats in 1-gen study)	M: 11–13 weeks F: 10–12 weeks (F)	M: 0, 5.5, 16.6, 45.3 F: 0, 7.7, 22.9, 45.2 (estimated TWA female doses based on reported 29 days premating, 21 days gestation, 14 days lactation)	BI, BW, CS, FI, GN, HE, HP, OF, OW	Bd wt	45.3 M 45.2 F			
					Hemato	45.3 M 45.2 F			
					Renal	16.6[c] M 45.2 F	45.3 M		Slight degeneration of proximal convoluted tubules
					Endocr	45.3 M 22.9 F	45.2 F		Decreased serum T3 and T4; increased TSH on GD 17 (LOAEL is for TWA maternal dose for premating through LD 14)
					Immuno	45.3 M 45.2 F			
					Neuro	45.3 M 45.2			
					Repro	45.3 M 45.2 F			
					Develop	24.7	49.4		9% depressed pup weight at PPD 22 (LOAEL is for TWA maternal dose for GD 0–LD 14)
Marty et al. 2013 – 2,4-D									
34	Rat (Sprague-Dawley) 12 F (satellite group in 1-gen study)	7–8 weeks (4 weeks before mating until GD 17) (F)	0, 7.21, 21.67, 42.04	BI, BW, CS, GN, HE, HP, OF, OW	Bd wt	42.04			
					Hemato	42.04			
					Renal	42.04			
					Endocr	42.04			
					Repro	42.04			
Marty et al. 2013 – 2,4-D									

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
35	Rat (Sprague-Dawley) 10 M, 10 F (set 1a pups of 1-gen study)	PPDs 21–70 (F) + gest/lact	M: 0, 9.24, 28.4, 76.6 F: 0, 9.56, 28.8, 57.9	GN, HP, OF	Bd wt	76.6 M 57.9 F			No effect
					Renal	28.4 M 28.8 F	76.6 M 57.9 F		M: Very slight/slight degeneration in proximal convoluted tubules (9/10 rats) F: 11% increased kidney weight; very slight degeneration in proximal convoluted tubules (5/10 rats)
					Endocr	76.6 M 57.9 F			
					Repro	76.6 M 57.9 F			
Marty et al. 2013 – 2,4-D									
36	Rat (Sprague-Dawley) 10 M, 10 F (set 1b pups of 1-gen study)	PPDs 21–60 (F) + gest/lact	M: 0, 9.88, 29.5, 81.7 F: 0, 10.1, 30, 59.2	GN, HP, OF	Neuro	81.7 M 59.2 F			
Marty et al. 2013 – 2,4-D									
37	Rat (Sprague-Dawley) 10 M, 10 F (set 2a pups of 1-gen study)	PPDs 21–70 (F) + gest/lact	M: 0, 9.15, 28.4, 75.3 F: 0, 9.66, 28.7, 58.4	GN, HP, OF	Immuno	75.3 M 58.4 F			No effect in SRBC AFC assay
Marty et al. 2013 – 2,4-D									

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
38	Rat (Sprague-Dawley) 10 M, 10 F (set 2b pups of 1-gen study)	PPDs 21–90 (F) + gest/lact	M: 0, 8.67, 25.8, 71.8 F: 0, 9.05, 26.7, 55.3	GN, HP, OF	Immuno	71.8 M 55.3 F			No effect in NK assay
Marty et al. 2013 – 2,4-D									
39	Rat (Sprague-Dawley) 23–27 M, 23–27 F (set 3 pups of 1-gen study)	PPD 21–139 (F) + gest/lact	M: 0, 6.83, 20.9, 55.6 F: 0, 7.59, 23.3, 46.7	GN, HP, OF	Endocr	55.6 M 46.7 F			
					Repro	55.6 M 46.7 F			
Marty et al. 2013 – 2,4-D									
40	Rat (Fischer 344) 15 M, 15 F	52 weeks (F)	0, 5, 75, 150	BW, GN, HP, OF	Bd wt	75	150		10% depressed terminal body weight
					Ocular	150 M 75 F		150 F	Retinal degeneration
Mattsson et al. 1997 – 2,4-D									
41	Rat (Albino) 10 F	GD 1–19 1 time/day (GO)	0, 100	DX, MX, TG	Bd wt			100	40–54% depressed maternal weight gain
					Develop			100	31% depressed fetal weight; morphological and skeletal defects
Mazhar et al. 2014 – 2,4-D									

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
42	Rat (Sprague-Dawley) 10 M	3 months (F)	0, 1.5, 7.1, 21.5, 64.6, 107.7, 215	BI, BW, HP, OW	Bd wt Hepatic	215 215			
Ozaki et al. 2001 – 2,4-D									
43	Rat (Wistar) NS F	GD 16–PPD 23 (F)	0, 70	BW, FI, DX, MX	Bd wt Develop	70 70			No effect on maternal body weight
Pochettino et al. 2016 – 2,4-D									
44	Rat (Wistar) 8 M	PPDs 24–45 following maternal exposure on GD 16–PPD 23 (F)	0, 70	BI, BW, DX, OF, OW	Develop		70		13% depressed body weight; effects on prostate effects (decreases in weight, epithelial thickness, and androgen receptor expression); decreased serum levels of testosterone, dihydroxytestosterone, IGF-1
Pochettino et al. 2016 – 2,4-D									
45	Rat (Wistar) 8 M	PPDs 24–60 following maternal exposure on GD 16–PPD 23 (F)	0, 70	BI, BW, DX, OF, OW	Develop		70		11% depressed body weight; decreased prostate weight; decreased serum levels of testosterone, dihydroxy-testosterone, IGF-1
Pochettino et al. 2016 – 2,4-D									
46	Rat (Wistar) 8 M	PPDs 24–90 following maternal exposure on GD 16–PPD 23 (F)	0, 70	BI, BW, DX, OF, OW	Develop		70		Prostate changes (increased weight, decreased alveolar epithelial thickness and cell number, increased androgen receptor expression); decreased serum IGF-1
Pochettino et al. 2016 – 2,4-D									

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
47	Rat (Sprague-Dawley) 10 M, 10 F	M: 71 days F: 96 days (4 weeks premating onward) (F)	0, 6, 25, 50, 75, 100 (maternal doses 0, 15, 58, 118, 140, 173 at LD 14)	BW, CS, GN, HE, HP, OW	Bd wt	100			
					Renal	50 M	75 M		Slight multifocal degeneration of proximal convoluted tubules in outer stripe of outer zone of medulla of males
					Repro	100			
					Develop	58		118	Up to 23% depressed pup weight during PPDs 14–21
Saghir et al. 2013a, 2013b – 2,4-D									
48	Rat (Fischer 344) 8 M	5 weeks 2 days/week 1 time/day (GO)	0, 20, 40, 80	BW, CS, OF	Bd wt	80			
Squibb et al. 1983 – 2,4-D									
49	Rat (Wistar) 6–8 F	PPD 1–16 (F)	0, 2.5, 5, 10, 15, 25, 50, 70	BH, BI, BW, DX, MX	Bd wt	70			No effect on maternal body weight
Stürtz et al. 2010 – 2,4-D									
50	Rat (Wistar) 6 F	GD 14–21 PPD 0–14 (W)	0, 126	BI, BW, CS, DX, MX	Bd wt	126			No effect on maternal body weight
					Hepatic		126		Increased maternal serum transaminases; liver histopathology
					Develop		126		Pups: 18% depressed PPD 14 body weight; decreased liver weight, increased serum transaminases, liver histopathology
Troudi et al. 2012a – 2,4-D									

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
51	Rat (Wistar) 6 F	GD 14–21 PPD 0–14 (W)	0, 126	BI, DX, HP, MX, OW	Bd wt	126			No effect on maternal body weight
					Develop		126		17% depressed PPD 14 pup weight; bone histopathology
Troudi et al. 2012b – 2,4-D									
52	Mouse (B6C3F1) 10 M, 10 F 12-month evaluation in 2-year study	12 months (F)	M: 0, 5, 62.5, 125 F: 0, 5, 150, 300	BI, BW, CS, FI, GN, HE, HP, LE, UR	Bd wt	125 M 300 F			
					Resp	125 M 300 F			
					Cardio	125 M 300 F			
					Gastro	125 M 300 F			
					Hemato	125 M 300 F			
					Musc/skel	125 M 300 F			
					Hepatic	125 M 300 F			
					Renal	5 M 5 F	62.5 M 150 F		M: increased kidney weight; vacuolation of proximal tubules; degeneration/regeneration in descending proximal tubule F: increased kidney weight; hypercellularity in descending proximal tubule
					Ocular	125 M 300 F			
					Endocr	125 M 300 F			
					Immuno	125 M 300 F			

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Neuro	125 M 300 F			
					Repro	125 M 300 F			
Charles et al. 1996a; EPA 1996b – 2,4-D									
53	Mouse (B6C3F1) 20 M, 20 F	13 weeks (F)	0, 5, 15, 45, 90	BI, BW, CS, GN, HE, HP, LE, OW	Bd wt	90			
					Resp	90			
					Cardio	90			
					Gastro	90			
					Hemato	90			
					Musc/skel	90			
					Hepatic	90			
					Ocular	90			
					Endocr	90			
					Immuno	90			
					Neuro	90			
					Repro	90			
EPA 1984 – 2,4-D									
54	Mouse (B6C3F1) 10 M, 10 F	52 weeks (F)	0, 1, 15, 45	BW, CS, FI, GN, HP, LE	Bd wt	45			
					Resp	45			
					Cardio	45			
					Gastro	45			
					Hemato	45			
					Musc/skel	45			
					Hepatic	45			
					Dermal	45			
					Ocular	45			
					Endocr	45			
					Immuno	45			

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Neuro	45			
					Repro	45			
EPA 1987a – 2,4-D									
55	Mouse (B6C3F1) 10 M	3 months (F)	0, 3.1, 7.2, 21.2, 63.9, 178.9, 429.4	BI, BW, HP, OW	Bd wt	178.9	429.4		18% depressed terminal body weight
					Hepatic	429.4			
					Renal	178.9	429.4		Renal tubule epithelium lesions
Ozaki et al. 2001 – 2,4-D									
56	Hamster (Golden Syrian) 10 M	3 months (F)	0, 1.1, 9.5, 47.4, 94.8, 474	BI, BW, HP, OW	Bd wt	474			
					Hepatic	474			
					Renal	474			
Ozaki et al. 2001 – 2,4-D									
CHRONIC EXPOSURE									
57	Rat (Fischer 344) 50 M, 50 F	2 years (F)	0, 5, 75, 150	BI, BW, CS, FI, GN, HE, HP, LE, UR	Bd wt	150 M 5 F	75 F	150 F	19 and 43% depressed weight gain at 75 and 150 mg/kg/day, respectively
					Resp	150			
					Cardio	150			
					Gastro	150			
					Hemato	5	75		M: decreased platelets F: decreases in platelets, erythrocyte counts, hematocrit
					Musc/skel	150			
					Hepatic	150			
					Renal	150			
					Ocular	75		150	Retinal degeneration, cataracts
					Endocr	5	75		Decreased serum T4 in males and females; increased thyroid weight in females
					Immuno	150			

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Neuro	150			
					Repro	150			
					Other noncancer	75	150		Atrophy of adipose tissue
Charles et al. 1996a; EPA 1996a – 2,4-D									
58	Rat (Osborne Mendel) 25 M, 25 F	2 years (F)	0, 0.37, 1.85, 9.25, 46.3, 92.5	BH, BW, CS, GN, HP	Bd wt	92.5			
					Resp	92.5			
					Cardio	92.5			
					Gastro	92.5			
					Musc/skel	92.5			
					Hepatic	92.5			
					Renal	92.5			
					Endocr	92.5			
					Immuno	92.5			
					Repro	92.5			
Hansen et al. 1971 – 2,4-D									
59	Mouse (B6C3F1) 50 M, 50 F	2 years (F)	M: 0, 5, 62.5, 125 F: 0, 5, 150, 300	BI, BW, CS, FI, GN, HE, HP, LE, UR	Bd wt	125 M 300 F			
					Resp	125 M 300 F			
					Cardio	125 M 300 F			
					Gastro	125 M 300 F			
					Hemato	125 M 300 F			
					Musc/skel	125 M 300 F			
					Hepatic	125 M 300 F			

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Renal	5 M 5 F	62.5[d] M 150 F		Proximal tubule degeneration/ regeneration
					Ocular	125 M 300 F			
					Endocr	125 M 300 F			
					Immuno	125 M 300 F			
					Neuro	125 M 300 F			
					Repro	125 M 300 F			
Charles et al. 1996a; EPA 1996b – 2,4-D									
60	Mouse (B6C3F1) 50 M, 50 F	2 years (F)	0, 1, 15, 45	BI, BW, CS, FI, GN, HE, HP, LE, UR	Bd wt	45			
					Resp	45			
					Cardio	45			
					Gastro	45			
					Hemato	45			
					Musc/skel	45			
					Hepatic	45			
					Dermal	45			
					Ocular	45			
					Endocr	45			
					Immuno	45			

Table 2-2. Levels of Significant Exposure to 2,4-D – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Neuro	45			
					Repro	45			
EPA 1987a – 2,4-D									

[a]The number corresponds to entries in Figure 2-3.
[b]Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[c]Used to derive an intermediate-duration oral minimal risk level (MRL) for 2,4-D; based on a NOAEL of 16.6 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability); see Appendix A for more detailed information regarding the MRL.
[d]Study result used to derive a chronic-duration oral MRL for 2,4-D; based on a $BMDL_{10}$ of 16.66 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability); see Appendix A for more detailed information regarding the MRL.

2,4-D = 2,4-dichlorophenoxyacetic acid; AFC = antibody forming cell; BC = serum (blood) chemistry; Bd wt or BW = body weight; BH = behavioral; BI = biochemical changes; BMDL = 95% lower confidence limit on the benchmark dose (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); Cardio = cardiovascular; CNS = central nervous system; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FSH = follicle-stimulating hormone; FX = fetal toxicity; (G) = gavage-not specified; (GO) = gavage-oil; (GW) = gavage in water; Gastro = gastrointestinal; GD = gestation day(s); gen = generation; gest/lact = gestational and lactational exposure; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LD = lactation day; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NK = natural killer; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; PPD = post-parturition day; Repro = reproductive; Resp = respiratory; SRBC = sheep red blood cell; T3 = triiodothyronine; T4 = thyroxine; TG = teratogenicity; TSH = thyroid-stimulating hormone; TWA= time-weighted average; UR= urinalysis; (W) = drinking water; WI = water intake

Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Acute (≤14 days)

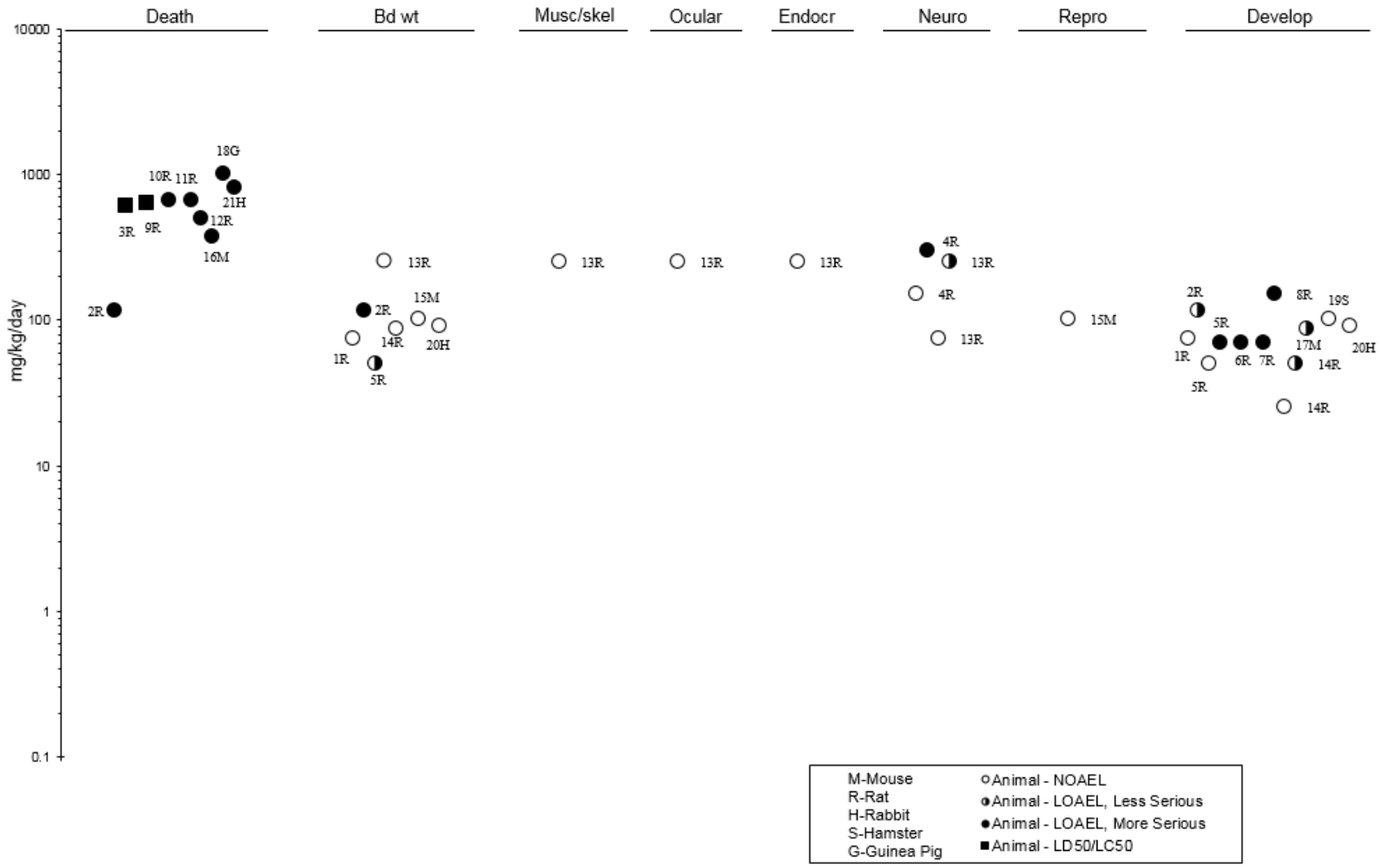


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Intermediate (15-364 days)

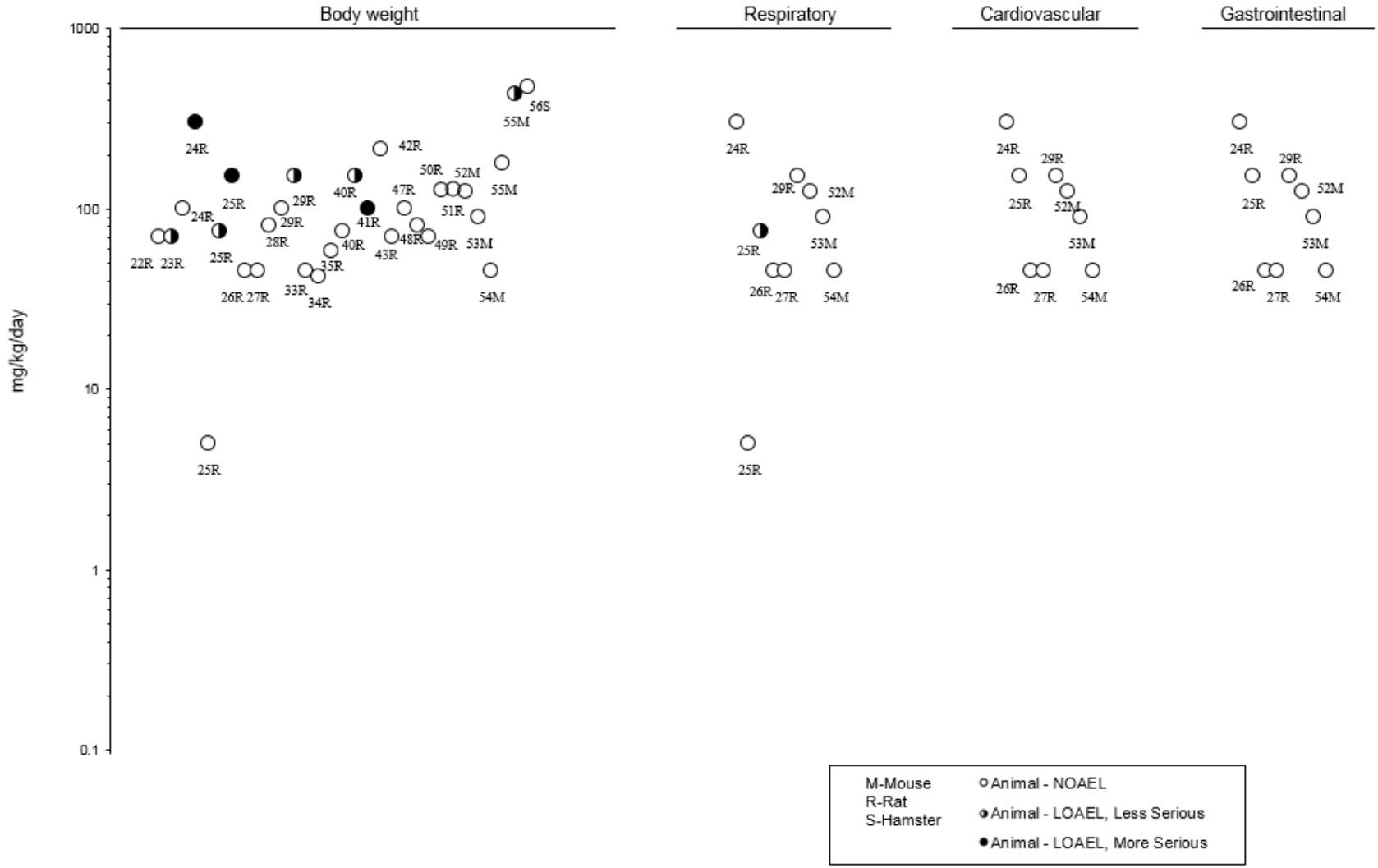


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Intermediate (15-364 days)

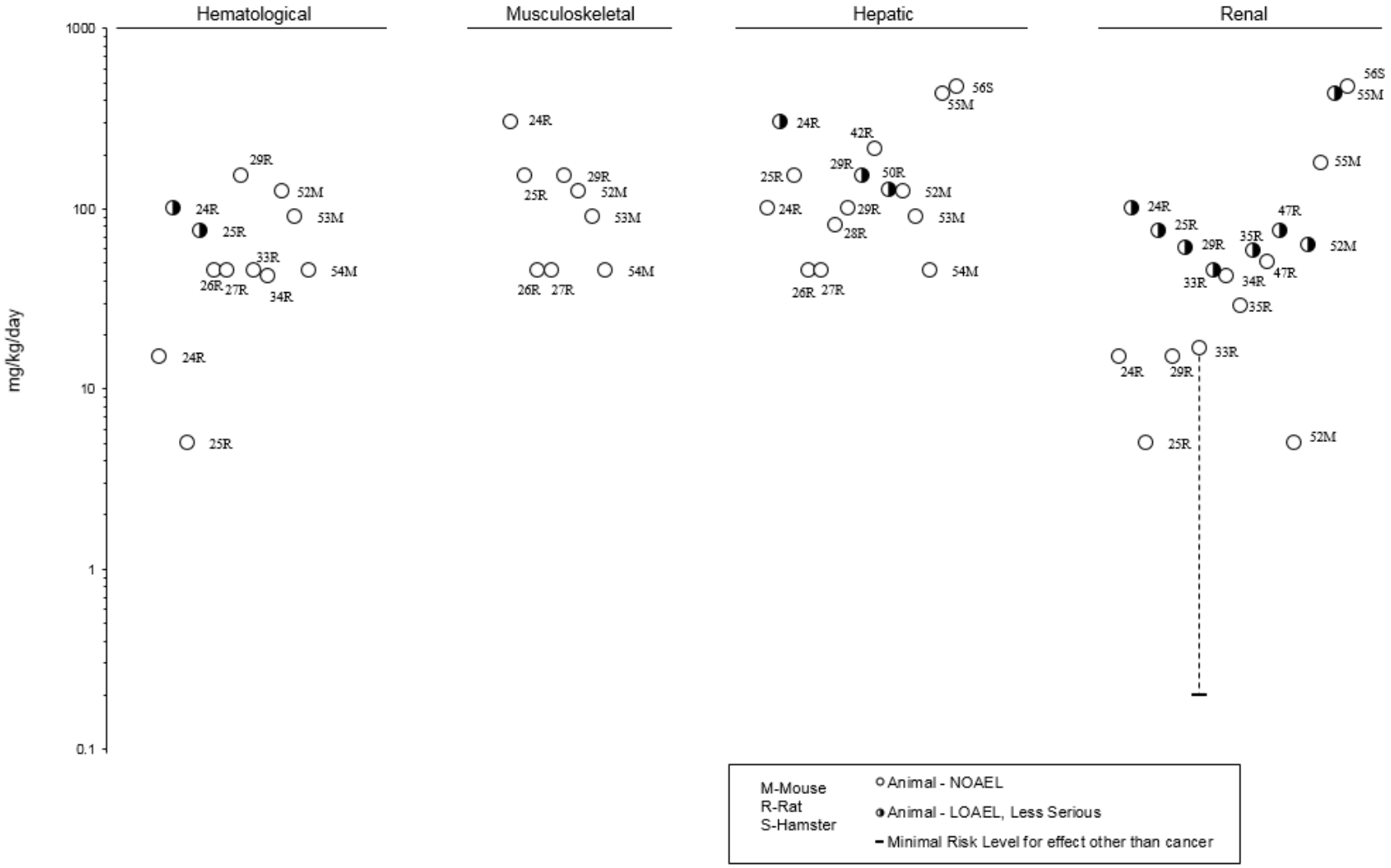


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Intermediate (15-364 days)

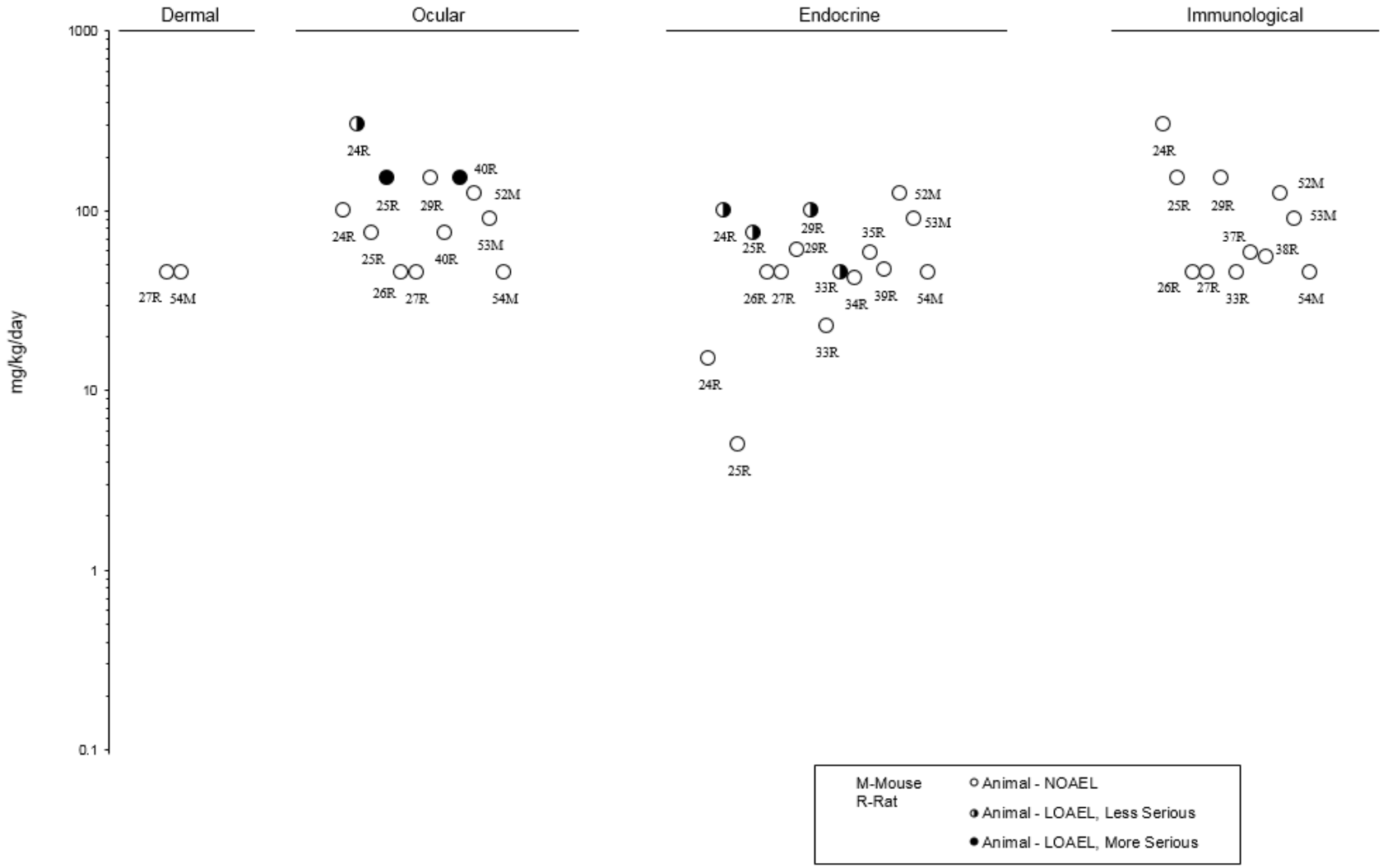


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Intermediate (15-364 days)

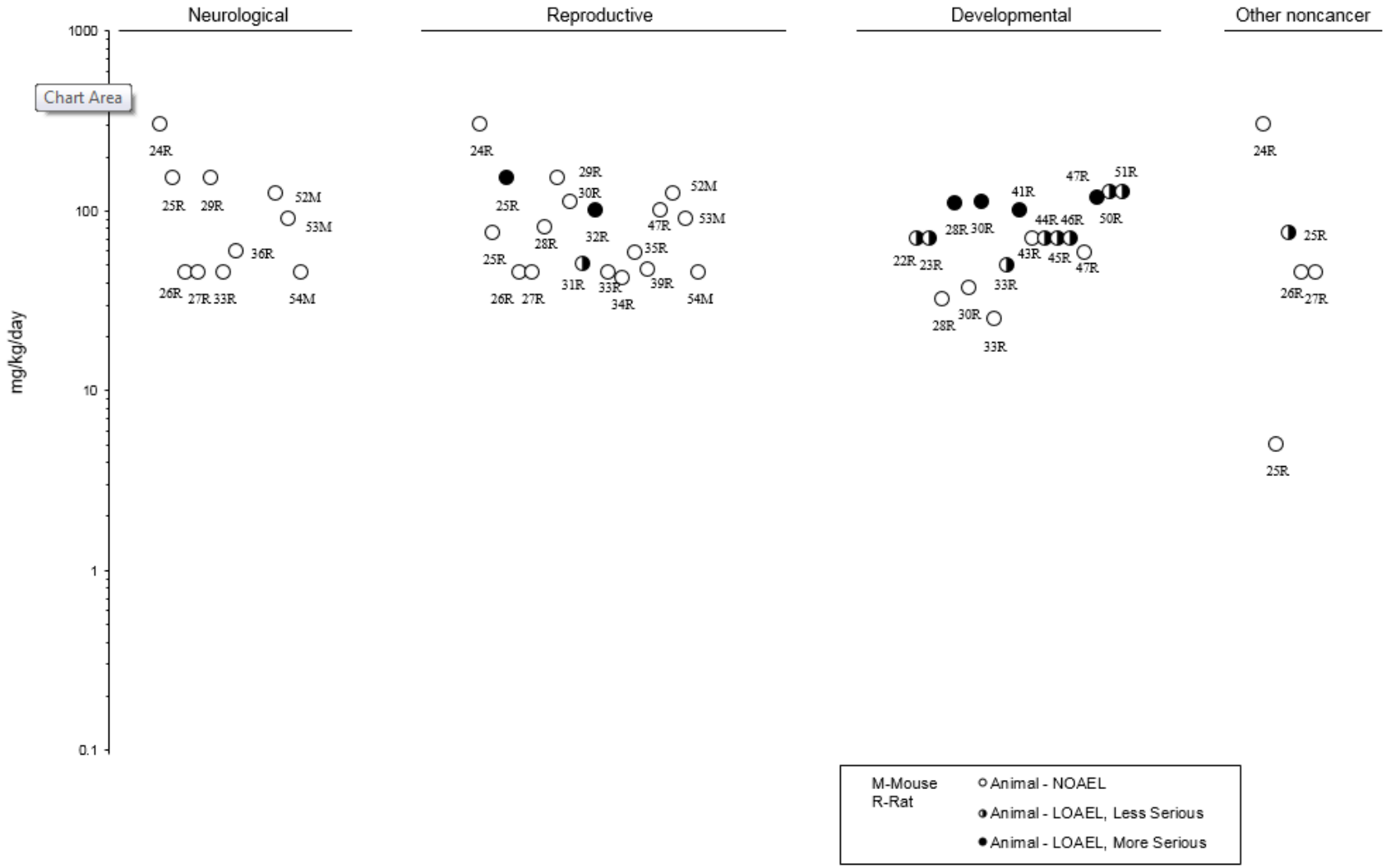


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Chronic (≥365 days)

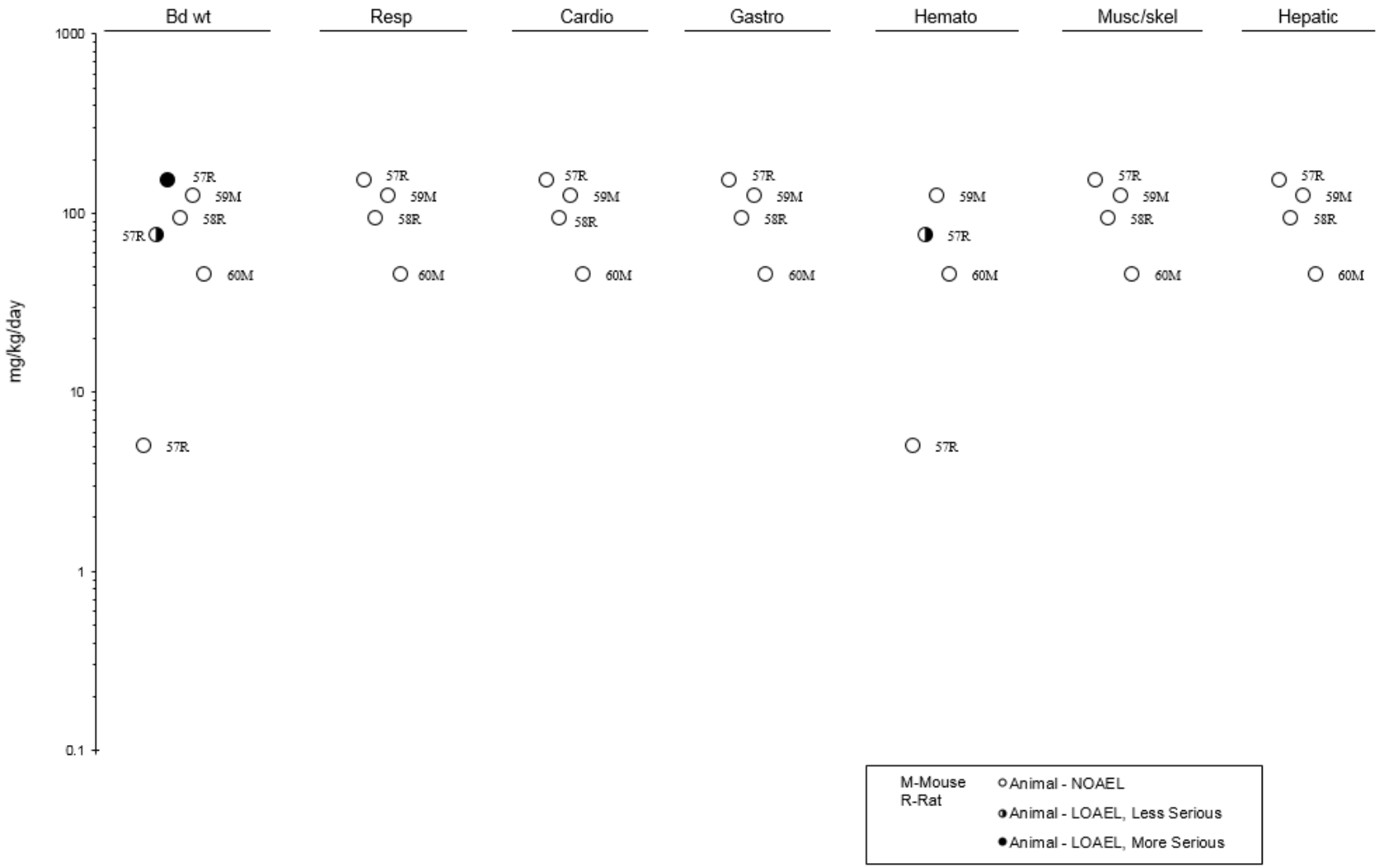


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Chronic (≥365 days)

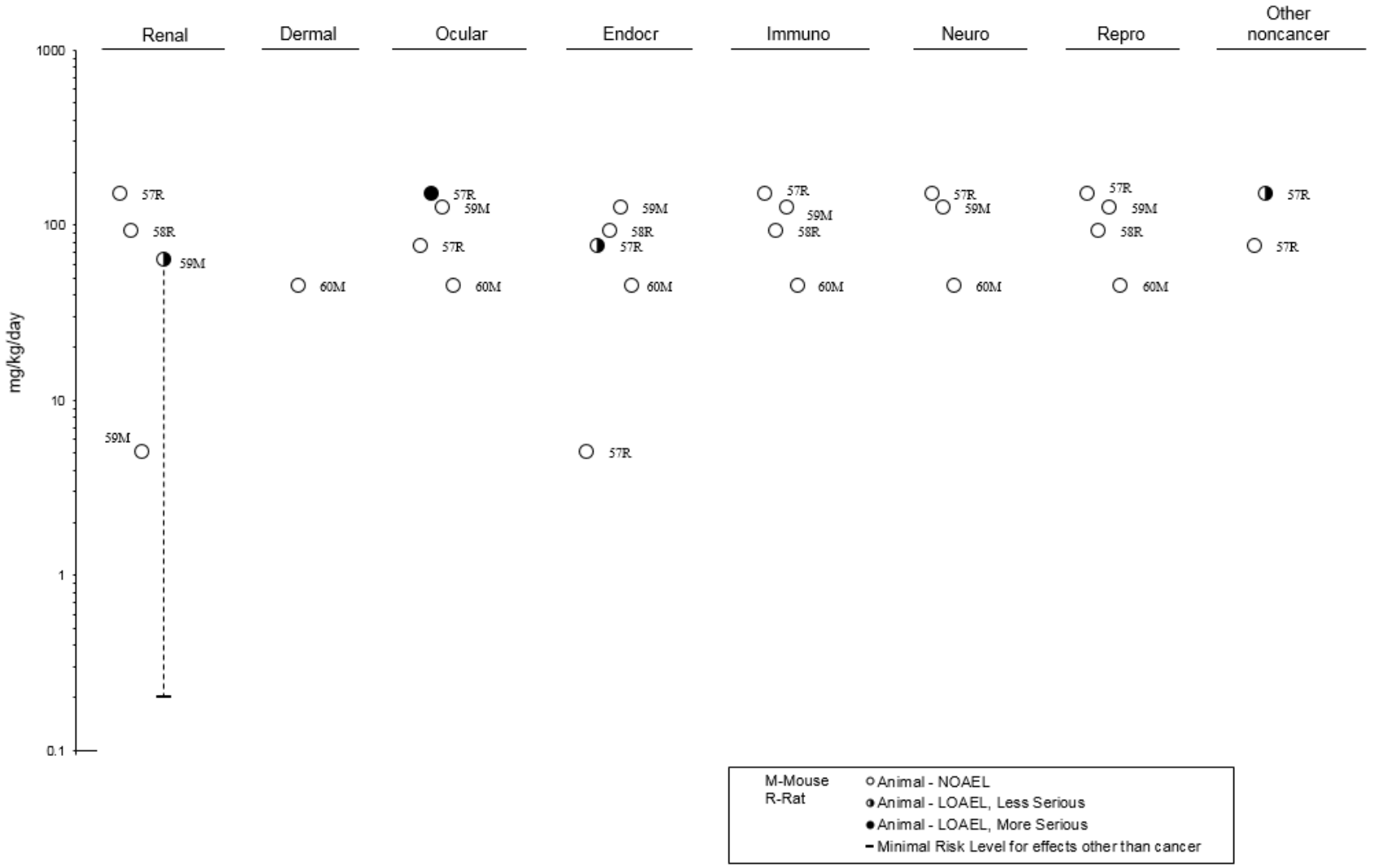


Table 2-3. Levels of Significant Exposure to 2,4-D – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
ACUTE EXPOSURE								
Mouse (BALB/c) 6–7 F	9 days (sensitization on days 1–3, 8–10, 15–17; challenge 2 weeks later)	25 µg for sensitization and dermal challenge 50 µL intratracheal challenge	BI, OF	Immuno		50 µL		Respiratory allergen
Fukuyama et al. 2009 – 2,4-D								
Dog (hairless) 3 NS	7 days 1 time/day (occluded application to abraded abdominal skin)	0.036 mg	GN, HP	Dermal		0.036 mg		Slight epidermal thickening and hyperplasia
Kimura et al. 1998 – 2,4-D								
Rabbit (New Zealand) 3 M, 3 F	Once for 4 hours (occluded application to intact skin)	500 mg	CS, GN	Dermal	500 mg			
EPA 1992 – 2,4-D								
INTERMEDIATE EXPOSURE								
Rabbit (New Zealand) 5 M, 5 F	21 days (daily 6-hour occluded dermal application)	0, 10, 100, 1,000 mg/kg/day	BI, BW, CS, FI, GN, HE, HP, LE, OW	Bd wt	1,000 (mg/kg/day)			
				Hemato	1,000 (mg/kg/day)			
				Hepatic	1,000 (mg/kg/day)			
				Renal	1,000 M 100 F (mg/kg/day)	1000 F (mg/kg/day)		Increased kidney weight

Table 2-3. Levels of Significant Exposure to 2,4-D – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
				Dermal		10 (mg/kg/day)		Very slight erythema in all treated groups
				Ocular	1,000 (mg/kg/day)			
EPA 1991a – 2,4-D								

2,4-D = 2,4-dichlorophenoxyacetic acid; Bd wt or BW = body weight; BI = biochemical changes; CS = clinical signs; F = female(s); FI = food intake; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight

2.2 DEATH

Cause-specific mortality was examined among employees engaged in the manufacture, formulation, or packaging of 2,4-D and related salts. Three studies were published: the original report (Bond et al. 1988), a 4-year follow-up (Bloemen et al. 1993), and a subsequent assessment of mortality to the end of 1994 (Burns et al. 2001). Various industrial plants were involved, and potential exposure to other chemicals was likely to have occurred based on the plant, the period, and the job; however, the common factor for the cohort was potential exposure to 2,4-D. Exposure data were provided in the first report and ranged from an estimated time-weighted average (TWA) of 0.18–3 mg/m^3 2,4-D for the various job categories. The first report included 878 chemical workers and the most recent report involved 1,515 male employees who contributed 39,799 person-years at risk for an average follow-up of 26.2 years. In none of the three studies were there patterns suggestive of a causal association between exposure to 2,4-D and any particular cause of death, including NHL, which has received the most attention in relation to exposure to phenoxy herbicides. Bloemen et al. (1993) calculated a standardized mortality ratio (SMR) of 196 (95% confidence interval [CI] 24–708) and Burns et al. (2001) calculated an SMR of 1.0 (95% CI 0.21–292) for NHL in the studies.

Many additional studies have examined mortality rates in subjects exposed to herbicides, particularly phenoxy herbicides that included 2,4-D, but did not conduct analyses for individual chemicals. Some examples of such studies include Becher et al. (1996), Bueno de Mesquita et al. (1993), Coggon et al. (1991), Gambini et al. (1997), Green (1991), Riihimäki et al. (1982), Saracci et al. (1991), Thörn et al. (2000), and Zahm (1997). Cohort sizes ranged from a few hundred subjects (Thörn et al. 2000) to >30,000 subjects in a study of employees of a lawn care service company (Zahm 1997). Except for the Zahm (1997) study, none of these studies found significantly elevated mortality risks for NHL. Zahm (1997) reported a significantly elevated SMR of 7.11 (95% CI 1.78–28.42) based on two cases of NHL among male applicators employed in the lawn care service company for >3 years. Although it could not be concluded that the NHL risk was related to exposure to pesticides or to a specific product such as 2,4-D, it was the only tumor with a duration effect; the SMR of 7.11 was similar to higher risk seen in frequent herbicide users in other studies (see Section 2.19, Cancer).

There have been deaths reported after intentional or accidental ingestion of products containing 2,4-D. Some examples are summarized below.

Nielsen et al. (1965) reported the case of a man who ingested an unknown amount of a commercial preparation containing the dimethyl amine salt of 2,4-D and died. An autopsy conducted on the same day of death showed acute congestion in all internal organs. Histological examination of the nervous system at various levels showed severe, degenerative changes of ganglion cells. Spots of acute emphysema were reported in the lungs, whereas the bronchioles contained presumed aspirated material. The total amount of 2,4-D measured in the various organs, blood, and urine was approximately 6 g (~80 mg/kg body weight). Dudley and Thapar (1972) reported the case of a man who died 6 days after ingestion of an unknown amount of 2,4-D. Signs observed prior to death included deep coma, altered respiration, hyperactive deep tendon reflexes, and moderate emphysema. Death was presumed to have been due to atrial fibrillation induced by muscle irritability associated with 2,4-D ingestion. Microscopic examination of tissues showed lesions in the brain, lungs, liver, and kidneys. Because the subject was 76 years old and autopsy was delayed for 36 hours, many of the histopathological alterations observed may not have been necessarily due to exposure to 2,4-D. Smith and Lewis (1987) reported a lethal case to have been due to ingestion of an unknown amount of an herbicide containing 2,4-D, based on the large amounts of 2,4-D found in the stomach and liver. No information was available regarding signs or symptoms preceding death. The only reported pathological findings were pulmonary edema and reddish watery fluid in the abdominal and thoracic cavities. An additional case of oral intoxication that ended up in death was reported by Keller et al. (1994). In this case, the subject had intentionally ingested an unknown amount of a commercial product that contained 500 g of 2,4-D/L. Based on levels of 2,4-D in blood, the investigators estimated that the amount of 2,4-D ingested was at least 25–35 g. Respiratory and kidney failure developed; death occurred after 48 hours of intensive care due to multiple organ failure.

An inhalation LC_{50} >1,790 mg/m^3 was reported for 2,4-D in rats (EPA 2005a); no further details were provided. No deaths were reported among Sprague-Dawley rats exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts 6 hours/day, 5 days/week for 28 days (EPA 2008). Rat oral LD_{50} values between 600 and 800 mg/kg for 2,4-D have been reported (Elo et al. 1988; Gorzinski et al. 1987; Hill and Carlisle 1947). In one study, males appeared to be slightly more sensitive than females (Gorzinski et al. 1987). An early study that tested various species reported oral LD_{50} values for 2,4-D sodium salt of 1,000, 800, 666, and 375 mg/kg for guinea pigs, rabbits, rats, and mice, respectively (Hill and Carlisle 1947); it was also reported that the sodium and ammonium salts had about the same toxicity as the acid. An oral LD_{50} of 100 mg/kg was reported for 2,4-D in mongrel dogs (Drill and Hiratzka 1953), although results from other acute studies in dogs do not support such a relatively low LD_{50} value (Dickow et al. 2000; Steiss et al. 1987). Common signs reported by Drill and Hiratzka (1953) included stiffness of the extremities with some muscular incoordination, lethargy, paralysis of the hindquarters, stupor, coma, and death. Hill and

Carlisle (1947) noted that some combination of some of these signs resembled myotonia congenita. Data regarding lethality in animals following dermal exposure are limited to a reported dermal LD_{50} >2,000 mg/kg for rabbits (Gorzinski et al. 1987).

In a developmental study, repeated oral doses of 115 mg/kg 2,4-D decreased survival of pregnant rats (Chernoff et al. 1990). In a repeated dose 13-week study, three out of four dogs administered capsules of 20 mg/kg/day 5 days/week died on days 18, 25, and 49 (Drill and Hiratzka 1953). Higher-than-normal muscle tonus in the hind limbs, particularly on passive extension, was described in these dogs; slight ataxia was also present. The days preceding death, the dogs showed difficulty in chewing or swallowing and there was also some oozing of blood from the gums and buccal mucosa.

2.3 BODY WEIGHT

A study that included 8,365 male pesticide applicator participants in the AHS examined the relationship between total cumulative exposure from age 20 years to the time of 5-year follow-up to classes of pesticides and individual components and body mass index (BMI) (LaVerda et al. 2015). Results from unadjusted and adjusted regression models that maintained all covariates in models estimating the association between exposure and amount of BMI associated with 100 cumulative exposure days between age 20 and age at follow-up showed a positive association for 2,4-D for Iowa applicators (p=0.0258 and 0.0183, respectively). However, after medical exclusions (cancer excluding non-melanoma skin cancer, diabetes, heart disease, lupus, and/or amyotrophic lateral sclerosis), no significant associations remained (p=0.2408).

Significant weight loss (~9 kg) was reported in two cases of dermal exposure to herbicide products containing 2,4-D (Goldstein et al. 1959). One of the cases had experienced nausea and vomiting for about 10 days after exposure, which could explain, at least in part, the weight loss. The other patient had been affected by anorexia while hospitalized due to adverse neurological symptoms.

Body weight of female rats intermittently exposed nose-only to 1,000 mg/m^3 2,4-D dusts for 28 days followed by a 4-week recovery period was significantly reduced (11–13%) from day 14 onward relative to controls (EPA 2008). Food consumption in this group was reduced approximately 10% during the study. No significant effects were reported in females exposed to ≤300 mg/m^3 2,4-D. In males, differences between exposed and control groups were either not statistically significant or were ≤10%.

Many oral animal studies monitored body weight, but making generalizations is difficult due to apparent inconsistencies between studies. Apparent inconsistencies may be due to testing animals of different ages (i.e., adults versus growing animals) or pregnant females, which could be more susceptible than nonpregnant females. Studies do not always provide data on food consumption. Even if they do, reduced food consumption in dietary studies may be due, in part, to poor palatability.

In rats administered a single gavage dose of 250 mg 2,4-D/kg, body weight was not affected over the next 15 days (Mattsson et al. 1997). Dosing of pregnant Wistar rats with ≥50 mg 2,4-D/kg/day by gavage on GDs 6–15 resulted in significant dose-related weight loss during pregnancy (Fofana et al. 2000), but dosing pregnant F-344 rats by gavage with ≤75 mg 2,4-D/kg/day or pregnant Sprague-Dawley rats with ≤87.5 mg 2,4-D/kg/day on GDs 6–15 did not significantly affect weight gain during treatment (Charles et al. 2001; Schwetz et al. 1971), suggesting that Wistar rats are more susceptible than F-344 rats. However, dosing pregnant Sprague-Dawley rats with 115 mg 2,4-D/kg/day on GDs 6–15 resulted in reduced weight gain during treatment (Chernoff et al. 1990). No effects were reported in pregnant rabbits dosed by gavage with 90 mg 2,4-D/kg/day on GDs 6–18 (Charles et al. 2001). Body weight was not significantly affected in mice dosed with 100 mg 2,4-D/kg via drinking water for 10 days (Dinamarca et al. 2007).

Intermediate-duration oral studies in rats provide a less-than-clear picture of 2,4-D treatment-related body weight effects. Three studies reported a NOAEL of 100 mg 2,4-D/kg/day (Charles et al. 1996b; Gorzinski et al. 1987; Saghir et al. 2013a, 2013b). Doses ≥150 mg 2,4-D/kg/day significantly decreased body weight gain (Charles et al. 1996b; Gorzinski et al. 1987; Mattsson et al. 1997). A 5-week study in rats reported a NOAEL of 80 mg 2,4-D/kg/day (Squibb et al. 1983), whereas a 13-week study reported no significant effects on body weight in rats dosed with 215 mg 2,4-D/kg/day (Ozaki et al. 2001). A study in pregnant rats reported a LOAEL of 100 mg 2,4-D/kg/day for significantly reduced weight gain during pregnancy (Mazhar et al. 2014), while another reported a NOAEL (5% difference between treated and controls) of 126 mg/kg/day (Troudi et al. 2012a). Male offspring from rats exposed to 70 mg 2,4-D/kg/day (only dose tested) during gestation and lactation and then directly showed an 11% reduction in body weight relative to controls at 90 days of age (Bortolozzi et al. 1999).

The highest NOAEL for body weight effects in intermediate-duration oral studies in mice was 178.9 mg 2,4-D/kg/day; the LOAEL was 429.4 mg/kg/day (Ozaki et al. 2001). Dogs exposed to 7.5 mg 2,4-D/kg/day for 52 weeks showed a 64% reduction in weight gain relative to controls; the NOAEL was 5 mg/kg/day (Charles et al. 1996c). Body weight was not significantly affected in hamsters exposed to 474 mg 2,4-D/kg/day for 3 months (Ozaki et al. 2001).

Body weight was not significantly affected in rabbits that received intermittent dermal applications of up to 1,000 mg 2,4-D/kg/day for 21 days (EPA 1991a).

Chronic-duration oral studies reported NOAEL and LOAEL values of 5 and 75 mg 2,4-D/kg/day, respectively, for body weight in rats (Charles et al. 1996a; EPA 1996a) and a NOAEL of 300 mg/kg/day for mice (Charles et al. 1996a; EPA 1996b).

2.4 RESPIRATORY

The AHS is a prospective cohort study of nearly 90,000 private pesticide applicators (mostly farmers), their spouses, and commercial pesticide applicators in Iowa and North Carolina. The AHS is sponsored by the National Institutes of Health (NIH 2014). In the study, exposure and outcome were assessed using two self-administered questionnaires that provided information regarding 40 specific chemicals (2,4-D among them) used in the year before enrollment, pesticide application methods, current agricultural activities, smoking history, medical history, and demographics. In the AHS, use of 2,4-D was associated with current rhinitis (odds ratio [OR] 1.34; 95% CI 1.09–1.64) (Slager et al. 2009). However, further analysis showed that rhinitis was associated only with current use of both 2,4-D and glyphosate, while current use of either herbicide alone was not associated with rhinitis when modeled separately (OR 0.99; 95% CI 0.63–1.54 for 2,4-D alone). In addition, analysis by days/years applied showed no dose-response relationship for 2,4-D. Use of 2,4-D was not associated with wheezing (OR 0.97; 95% CI 0.86–1.10 for farmers; OR 0.99; 95% CI 0.73–1.34 for applicators) (Hoppin et al. 2006a, 2006b).

Hoppin et al. (2017) evaluated risk of allergic and non-allergic wheeze among male participants in the AHS who completed follow-up interviews for the years 2005–2010. In this study, current use of 2,4-D was associated with allergic wheeze (OR 1.46; 95% CI 1.19–1.79), but not non-allergic wheeze (OR 1.12; 95% CI 0.99–1.26). The association was strongest among the men reporting highest use of 2,4-D (16–365 days/year).

In a group of 583 farm women (wives of pesticide applicators) in the AHS, prevalence of self-reported history of doctor-diagnosed chronic bronchitis was associated with lifetime exposure to 2,4-D in models adjusted for age and state (OR 1.29; 95% CI 1.02–1.63) (Valcin et al. 2007). No association was found following multivariate adjustment that added variables within the herbicide group (OR 1.20: 95% CI 0.89–1.63). A similar study of farm women in the AHS found that use of 2,4-D was associated with self-

reported history of atopic asthma (OR 1.53; 95% CI 1.12–2.10), but not with nonatopic asthma (OR 1.07; 95% CI 0.82–1.41) (Hoppin et al. 2008).

Tachypnea was reported in a person who drank 100–200 mL of a 40% solution of 2,4-D (40–80 g) (Durakovic et al. 1992). Emphysema in the lungs was reported in two lethal cases reported by Nielsen et al. (1965) and Dudley and Thapar (1972). A subject who ingested approximately 110 mg 2,4-D/kg from a commercial herbicide product complained of breathing difficulties 24 hours after admission to the hospital (Berwick 1970). Pulmonary edema was noted in a lethal case reported by Smith and Lewis (1987) and respiratory failure was noted in the case reported by Keller et al. (1994).

Labored breathing was reported in rats exposed intermittently nose-only to 2,4-D dust at 1,000 mg/m^3 in a 28-day inhalation study (EPA 2008). The effect was first seen on the 12th exposure; no such effect was seen in rats exposed at ≤300 mg/m^3. Microscopic examination of the respiratory tract of the rats at termination showed lesions restricted to the larynx in all exposed groups (50, 100, 300, and 1,000 mg/m^3). The lesions consisted of squamous/squamoid epithelial metaplasia with hyperkeratosis, hyperplasia of the arytenoid epithelium, and increased number of mixed inflammatory cells and showed dose-related severity. Examination of rats from the highest exposure group during a 4-week recovery period showed that the lesions persisted, but with reduced severity. It should be noted that the exposure level associated with labored breathing in the rats (1,000 mg/m^3) is several orders of magnitude higher than the highest level that has been measured in outdoor air (4 µg/m^3; see Section 5.5.1).

With one exception, oral studies in animals that conducted gross and microscopic examination of the respiratory tract did not report alterations attributed to exposure to 2,4-D. No significant effects were reported in an acute-duration study in dogs exposed once at ≤125 mg/kg (Steiss et al. 1987) and in intermediate-duration studies in rats exposed to ≤300 mg/kg/day (Charles et al. 1996b; EPA 1984, 1985; Gorzinski et al. 1987), mice exposed to ≤90 mg/kg/day (EPA 1984, 1987a), and dogs exposed to 7.5 mg/kg/day (Charles et al. 1996c). Similar results were reported in chronic-duration studies in rats exposed to up to 150 mg/kg/day (Charles et al. 1996a; EPA 1996a; Hansen et al. 1971), mice exposed to ≤300 mg/kg/day (Charles et al. 1996a; EPA 1987a, 1996b), and dogs exposed to 10 mg/kg/day (Hansen et al. 1971). The only effect attributed to exposure to 2,4-D was the finding of pale foci in the lungs from four out of five female rats exposed to 150 mg/kg/day for 52 weeks; no alterations were seen at 75 mg/kg/day (Mattsson et al. 1997).

No definite conclusions can be drawn regarding respiratory effects after oral exposure to 2,4-D based solely on morphological evaluations of the respiratory tract in animal studies; it does not seem that the lungs are a particularly sensitive organ for ingested 2,4-D in animals at doses that do not induce overt effects.

2.5 CARDIOVASCULAR

Tachycardia was reported in two of the four cases of intoxication with an herbicide containing 2,4-D reported by Durakovic et al. (1992). One person had ingested approximately 100 mL of a 40% solution of 2,4-D (40 g); the other individual had ingested 400 mL of a 40% solution of a commercial herbicide (140 g). Tachycardia was also reported in the fatal case reported by Keller et al. (1994). Normal blood pressure and electrocardiogram (except for a sinus tachycardia) were observed in a subject who ingested approximately 110 mg 2,4-D/kg from a commercial herbicide product (Berwick 1970).

Information regarding cardiovascular effects in animals is limited to results of morphological examination of the heart. No gross or microscopic lesions were reported in the heart or thoracic aorta from rats intermittently exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days (EPA 2008). No alterations were reported in the heart from dogs following oral administration of a single dose of ≤125 mg 2,4-D/kg (Steiss et al. 1987). In intermediate-duration oral studies, no effects were reported in rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1984, 1985; Gorzinski et al. 1987), mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a), or dogs exposed to 7.5 mg 2,4-D/kg/day (Charles et al. 1996c).

Similar negative results were reported in chronic-duration oral studies in rats exposed to ≤150 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1996; Hansen et al. 1971), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1987a), and dogs exposed to ≤10 mg 2,4-D/kg/day (Hansen et al. 1971).

Based on the information available, it does not appear that the cardiovascular system is a sensitive target for 2,4-D.

2.6 GASTROINTESTINAL

Nausea and vomiting have been reported following ingestion of products containing 2,4-D (Berwick 1970; Keller et al. 1994; Nielsen et al. 1965). Abdominal sonography and gastroscopy performed in the

case reported by Keller et al. (1994) revealed massive damage of the esophagus and accumulation of blood in the stomach. Furthermore, the stomach mucosa indicated signs of massive hemorrhage and mild necrosis. Autopsy performed on the lethal case studied by Dudley and Thapar (1972) showed markedly hyperemic stomach, duodenum, and proximal jejunum. Light microscopy of the esophagus, stomach, and duodenum showed severe congestion of vessels throughout the mucosa and submucosa. This limited information suggests that bolus ingestion of commercial products containing 2,4-D can produce severe irritation to mucosal membranes. Nausea and vomiting were reported in two cases of intoxication due to dermal contact with an herbicide containing 2,4-D (Goldstein et al. 1959). No further relevant information was located.

Intermittent nose-only exposure of rats to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not induce gross or microscopic lesions in the gastrointestinal tract, including the pancreas (EPA 2008). No alterations were reported in the gastrointestinal tract from dogs following administration of a single dose of ≤125 mg 2,4-D/kg in a gelatin capsule (Steiss et al. 1987). Another acute-duration study reported that vomiting was observed in two out of six female dogs given a dose of 200 mg 2,4-D/kg in a gelatin capsule, and all six dogs had diarrhea (Dickow et al. 2000).

No significant morphological alterations in the gastrointestinal tract were reported in intermediate-duration studies in rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1984, 1985, 1996; Gorzinski et al. 1987), mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a), or dogs exposed to 7.5 mg 2,4-D/kg/day (Charles et al. 1996c).

Similar results were reported in chronic-duration studies in rats exposed to ≤150 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1996a; Hansen et al. 1971), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1987a), and dogs exposed to 10 mg 2,4-D/kg/day (Hansen et al. 1971).

The data in animals suggest that relatively high doses of 2,4-D are unlikely to cause gastrointestinal irritation if 2,4-D is mixed in the food.

2.7 HEMATOLOGICAL

Limited human data are available. Hemoglobin concentration and erythrocyte and leukocyte counts were within normal limits in three cases of intoxication due to dermal contact with an herbicide containing 2,4-D (Goldstein et al. 1959). Apparent leukocytosis was reported in two of four cases of intoxication

with products containing 2,4-D described by Durakovic et al. (1992). No other relevant human data were located.

No information was located regarding hematological effects in animals following acute-duration inhalation, oral, or dermal exposure to 2,4-D.

Hematology tests conducted on male and female rats intermittently exposed nose-only to ≥300 mg/m^3 2,4-D dusts for 28 days showed a significant decrease (20–26%) in reticulocytes (EPA 2008). This effect persisted during a 4-week recovery period in females exposed to 1,000 mg/m^3 2,4-D dusts. The study also reported a reversible decrease in leukocyte counts (~31%) in female rats exposed to 1,000 mg/m^3 2,4-D dusts. However, because this did not occur in males, pre-exposure values were not established, and there was no correlating pathology, it was not considered toxicologically significant.

Intermediate- and chronic-duration oral studies reported some statistically significant differences in hematological parameters between treated and control rats. Significantly decreased platelet counts were reported in male and female rats exposed to ≥100 mg 2,4-D/kg/day for 13 weeks; the NOAEL was 15 mg/kg/day (Charles et al. 1996b). Hemoglobin and red blood cell counts were also decreased in male and female rats exposed to 300 mg 2,4-D/kg/day for 13 weeks (Charles et al. 1996b). EPA (1984) reported that male rats showed significant decreases in hemoglobin in rats exposed to ≥1 mg 2,4-D/kg/day for 13 weeks, but the values were well within the normal range. Another 13-week study reported a NOAEL of 150 mg/kg/day (highest dose tested) for hematological effects, but platelet counts were not determined (Gorzinski et al. 1987). No significant hematological alterations were reported in mice exposed to ≤90 mg 2,4-D/kg/day for 13 weeks (EPA 1984) or ≤45 mg/kg/day for 52 weeks (EPA 1987a), or in dogs exposed to ≤7.5 mg 2,4-D/kg/day for 52 weeks (Charles et al. 1996c). Exposure of rats to ≥75 mg 2,4-D/kg/day for 2 years induced significant decreases in platelet counts, erythrocyte counts, and hematocrit in females; the NOAEL was 5 mg/kg/day (Charles et al. 1996a; EPA 1996a). In contrast, no significant hematological alterations were reported in mice exposed to ≤300 mg 2,4-D/kg/day for 2 years (Charles et al. 1996a), suggesting that mice are less susceptible than rats to 2,4-D-induced hematological effects.

Intermittent application of up to 1,000 mg 2,4-D/kg/day onto the back of rabbits for 21 days did not induce treatment-related alterations in hematological parameters (EPA 1991a).

2.8 MUSCULOSKELETAL

Spontaneous fibrillary twitching in the muscles of the upper extremities was reported in a subject 24 hours after ingestion of approximately 110 mg 2,4-D/kg (Berwick 1970). The only additional relevant information is that an autopsy of a man who died after consuming an unknown amount of 2,4-D did not reveal abnormalities in the musculoskeletal system (Dudley and Thapar 1972).

Limited information is available from acute-duration studies. A single gavage dose of 250 mg 2,4-D/kg (highest dose tested) did not induce gross or microscopic alterations in skeletal muscle from rats (Mattsson et al. 1997). However, 200 mg 2,4-D/kg administered in a gelatin capsule to six female dogs induced prolonged insertional electrical activity (electromyography [EMG]) in all dogs and fibrillation potentials in one dog, indicating possible muscle pathology (Dickow et al. 2000). Mean total and unbound concentrations of 2,4-D in plasma at the time of the electromyographic evaluation were 511 and 129 mg/L, respectively. Transient myotonia was reported in female dogs given a single dose of ≥50 mg 2,4-D/kg; however, no histological alterations were reported in skeletal muscles examined 28 days after administration of a single dose of ≤125 mg 2,4-D/kg (Steiss et al. 1987).

Intermittent nose-only exposure of rats to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not induce gross or microscopic lesions in bone or skeletal muscle (EPA 2008). Intermediate-duration oral studies provide information on skeletal muscle and bone morphology. No significant effects were reported in rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1984, 1985; Gorzinski et al. 1987), mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a), or dogs exposed to ≤7.5 mg 2,4-D/kg/day (Charles et al. 1996c).

Similar results were reported in chronic-duration oral studies in rats exposed to ≤150 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1996a; Hansen et al. 1971), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1987a), and dogs exposed to ≤10 mg 2,4-D/kg/day (Hansen et al. 1971).

Although animals tested in the long-term oral studies did not exhibit clinical signs (i.e., altered posture or gait) that could suggest skeletal muscle alterations, it would be helpful to have information on muscle physiology following prolonged exposure to 2,4-D.

2.9 HEPATIC

Schreinemachers (2010) conducted a study of a subset of 727 healthy participants from the cross-sectional National Health and Nutrition Examination Survey (NHANES), 1988–1994, 20–59 years of age, to investigate risk factors that are linked to the pathogenesis of acute myocardial infarction and type-2 diabetes soon after exposure to 2,4-D. Only 14% of the subjects had urinary 2,4-D levels above the limit of detection (1 mg/dL). Subjects with urinary 2,4-D level above and below the detection level were compared. The results showed that subjects with detectable urinary 2,4-D had significantly lower serum high-density lipoprotein (HDL) than subjects with undetectable 2,4-D in the urine, although still within the normal range. No significant differences were observed between the groups for serum triglycerides and non-HDL cholesterol levels. The investigators also noted that in susceptible populations characterized by high serum glucose and low T4, 2,4-D was associated with increased levels of serum triglycerides. Because no formal statistical sampling procedure was used to recruit the subset of NHANES volunteers, the cohort was not representative of the U.S. population. In addition, it was not clearly indicated in the study when the urine and serum samples were collected in relation to the exposure to 2,4-D or whether there could have been exposure to other chemicals.

Liver congestion was observed at autopsy in the fatal intoxication case reported by Nielsen et al. (1965). Gross necropsy of the liver in the lethal case reported by Dudley and Thapar (1972) showed hyperemic liver; microscopic examination showed diffuse acute necrosis. Significant increases in liver enzymes were reported in a man who ingested approximately 110 mg 2,4-D/kg from a commercial herbicide product and survived (Berwick 1970). No general conclusions regarding hepatic effects of ingested 2,4-D in humans can be made based on only these two case reports.

Results from a sulfobromophthalein test for liver function performed in one of the cases of dermal intoxication reported by Goldstein et al. (1959) were normal. It is unclear whether liver tests were performed on the two other cases described in the report.

Limited data from acute-duration oral studies in animals showed that in dogs, a single dose of 125 mg 2,4-D/kg in a gelatin capsule did not induce histological alterations in the liver (Steiss et al. 1987) and a dose of 200 mg/kg did not significantly alter clinical chemistry parameters used to assess liver function (Dickow et al. 2000).

Female rats intermittently exposed nose-only to 1,000 mg/m^3 2,4-D dusts for 28 days had a significant increase in serum alkaline phosphatase activity (40%) and aspartate aminotransferase activity (35%) relative to controls at termination of exposure (EPA 2008). Females exposed to 300 mg/m^3 2,4-D dusts also showed a significant increase in alkaline phosphatase activity (24%). These values tended to return to control levels at the end of a 4-week recovery period; no significant effects were reported at 100 mg/m^3 2,4-D. Male rats exposed to 1,000 mg/m^3 2,4-D showed a significant increase in serum alanine aminotransferase (ALT) activity at termination of exposure, which appeared to be due to an outlier value nearly 4 times greater than the other values. No other treatment-related alterations in clinical chemistry parameters used to assess liver function were reported. Gross and microscopic examination of the liver did not show treatment-related alterations.

In general, results from intermediate-duration oral studies suggest species differences in sensitivity, with dogs being more sensitive than rodents. Increased absolute liver weight, liver histopathology, increased serum transaminases, and oxidative stress were reported in Wistar rats exposed to 126 mg 2,4-D/kg/day (only dose tested, administered in drinking water) on GDs 14–21 and on postnatal days (PNDs) 0–14 (Troudi et al. 2012a). However, dietary doses of approximately 215 mg 2,4-D/kg/day (highest dose tested) did not cause histological alterations in the liver from Sprague-Dawley rats in a 13-week study (Ozaki et al. 2001). In three additional 13-week dietary studies in F-344 rats, 2,4-D doses ≥150 mg 2,4-D/kg/day induced histological alterations in the liver and the NOAEL was 100 mg/kg/day (Charles et al. 1996b; EPA 1984; Gorzinski et al. 1987). A 2-generation reproductive study that employed dietary exposure to 2,4-D reported a NOAEL of 80 mg 2,4-D/kg/day for liver histopathology in the parental and F1 generations (EPA 1986).

In mice, dietary exposure to ≤429 mg 2,4-D/kg/day for 13 weeks (EPA 1984; Ozaki et al. 2001) or ≤45 mg/kg/day for 52 weeks (EPA 1987a) did not induce histological alterations in the liver. Similarly, hamsters exposed via the diet to ≤474 mg 2,4-D/kg/day for 13 weeks did not show treatment-related lesions in the liver (Ozaki et al. 2001). In dogs, however, dietary doses of ≤7.5 mg 2,4-D/kg/day for 13 weeks induced what was described as perivascular active inflammation in the liver; the NOAEL was 3.75 mg/kg/day (Charles et al. 1996c).

Repeated dermal application of up to 1,000 mg 2,4-D/kg/day onto the skin of rabbits for 21 days did not induce treatment-related alterations in clinical chemistry tests or histological alterations in the liver (EPA 1991a).

Chronic-duration oral studies in rats showed that increasing the duration of exposure from 13 weeks to 2 years did not result in increased incidence or severity of the liver alterations reported at a 2,4-D dose of 150 mg/kg/day in the 13-week study (Gorzinski et al. 1987). Rats treated for 2 years at 150 mg/kg/day showed only increased incidence of "minimal panlobular tinctorial properties" and treatment at 75 mg/kg/day resulted in increased serum ALT activity (Charles et al. 1996a; EPA 1996a). In mice, 2,4-D treatment for 2 years at up to 300 mg/kg/day did not induce liver histopathology (Charles et al. 1996a) and the same was reported in dogs exposed for 2 years at up to 10 mg/kg/day (Hansen et al. 1971).

Results from animal studies suggest that minimal liver pathology occurs in animals at exposure levels considerably higher than would be encountered by humans due to environmental exposures (in the µg 2,4-D/kg/day range).

2.10 RENAL

In the NHANES cross-sectional study of 727 participants described above in Section 2.9 (Schreinemachers 2010), subjects with measurable urinary levels of 2,4-D had significantly higher levels of urinary creatinine than subjects with undetectable levels, but still within the normal range. In the absence of additional renal function tests, the biological significance of this finding is unknown. In a study designed to evaluate risk of end-stage renal disease among wives of pesticide applicators enrolled in the AHS (Lebov et al. (2015), no increased risk was observed for wives reporting ever use of 2,4-D or for wives who did not apply pesticides.

Urinalysis was normal in one of the cases of dermal exposure to an herbicide containing 2,4-D described by Goldstein et al. (1959). In another case, urinalysis showed persistent albuminuria and occasional casts (Goldstein et al. 1959).

Renal congestion, but no degenerative changes in the kidneys, was observed in a fatal case reported by Nielsen et al. (1965). Acute kidney failure preceding death was reported in a case described by Keller et al. (1994) and in a case of a person who survived intoxication as described by Durakovic et al. (1992). In a fatal case of intoxication with 2,4-D reported by Dudley and Thapar (1972), autopsy revealed a hyperemic renal medulla. Microscopic examination of the kidneys showed mildly active chronic pyelonephritis, moderate arteriolar sclerosis, congestion of the capillaries of the medulla, and dilated collecting tubules.

Acute-duration studies that evaluated renal endpoints are limited to the oral exposure route. No significant histopathological alterations were reported in the kidneys from dogs administered a single dose of 2,4-D at 125 mg/kg (highest dose tested) (Steiss et al. 1987). A single dose of 200 mg/kg (only dose tested) did not significantly affect clinical chemistry parameters normally used to monitor kidney function; no histopathological assessment was conducted in this study of dogs (Dickow et al. 2000).

Intermittent nose-only exposure of rats to 2,4-D dusts at ≤1,000 mg/m^3 for 4 weeks did not induce gross or microscopic alterations in the kidneys (EPA 2008). Serum creatinine and blood urea nitrogen (BUN) values were also not significantly affected by exposure to 2,4-D. No urinalysis was performed in the study.

Alterations in the kidneys have been reported in intermediate-duration oral studies in rats, but there are some apparent inconsistencies between studies. The lowest 2,4-D dose resulting in morphological changes in the kidney was approximately 7.1 mg/kg/day reported in a 13-week dietary study (Ozaki et al. 2001). The alterations were diagnosed as simple hyperplasia. The lesion was located in the outer stripe of the outer medulla and consisted of a few scattered foci of tubules with prominent basophilia due to high nuclear density and decreased cytoplasmic volume of the epithelial cells. A NOAEL of 474 mg/kg/day was reported for hamsters administered 2,4-D in the diet for 13 weeks (Ozaki et al. 2001). Other ≤13-week dietary studies in rats reported histopathological alterations in the kidneys at 2,4-D doses in the range of 40–75 mg/kg/day (EPA 1984; Gorzinski et al. 1987; Marty et al. 2013; Saghir et al. 2013a). Renal clearance of 2,4-D is saturated in rats at different levels in adult females (14–27 mg/kg/day) and adult males (approximately 63 mg/kg/day) (Saghir et al. 2013a). Charles et al. (1996b) reported kidney histopathology in male and female rats receiving 2,4-D from the diet at 300 mg/kg/day for 13 weeks, but not at 100 mg/kg/day. Altered kidney histology was reported at 20 mg/kg/day in a 2-generation dietary study of rats (EPA 1987b). In a 52-week oral rat study, increased incidence of tubular cell brown pigment was reported in males and females receiving 2,4-D from the food at 15 mg/kg/day; females also showed fine vacuolization of the cytoplasm in the renal cortex at 15 mg/kg/day (EPA 1985). Chronic-duration studies did not report kidney lesions in rats receiving 2,4-D from the food at up to 150 mg/kg/day for 2 years (Charles et al. 1996a; EPA 1996a; Hansen et al. 1971). However, at 52-week interim assessment of rats from a 2-year dietary study (reported by Charles et al. 1996a and EPA 1996a), degeneration in descending proximal convoluted tubules was reported in males and females at 2,4-D dose levels of 75 and 150 mg/kg/day.

Changes described as increased homogeneity and altered tinctorial properties of the cytoplasm and decreased intracellular/intraluminal vacuolization in the cortex were reported in male mice receiving 2,4-D from the food at 15 mg/kg/day for 13 or 52 weeks (EPA 1984, 1987a). However, in another 13-week study, kidney lesions were reported in male mice dosed at 430 mg/kg/day, but not at 179 mg/kg/day (Ozaki et al. 2001). No histological alterations were seen in the kidneys from dogs receiving 2,4-D from the food at doses ≤7.5 mg/kg for intermediate durations, but there was some indication of altered kidney function assessed as increased BUN and serum creatinine (Charles et al. 1996c).

In a 2-year dietary mouse study, increased relative kidney weight and histopathologic kidney lesions (proximal tubule degeneration/regeneration) were reported in males and females at 62.5 and 150 mg/kg/day, respectively (Charles et al. 1996a; EPA 1996b). EPA (1987a) reported reduced cytoplasmic vacuoles in renal tubule epithelium from mice receiving 2,4-D from the food for 2 years at 15 mg/kg/day.

Hansen et al. (1971) did not find morphological alterations in the kidneys from dogs receiving 2,4-D from the food at up to 10 mg/kg/day for 2 years; however, clinical chemistry tests were not conducted in this study, so kidney function was not addressed. Dogs appear to be more sensitive than other species (including humans) to 2,4-D toxicity due to a significantly lower capacity to eliminate 2,4-D via the kidneys (Timchalk 2004). Although available dog studies are summarized in Table 2-2 and Figure 2-3, the results were not considered appropriate for deriving oral MRLs for 2,4-D.

Data evaluation records (DERs) from the dietary rat and mouse studies submitted to EPA in the 1980s (EPA 1984, 1985, 1986, 1987a, 1987b) provide inadequate descriptions of the kidney lesions reported. Thus, the degenerative nature of the described lesions is in question and the results are not included in Table 2-2. Furthermore, the kidney results were not considered appropriate candidates for potential MRL derivation due to a lack of convincing evidence that the histological changes should be considered adverse.

Application of 2,4-D at 1,000 mg/kg/day onto the skin of male and female rabbits for 21 days resulted in significantly increased absolute and relative kidney weight in females (EPA 1991a). However, there were no treatment-related alterations in clinical chemistry for kidney function nor histological changes in the kidneys.

2.11 DERMAL

The only relevant human information is that of a case in which a farmer who accidentally wetted his legs with an herbicide containing 2,4-D developed desquamation of the skin of the palms and soles (Goldstein et al. 1959).

Examination of the skin of rats exposed intermittently nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not show gross lesions (EPA 2008).

The only information regarding dermal effects in animals following oral exposure to 2,4-D is that no histological alterations were seen in the skin of rats and mice exposed to ≤45 mg 2,4-D/kg/day for 52 weeks (EPA 1985, 1987a) or mice exposed to ≤45 mg/kg/day for 2 years (EPA 1987a).

Limited information is available regarding dermal effects of 2,4-D in animals. Hairless dogs that received daily application of a 0.036 mL of a 0.1% solution of 2,4-D for 7 days showed no inflammation or pigmentation at the application site 1 day after termination of dosing (Kimura et al. 1998). No gross changes were seen 14 days after cessation of dosing. One day after cessation of treatment, light microscopy showed slight epidermal thickening and hyperplasia; no significant changes were seen 14 days after termination of treatment. The skin of rabbits that received an application of 0.5 g of 2,4-D onto a shaved area of the skin for 4 hours did not show signs of irritation (EPA 1992). Repeated application of ≥10 mg 2,4-D/kg/day to the skin of rabbits for 21 days resulted in slight erythema and epidermal scaling at various times during the study, but no edema was observed (EPA 1991a).

2.12 OCULAR

The only information regarding ocular effects in humans exposed to 2,4-D is that from a study of 31,173 wives whose husbands were licensed pesticide applicators participating in the AHS (Kirrane et al. 2005). Using logistic and hierarchical logistic regression analyses after adjusting for potential effect modifying and potential confounders, an OR of 1.1 (95% CI 0.7–1.8) was reported for use of 2,4-D and retinal degeneration or other eye disorders.

Ophthalmoscopic examination of the eyes from rats intermittently exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not show changes compared to pre-exposure test results (EPA 2008).

Chromodacryorrhea (red lacrimation caused by excessive secretion of porphyrins with tears) occurred on day 12 and intermittently thereafter.

Ocular effects were reported in rats in intermediate- and chronic-duration oral studies; no ocular effects were reported in other animal species tested. Acute administration of a single doses of ≤250 mg 2,4-D/kg to rats did not induce histological alterations in the eye, but 150 mg/kg/day given chronically for 52 weeks induced bilateral retinal degeneration in five out of five females; no treatment-related lesions were seen at 75 mg/kg/day (Mattsson et al. 1997). The degeneration was characterized by a complete loss of the rod and cone layer and the outer and inner nuclear layers. Thirteen-week studies established a NOAEL of 150 mg/kg/day for ocular lesions in rats (Gorzinski et al. 1987), but exposure to 300 mg 2,4-D/kg/day induced retinal degeneration and cataract formation in female rats (Charles et al. 1996b).

Chronic-duration studies confirmed the existence of an exposure-duration factor evident in intermediate-duration studies as exposure to 150 mg 2,4-D/kg/day for 2 years caused constriction of blood vessels and hyperreflectivity of the fundus in male rats and lens opacity in female rats (Charles et al. 1996a; EPA 1996a). Microscopically, both sexes showed retinal degeneration and cataracts; the incidence of ocular lesions was not significantly elevated in rats exposed to ≤75 mg 2,4-D/kg/day.

Though rat studies indicate that ocular lesions/degeneration is possible from 2,4-D exposure, the significance of this finding to humans is unknown. It should be noted also that the lesions appear to occur at exposure levels much higher than from exposure to environmental levels of 2,4-D.

The only relevant information in animals exposed dermally is that application of up to 1,000 mg 2,4-D/kg/day onto the skin of rabbits for 21 days did not induce histological alterations in the eyes (EPA 1991a).

2.13 ENDOCRINE

Goldner et al. (2010) examined 16, 529 female spouses of pesticide applicators who had thyroid data, pesticide use data, and all covariates data. Among this group, 2.2% were classified as hyperthyroid, 6.7% as hypothyroid, 3.4% as having other thyroid disease, and 87.6% as having no thyroid disease. Regression analyses showed elevated ORs for hypothyroid disease if the spouse ever worked or lived on a farm (OR 1.3; 95% CI 0.87–2.0). Analyses of individual pesticides yielded an OR of 0.93 (95% CI 0.68–1.3) for ever-use of 2,4-D and hyperthyroidism, an OR of 0.96 (95% CI 0.8–1.1) for hypothyroidism, and

an OR of 1.2 (95% CI 0.95–1.5) for other thyroid disease. In a subsequent study of male participants in the AHS, Goldner et al. (2013) reported an association between ever-use of 2,4-D and hypothyroid disease (OR 1.35; 95% CI 1.04–1.76). Exposure-response analyses using the intensity-weighted measure showed a monotonic exposure-response for 2,4-D. The seemingly conflicting results between the study of women and the one of men may reflect, at least in part, the fact that male pesticide applicators use a larger number of pesticides and often apply larger amounts of individual pesticides than their female spouses, as noted by Goldner et al. (2013). Acute congestion was seen in the adrenals in the lethal case reported by Nielsen et al. (1965). The endocrine system appeared normal at autopsy in a case reported by Dudley and Thapar (1972).

Mean serum levels of T4, thyroid-stimulating hormone (TSH), insulin, and C-peptide (a marker of endogenous production of insulin) in a group of 102 subjects with detectable levels of 2,4-D in the urine were not different from those in 625 subjects with urinary 2,4-D below the limit of detection (1 mg/dL) (Schreinemachers 2010). However, in subjects with low HDL, 2,4-D was associated with increased levels of C-peptide ($p \leq 0.05$), insulin ($p \leq 0.01$), and TSH ($p \leq 0.05$), especially in populations with high serum glucose and low T4 levels.

Studies in animals provide information on gross and microscopic morphology of endocrine glands following long-term oral exposure to 2,4-D. Results from some studies showed alterations in serum levels of thyroid hormones and prolactin.

Gross and microscopic examination of the pituitary, adrenal, thyroid, and parathyroid glands from rats exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts intermittently for 28 days did not reveal treatment-related alterations (EPA 2008).

Stürtz et al. (2008, 2010) reported serum levels of prolactin were significantly decreased in maternal rats administered 2,4-D at doses ≥2.5 mg/kg/day on postpartum days 1–16. This effect was attributed in part to decreased levels of serotonin and increased levels of dopamine in the arcuate nucleus of the brain. However, the toxicological significance of these results is uncertain. Therefore, the results are not included in Table 2-2 or Figure 2-3.

Alterations in thyroid hormone levels have been reported in rats in long-term studies. For example, serum T4 and T3 were significantly reduced in female rats following exposure to 100 mg 2,4-D/kg/day for 13 weeks; the NOAEL was 15 mg/kg/day (Charles et al. 1996b). Decreased serum T4 was also reported

in females exposed to 100 mg 2,4-D/kg/day in another 13-week study (Gorzinski et al. 1987). In contrast, T4 was elevated in male rats at 300 mg 2,4-D/kg/day (Charles et al. 1996b) and EPA (1984) reported that serum T4 was increased in male rats exposed to 5 or 15 mg 2,4-D/kg/day for 13 weeks, but no significant change was seen in rats exposed to 45 mg 2,4-D/kg/day. Also, EPA (1985) reported that female rats exposed to ≥15 mg 2,4-D/kg/day for 27 weeks had significantly increased serum T4, but no increase was evident after 52 weeks of exposure and no alterations were seen in males exposed to ≤45 mg 2,4-D/kg/day at either time point. In none of these studies were there histological alterations in the thyroid. Pregnant rats exposed to approximately 50 mg 2,4-D/kg/day from pre-breeding through GD 17 had nonsignificant decreased serum T3 and T4 and increased TSH on GD 17 (Marty et al. 2013). The investigators also noted that 3 out of 12 females had histological alterations consisting of smaller thyroid follicles with small vacuoles in the colloid, which suggested colloid resorption. Because there were no adverse pathological alterations and thyroid changes in dams exposed similarly and examined on lactation day 21, the investigators suggested that the changes were transient, and therefore, were considered adaptive, yet exposure related. Dose-related decreases in serum T4 were also reported in male and female rats exposed to ≥75 mg 2,4-D/kg/day; the NOAEL was 5 mg/kg/day (Charles et al. 1996a; EPA 1996a). There were no histopathological alterations in either sex exposed to ≤150 mg 2,4-D/kg/day.

Adrenal cortex hypertrophy was reported in female rats exposed to 100 mg 2,4-D/kg/day for 13 weeks (Charles et al. 1996b). Male mice exposed to ≥1 mg 2,4-D/kg day for 52 weeks showed significant decreases in absolute and relative adrenals weight, but exposure to ≥15 mg 2,4-D/kg/day for 104 weeks resulted in significant increases in absolute and relative adrenals weight (EPA 1987a). In the absence of histopathology, the toxicological significance of these changes in adrenal weight is unknown.

Alterations in thyroid hormones in rats unaccompanied by pathological changes in the thyroid gland occur at exposure levels unlikely to be found in the environment. Neal et al. (2017) performed a weight-of-the-evidence evaluation of potential for 2,4-D to interact with estrogen, androgen, and thyroid pathways and steroidogenesis. The evaluation found no evidence of 2,4-D-mediated endocrine effects in experimental animals at dose levels below renal saturation; levels above the renal saturation limit were considered irrelevant to humans because they were many times higher than those reported in human exposure and biomonitoring studies. It was concluded that 2,4-D is unlikely to disrupt endocrine activity in wildlife or humans at environmentally-relevant potential exposure levels.

2.14 IMMUNOLOGICAL

No studies were located that examined a potential association between exposure specifically to 2,4-D and immunological parameters in humans. A small study of 10 Italian farmers reported that exposure (assumed to have been acute) to unidentified commercial mixtures containing 2,4-D and 4-chloro-2-methylphenoxy acid (MCPA) resulted in transient alterations in lymphocyte subsets, natural killer cells, and lymphoproliferative response to mitogen stimulations (Faustini et al. 1996). Another study of 47 workers in a plant producing herbicides (2,4-D among them), fungicides, and seed dressings reported alterations in lymphocyte subsets and immunoglobulin A levels compared to unexposed control individuals (Kluciński et al. 2001). However, neither of these studies provided specific information regarding 2,4-D. A nested case-control study of female spouses of participants in the AHS reported an OR of 0.5 (95% CI 0.3–0.9) for exposure to 2,4-D and rheumatoid arthritis (De Roos et al. 2005). There was no explanation for the apparent inverse association.

Significant increases in absolute and relative (to body weight and brain) spleen weight occurred in male rats intermittently exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days and allowed to recover for 4 additional weeks (EPA 2008). In females, absolute spleen weight was significantly decreased after recovery. Because gross and microscopic examination of the spleen, thymus, and lymph nodes from exposed rats did not show treatment-related alterations, the biological significance of the changes in spleen weight are unknown.

For the most part, oral studies in animals only provide information on gross and microscopic morphology of lymphoreticular organs and tissues; limited information is available regarding immunocompetence. No morphological alterations were observed in the spleen and lymph nodes from dogs treated once with up to 125 mg 2,4-D/kg (Steiss et al. 1987).

Intermediate-duration oral studies did not report morphological alterations in lymphoreticular tissues from rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1984, 1985; Gorzinski et al. 1987; Marty et al. 2013). An F1-extended 1-generation study did not find altered immunocompetence (assessed by the sheep red blood cell [SRBC] antibody plaque forming cell assay) in the F1 generation that had been exposed directly to ≤75.3 mg 2,4-D/kg/day and indirectly during gestation and lactation (Marty et al. 2013). Results from a natural killer cells assay were also negative. No morphological alterations were reported in mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a) and in dogs exposed to ≤7.5 mg 2,4-D for up to 1 year (Charles et al. 1996c).

Chronic-duration oral exposure of rats to ≤150 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1996a; Hansen et al. 1971), mice to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1987a), or dogs to ≤10 mg 2,4-D/kg/day (Hansen et al. 1971) did not result in gross or microscopic alterations in lymphoreticular organs or tissues.

The available animal data, although rather limited, suggest that immunological alterations should not be a concern for humans exposed to environmental levels of 2,4-D.

2,4-D was a respiratory allergen in mice as assessed by a significant increase in total IgE levels and IgE-expressing B-cell populations following repeated dermal applications of 25 µL of a 5% solution of 2,4-D in acetone/saline (doses of approximately 62.5 mg 2,4-D/kg) and then challenged intratracheally with 50 µL of a 0.5% solution of the chemical (Fukuyama et al. 2009).

2.15 NEUROLOGICAL

Information regarding neurological effects in humans exposed to 2,4-D is limited to a few epidemiological studies and case reports. The epidemiological studies examined the association between pesticide exposure and Parkinson's disease; the results do not suggest a causal association. In the AHS, the OR for ever-use of 2,4-D and prevalent cases of Parkinson's disease was 0.9 (95% CI 0.5–1.8), and the OR for incident cases of Parkinson's disease was 1.0 (95% CI 0.5–2.1) (Kamel et al. 2007). Prevalent cases were self-reported cases at enrollment in the AHS, whereas incident cases were self-reported cases at follow-up. A much smaller case-control study of Parkinson's disease in East Texas (100 cases, 84 controls) reported an OR of 1.2 (95% CI 0.6–2.8) for "ever personally used/mixed or applied" 2,4-D and Parkinson's disease (Dhillon et al. 2008). A case-control study of 319 cases of Parkinson's disease and 296 relative and other controls reported an OR of 2.07 (95% CI 0.6–6.23) for ever-use of 2,4-D and Parkinson's disease (Hancock et al. 2008). A significant association (OR 2.59; 95% CI 1.03–6.48) between use of 2,4-D and risk of parkinsonism was reported in a multicenter case-control study of 519 cases and 511 controls based on 16 cases among exposed subjects and 7 among controls (Tanner et al. 2009).

Studies of female spouses of pesticide applicators in the AHS reported that depression (physician-diagnosed or self-reported) was not associated with 2,4-D (Beseler et al. 2006 [OR 1.05, 95% CI 0.99–

1.11]; Beard et al. 2013 [risk ratio 0.71; 85% CI 0.58–0.89]). The inverse association reported by Beard et al. (2013) was attributed by the authors to reverse causality or just chance.

Limited data from case reports provide additional information. Goldstein et al. (1959) described three cases of dermal exposure to an herbicide product containing an ester of 2,4-D. In the three cases, there was contact of the product with unprotected skin; symptoms and signs involved the peripheral nervous system and started hours after skin contact with the product containing 2,4-D. In one case, there was a second exposure about 2 months after the first exposure. In general, symptoms consisted of pain, paresthesias (abnormal sensations), and paralysis that were severe enough to require hospitalization of the three patients. Recovery was slow and some symptoms persisted for years after exposure had occurred. Berkley and Magee (1963) also reported a case of primary sensory neuropathy in a farmer who had dermal contact with a 40% solution of the dimethylamine salt of 2,4-D and water.

Neurological effects have been reported in most cases of intoxication with commercial products containing 2,4-D. For example, coma and absence of reflexes were reported on admission in three out of the four nonlethal cases of intoxication described by Durakovic et al. (1992). The lethal case reported by Dudley and Thapar (1972) was described as comatose upon admission to the emergency room. Autopsy revealed multiple petechiae throughout the white matter of the brain. However, microscopic examination of the brain showed changes (i.e., senile plaques, lipofuscin accumulation) that appeared consistent with senile dementia (the subject was 76 years old) and not caused by the acute intoxication. Internal examination of another lethal case showed slight edema of the brain and pia-arachnoid (Nielsen et al. 1965). Histological examination showed marked congestion at all brain levels examined as well as severe degenerative changes in ganglion cells. Information regarding signs and symptoms before death was not available because the subject was found dead in an uninhabited area. Because the time elapsed between death and the postmortem examination was unknown, it is impossible to determine with certainty whether the histological alterations seen in the brain were caused by the product ingested or represented normal postmortem changes. Neurological examination of a man 24 hours after ingesting approximately 110 mg 2,4-D/kg from a commercial herbicide product showed hyperactive biceps and triceps, but no other abnormal reflexes; the subject, however, did complain of hyperesthesia of the upper part of his torso (Berwick 1970).

Numerous studies in animals provide information on gross and microscopic morphology in the nervous system following exposure to 2,4-D; a few studies also examined neurobehavioral parameters. In general,

the results show lack of adverse morphological effects at the exposure levels tested, but some studies reported neurobehavioral and neurochemical alterations.

An acute-duration oral study reported that a single gavage dose of 300 mg 2,4-D/kg induced vascular damage in the central nervous system in rats; no such effect was observed at 150 mg 2,4-D/kg (Elo et al. 1988). The effect was attributed to 2,4-D-induced damage to the blood brain barrier, caused in turn by saturation of the organic acid transport out of the brain. A single lower dose of 250 mg 2,4-D/kg administered to rats did not induce morphological alterations in the brain, spinal cord, or trigeminal nerve (Mattsson et al. 1997). Also, no morphological alterations were reported in the brain or spinal cord from dogs given a single oral dose of up to 125 mg 2,4-D/kg in a capsule (Steiss et al. 1987).

No treatment-related gross or microscopic alterations were reported in the brain, spinal cord, or peripheral nerves from rats intermittently exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days (EPA 2008). Intermediate-duration oral studies in rats did not report morphological alterations in tissues of the nervous system even with the highest doses tested, 300 mg 2,4-D/kg/day (Charles et al. 1996b). Other studies that examined this endpoint in rats include EPA (1984, 1987a), Gorzinski et al. (1987), Marty et al. (2013), and Mattsson et al. (1997). No significant morphological alterations in the nervous system were reported in mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a) or dogs exposed to ≤7.5 mg 2,4-D/kg/day (Charles et al. 1996c).

No morphological alterations in the nervous system were reported in chronic-duration studies of rats administered ≤150 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1996a), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1987a), or dogs exposed to ≤10 mg 2,4-D/kg/day (Hansen et al. 1971).

Studies have also examined neurobehavioral parameters in animals following oral exposure to 2,4-D. In fact, the lowest LOAEL for neurological effects in animals was 15 mg 2,4-D/kg (lowest dose tested) for alterations in maternal behavior in rats dosed via the food on postpartum days 1–7 (Stürtz et al. 2008). Specifically, the effects consisted of increased latency of retrieval of pups, increased latency of crouching, decreased percent dams licking the pups, decreased percent dams licking the anogenital region of the pups, increased percent of dams leaving the nest, and increased time spent out of the nest. These behaviors were associated with a decrease in serotonin and an increase in dopamine in the arcuate nucleus of the brain. The relevance of these behavioral effects to humans is unknown. Furthermore, such higher doses (250 mg 2,4-D/kg, but not 75 mg/kg) induced altered gait and increased motor activity in rats 1 day

after dosing (Mattsson et al. 1997), and a single dose of 125 mg 2,4-D/kg (highest dose tested) did not affect motor nerve conduction velocity in dogs (Steiss et al. 1987). Therefore, the reported neurobehavioral results of Stürtz et al. (2008) are not included in Table 2-2 or Figure 2-3.

In intermediate-duration oral studies, results from tests for motor activity, acoustic startle response, and a functional observational battery (FOB) administered to 54–56-day-old rats exposed to 59.2–81.7 mg 2,4-D/kg/day in the diet from PND 21 were not significantly different from controls (Marty et al. 2013). It should be mentioned that these rats also had been exposed to 2,4-D *in utero* and through maternal milk. However, higher dietary doses (150 mg 2,4-D/kg/day) administered to adult rats for at least 3 months significantly increased forelimb grip strength; no significant effect was reported at 75 mg/kg/day (Mattsson et al. 1997). In this study, no significant alterations were reported in tests of motor activity or on an FOB. Increased grip strength had also been reported in an earlier study in rats dosed by gavage with ≥20 mg 2,4-D/kg 2 days/week for 5 weeks (Squibb et al. 1983). This result is not included in Table 2-2 or Figure 2-3 because other studies did not find a similar effect at exposure levels <150 mg/kg/day and the toxicological significance of increased grip strength in the absence of other signs of neurotoxicity is questionable. No neurobehavioral tests were conducted in chronic-duration studies.

Standard tests for neurotoxicity do not suggest that the nervous system is very sensitive to exposure to 2,4-D. The available information also indicates that neurobehavioral effects can be detected before morphological alterations can be observed.

2.16 REPRODUCTIVE

Limited information is available regarding reproductive effects in humans following exposure to 2,4-D. An early study of 32 male farm sprayers who were exposed to 2,4-D for 1–2 months and 25 controls reported significant differences ($p<0.01$) in various sperm parameters between the exposed and control group, which tended to disappear following a short recovery period; regression analyses were not conducted in this study (Lerda and Rizzi 1991). Although not totally clear, it appears that sperm analyses were conducted 6 months (March) after the exposure period (August–September) and again 3 months later (July) to examine possible recovery. No information was provided regarding possible exposures to other chemicals. A more recent nested case-control study of 50 men with low semen quality and 36 men with sperm parameters within normal limits from Missouri and Minnesota reported an OR of 0.8 (95% CI 0.2–3.0) for levels of 2,4-D in urine (≥0.1 μg/g creatinine) and semen quality (Swan et al. 2003). 2,4-D was not associated with serum total testosterone (adjusted regression coefficient [R^2] -0.084; 95%

CI -0.167, -0.001) in group of 68 male farmers residing in the Inthakhin district of the Thai province of Chiang Mai (Panuwet et al. 2018). However, the small number of subjects precludes meaningful conclusions regarding associations between 2,4-D and serum total testosterone.

A nested case-control study of 2,110 women participants in the Ontario Farm Family Health Study that contributed 3,936 pregnancies including 395 spontaneous abortions found no association between spontaneous abortion and use of 2,4-D during the preconception period (OR 1.2; 95% CI 0.8–1.6) or the post-conception period (OR 1.0; 95% CI 0.7–1.6) (Arbuckle et al. 2001). However, when models were constructed with exposure window as the outcome, preconception exposure to 2,4-D was associated with increased risk of early abortion (<12 weeks) (OR 2.9; 95% CI 1.1–8.0), but not with risk of late spontaneous abortion (OR 0.5; 95% CI 0.2–1.1). A prior study of this population, which did not control for history of prior spontaneous abortion, did not find associations between exposure to 2,4-D and spontaneous abortions (Arbuckle et al. 1999); the OR for preconception exposure adjusted for maternal age, education, and alcohol intake was 0.9 (95% CI 0.5–1.8) and the OR for postconception exposure was 1.1 (95% CI 0.5–2.4). The available data are insufficient due to multiple factors, one being the likelihood of being exposed to a mixture of pesticides, to determine whether exposure to 2,4-D can adversely affect reproductive function in humans.

No significant gross or histological alterations were reported in the prostate and testes from a man who died after ingesting at least 80 mg 2,4-D/kg from a commercial herbicide consisting of the dimethylamine salt of 2,4-D (Nielsen et al. 1965).

Gross and microscopic examination of primary or secondary reproductive organs of male and female rats intermittently exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not show treatment-related alterations (EPA 2008).

Numerous oral studies in animals provide information regarding gross and microscopic appearance of reproductive organs following exposure to 2,4-D, but relative few studies provide information regarding other reproductive endpoints. Overall, the reproductive system does not appear to be a particularly sensitive target for 2,4-D toxicity.

Only one acute-duration oral study was located (Dinamarca et al. 2007). In that study, administration of ≤100 mg 2,4-D/kg given to pregnant mice on GDs 0–9 did not significantly affect the numbers of corpora lutea, implantation sites, resorptions, or live embryos.

Intermediate-duration oral studies in which rats were exposed to 2,4-D via the diet did not report gross or microscopic alterations in the reproductive organs from male or female animals (Charles et al. 1996b; EPA 1984, 1985; Gorzinski et al. 1987; Marty et al. 2013). The highest dose tested was 300 mg 2,4-D/kg/day in a 13-week study (Charles et al. 1996b). A study in which rats were administered 2,4-D daily by gavage for 30 days reported histological alterations in Sertoli and Leydig cells even with the lowest dose tested (50 mg/kg/day) (Joshi et al. 2012). The only plausible explanation for the discrepancy in results from Joshi et al. (2012) and those reported in other studies is the different mode of administration of 2,4-D (gavage versus diet).

Fertility was not affected in male or female rats exposed to up to 111 mg 2,4-D/kg/day in intermediate-duration oral studies (EPA 1986; Hansen et al. 1971; Marty et al. 2013; Saghir et al. 2013a, 2013b), and neither were mating index, time to mating, gestation length, pre- and postimplantation losses, or number of corpora lutea in rats exposed to ≤50 mg 2,4-D/kg/day (Marty et al. 2013). Sperm parameters were also not affected in the latter study, but sperm count and motility were significantly reduced in rats exposed to ≥50 mg 2,4-D/kg/day in the 30-day gavage study mentioned above (Joshi et al. 2012). In addition, serum levels of testosterone, follicle-stimulating hormone, and luteinizing hormone were significantly reduced in male rats (only males tested) from the Joshi et al. (2012) study. Testicular atrophy was reported in male rats dosed at 150 mg/kg/day for 52 weeks during a chronic study, but not at 2-year terminal sacrifice (Charles et al. 1996a; EPA 1996a).

Additional intermediate-duration oral studies did not report morphological alterations in the reproductive organs from mice exposed via the diet to up to 45 mg 2,4-D/kg/day for 52 weeks (EPA 1987a) or 90 mg 2,4-D/kg/day for 13 weeks (EPA 1984), or in dogs exposed to up to 7.5 mg 2,4-D/kg/day for 1 year (Charles et al. 1996c).

Two-year dietary studies also did not report morphological alterations in the reproductive organs from rats exposed to up to 150 mg 2,4-D/kg/day (Charles et al. 1996a; Hansen et al. 1971), mice exposed to up to 300 mg 2,4-d/kg/day (Charles et al. 1996a; EPA 1987a), or dogs exposed to up to 10 mg 2,4-D/kg/day (Hansen et al. 1971).

2,4-D did not induce adverse reproductive effects in animals when administered via the diet, at the dietary levels tested. However, a gavage study reported histopathology of the testes and alterations in sperm parameters and serum levels of reproductive hormones (Joshi et al. 2012). The available data suggest that

exposure to environmental levels of 2,4-D by a relevant route is unlikely to cause adverse reproductive effects in humans.

2.17 DEVELOPMENTAL

A case-control study of 3,412 pregnancies and 118 malformations nested in the Ontario Farm Family Health Study did not find associations between exposure to 2,4-D and birth defects (Weselak et al. 2008). The investigators performed separate analyses for reported use of 2,4-D during the preconception period (OR 1.07; 95% CI 0.55–2.08) and during the post-conception period (OR 0.97; 95% CI 0.42–2.25), and for couples who lived on farms where the father had reported direct chemical activity during a relevant period of time and there was reported use of 2,4-D (OR 0.60; 95% CI 0.25–1.46). A similar study examined the potential associations between women's residential proximity to agricultural pesticide applications in the San Joaquin Valley of California during early pregnancy and risk of neural tube defects and orofacial clefts (Yang et al. 2014). Evaluation of the association between exposure to a mixture of 2,4-D and dichlorprop and risk of anencephaly yielded an OR of 2.0 (95% CI 0.8–51), whereas that between exposure to the mixture and incidence of cleft lip with or without cleft palate produced an OR of 1.1 (95% CI 0.6–2.1). There were too few cases of spina bifida and cleft palate alone for meaningful analyses. A study of 4,935 births to 34,772 state-licensed, private pesticide applicators in Minnesota found that in regions where chlorophenoxy herbicides and/or fungicides were frequently used, infants conceived in spring, when application of the chemicals routinely occurred, showed an increase in birth defects compared to infants conceived in other seasons (OR 1.36; 95% CI 1.10–1.69) (Garry et al. 1996); chemical-specific analyses were not conducted in this study. The same group of investigators conducted a follow-up study of 695 farm families and 1,532 children from the same area in Minnesota during 1997–1998. This study confirmed the earlier finding that conceptions in the spring led to significantly more children with birth defects compared with children conceived in any other season ($p=0.02$; ORs were not estimated), but chemical-specific analyses were not conducted (Garry et al. 2002).

Evaluation of morbidity among children born to participants in the Ontario Farm Family Health Study reported an increased risk of hay fever or allergies associated with maternal exposure to 2,4-D during pregnancy (Weselak et al. 2007). ORs were estimated as 1.84 (95% CI 1.08–3.04) for male offspring and 1.26 (95% CI 0.70–2.28) for female offspring. No increased risks were reported for asthma or persistent cough or bronchitis. Evaluation of birth weight among 2,246 farm women in the AHS whose most recent singleton birth occurred within 5 years of enrollment (1993–1997) showed that ever-use of 2,4-D during early pregnancy was associated with a reduction of 38 grams in birth weight(95% CI [-103]–27)

(Sathyanarayana et al. 2010). The limited data available, with mostly mixtures or unclear exposure to 2,4-D, do not suggest a role for 2,4-D in birth defects or other developmental effects in humans.

Developmental effects have been observed in rodents following perinatal exposure to 2,4-D. For the most part, results from acute-duration oral studies suggest that effects might be observed at doses that caused maternal effects, mainly reduced maternal weight. For example, exposure of rats to 75 mg 2,4-D/kg/day on GDs 6–15 did not result in significant maternal toxicity or developmental effects in fetuses examined on GD 20 (Charles et al. 2001). However, similar exposure of rats to 100–115 mg 2,4-D/kg/day significantly reduced maternal weight gain during treatment and significantly increased the incidence of morphological and skeletal defects in fetuses examined on GD 20 (Chernoff et al. 1990; Mazhar et al. 2014). In other rat studies, doses of 70 mg 2,4-D/kg/day during gestation caused maternal weight loss during treatment and induced renal malformations and offspring lethality during the first 2 weeks of life (Fofana et al. 2000, 2002). One study in rats reported significantly reduced fetal weight and increased incidence of soft-tissue and skeletal anomalies on GD 20 following maternal exposure to ≥50 mg 2,4-D/kg/day on GDs 6–15; the NOAEL was 25 mg 2,4-D/kg/day (Schwetz et al. 1971). However, neither growth nor viability were affected in offspring from dams that were allowed to give birth and had been exposed to up to 87.5 mg 2,4-D/kg/day (Schwetz et al. 1971).

Oral exposure of maternal mice to 87.5 mg 2,4-D/kg/day (only dose level tested) on GDs 8–12 resulted in significantly reduced offspring weight on PND 1, but not PND 3 (Kavlock et al. 1987). While it was noted that there were no significant increases in maternal mortality or resorptions, no information was provided regarding changes in maternal weight during treatment.

No significant developmental effects were reported in hamsters following maternal oral exposure to up to 100 mg 2,4-D/kg/day on GDs 6–10 (Collins and Williams 1971) or rabbits following maternal exposure to up to 90 mg 2,4-D/kg/day on GDs 6–18 (Charles et al. 2001).

Several intermediate-duration oral studies provide information on developmental endpoints; all of the available studies were conducted in rats. The lowest maternal dose level at which developmental effects were reported was 2.5 mg 2,4-D/kg/day (the lowest dose tested) and this caused a significant reduction in body weight (5–7% on lactation days 10–16) for pups from dams exposed to 2,4-D in the diet on postpartum days 1–16 (Stürtz et al. 2010). This effect was attributed to inhibition of suckling-induced hormone release and milk transfer to the litter by an action of 2,4-D at the central level. The study also reported that maternal exposure to 2,4-D altered the contents of lipids (30% decreased at 25 mg

2,4-D/kg/day) and of some proteins in the milk. With the changes in milk content, it is possible that nutritional deficiency resulted in hindered growth of the pups. Limitations to this study include a lack of supporting data from other animal studies designed to evaluate developmental toxicity endpoints (EPA 1986; Marty et al. 2013). Therefore, the report of depressed pup body weight at the maternal dose level of 2.5 mg/kg/day is not included in Table 2-2 or Figure 2-3 and was not considered appropriate to serve as a critical effect for the purpose of MRL derivation.

Other studies have also reported effects on pup body weight, but at significantly higher 2,4-D doses. For example, in a 2-generation reproductive study, pup body weight was reduced significantly on PND 28 at maternal doses ≥35 mg 2,4-D/kg/day during lactation, but not at 10 mg 2,4-D/kg/day (EPA 1986). In another study, significantly reduced pup body weight (about 10%) was reported for PND 22 pups following perinatal exposure at the lowest 2,4-D concentration tested (approximate maternal dose of 9 mg 2,4-D/kg/day (Marty et al. 2013). However, Marty et al. (2013) considered the pup body weight changes at the low- and mid-dose levels to be spurious due to artifactual differences in PND 22 male pup body weights during group assignment and a lack of a dose-response relationship. The study authors considered the highest exposure level (600 ppm, estimated maternal dose of approximately 50 mg/kg/day) to represent a LOAEL for depressed pup body weight. Marty et al. (2013) reported significant decreases in the weight of the adrenals, kidneys, liver, spleen, and testes from pups at the maternal exposure level of approximately 60 mg 2,4-D/kg/day during lactation and sacrificed on PND 22; however, no histological alterations were observed in these organs. Monitoring of developmental landmarks in additional pups born to dams exposed to up to 50 mg 2,4-D/kg/day showed no significant effects on nipple retention in males, age at vaginal opening, or mean estrous cycle length (Marty et al. 2013). There was, however, a slight delay (1.6 days) in the age at preputial separation in male pups, which was attributed to body weight decrement and slightly delayed growth. Saghir et al. (2013a, 2013b) reported up to 23% depressed pup body weight on PNDs 14–22 in a study of male and female rats receiving 2,4-D from the food during 4 weeks premating and throughout gestation and lactation periods; the estimated maternal dose was 50 mg/kg/day. However, it is likely that the pups received some 2,4-D from the maternal food during latter stages of the lactation period.

Other studies that reported reduced offspring weight at much higher maternal oral doses include Bortolozzi et al. (1999), Hansen et al. (1971), Mazhar et al. (2014), and Troudi et al. (2012a, 2012b). Mazhar et al. (2014) also reported that maternal exposure to 100 mg 2,4-D/kg/day (only dose level tested) on GDs 1–19 significantly increased the incidence of morphological and skeletal defects in fetuses examined on GD 20. Further, exposure to 2,4-D significantly reduced maternal weight gain (40–54%)

during treatment and caused decreased activity, rapid breathing, loss of appetite, weakness, nasal hemorrhage, and slight diarrhea.

Other effects that have been reported in intermediate-duration oral studies in rats include neurobehavioral alterations in male and female pups and delayed vaginal opening in females following maternal exposure to 70 mg 2,4-D/kg/day (only dose level tested) (Bortolozzi et al. 1999) and histological alterations in pups' liver and bone following maternal exposure to 126 mg 2,4-D/kg/day (only dose level tested) (Troudi et al. 2012a, 2012b). In the latter two studies, developmental effects were associated with increased markers of oxidative stress and reduced antioxidant enzyme levels in dams and pups.

Overall, studies in animals suggest that 2,4-D does not induce teratogenicity, but it has caused alterations in neurobehavioral effects in one study (Bortolozzi et al. 1999).

2.18 OTHER NONCANCER

Charles et al. (1996a) reported atrophy of adipose tissue among female rats receiving 2,4-D from the food for 52 weeks at 75 mg/kg/day; the reported NOAEL was 5 mg/kg/day.

2.19 CANCER

Cancers Affecting the Lymphatic System. Many studies, mostly population-based, case-control design, have examined possible relationships between phenoxy herbicides and cancers affecting the lymphatic system, especially NHL. However, only a relatively small number provided information regarding specific products such as 2,4-D.

NHL. Several studies reported increased risk of NHL associated with exposure to 2,4-D. In a population-based, case-control study in Kansas, ever-use of phenoxyacetic acids, mostly 2,4-D, was associated with an OR of 2.2 (95% CI 1.2–4.1) based on 24 cases and 78 controls (Hoar et al. 1986). Use of 2,4-D only was associated with an OR of 2.6 (95% CI 1.4–5.0) based on 21 cases and 60 controls. Stratification by duration of use, frequency of use, and latency did not show consistent dose-responses, but those with the highest frequency of use (≥21 days/year) had the highest OR of 7.6 (95% CI 1.8–32.3), although stratification resulted in small number of cases and controls.

A Canadian multicenter population-based, case-control study of 517 cases and 1,506 controls reported an increased OR for phenoxyherbicides and specifically for exposure to 2,4-D (OR 1.32; 95% CI 1.01–1.73) and mecoprop (MCPP), but not for other phenoxyherbicides (McDuffie et al. 2001). Stratification of the subjects by the number of days per year of exposure, however, did not show a dose-response relationship.

A nested case-control study embedded in a cohort of 139,000 ever-members of a farm worker labor union in California reported an increased risk of NHL and high use of 2,4-D (OR 3.80; 95% CI 1.85–7.81) (Mills et al. 2005). Prevalence of exposure, however, was low (only 15% for 2,4-D). The investigators noted also that since cases and controls were not interviewed in the study and only work histories were available, no information was collected for parameters that may be involved in the etiology of lymphohematopoietic cancers such as smoking history, diet, or medical history.

Hardell et al. (1994) also reported an increased risk of NHL with exposure to 2,4-D (OR 13; 95% CI 1.2–360) in a case-control study of 105 NHL cases and 335 controls based on only three cases and one control.

An Italian multicenter case-control study of 1,145 NHL cases and 1,232 controls found that overall use of 2,4-D was not associated with NHL (OR 0.9; 95% CI 0.5–1.8) (Miligi et al. 2006). However, an increased risk (OR 4.4; 95% CI 1.1–29.1) was reported among subjects who used 2,4-D but never used protective equipment, based on nine cases and three controls, suggesting that they actually had the highest exposure in this study (Miligi et al. 2006).

A meta-analysis that included 12 observational studies, 11 case-control studies, and 1 cohort study reported increased risk of NHL (summary relative risk 1.38; 95% CI 1.07–1.77) when comparing subjects who were ever exposed versus never exposed to 2,4-D (Smith et al. 2017). Analyses focusing on results from highly exposed groups resulted in a summary relative risk of 1.73 (95% CI 1.10–2.72).

Some studies have not found associations between NHL and agricultural exposure to 2,4-D (Cantor et al. 1992 [OR 1.2; 95% CI 0.9–1.6]; De Roos et al. 2003 [OR 0.8; 95% CI 0.6–1.1]; Lee et al. 2004b [OR 1.0; 95% CI 0.8–1.3]; Weisenburger 1990 [OR 1.5; 95% 0.9–2.4]; Woods et al. 1987 [OR 0.68; 95% CI 0.3–1.4]; Zahm et al. 1990 [OR 1.5; 95% CI 0.9–2.5]), residential use of 2,4-D (relative risk 0.89; 95% CI 0.49–1.59) (Hartge et al. 2005), exposure during manufacture (Burns et al. 2011; standardized incidence ratio [SIR] 1.36 [95% CI 0.74–2.29]), or in children from parents in Iowa participating in the AHS (Flower et al. 2004 [OR 1.18; 95% CI 0.29–4.70]). However, in the Burns et al. (2011) study, duration

and cumulative exposure to 2,4-D elevated the relative risk 2–3-fold. No associations were reported in a few studies that did not assess 2,4-D alone, but assessed the combination of 2,4-D and other phenoxy acids such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Eriksson et al. 2008 [OR 1.61; 95% CI 0.87–2.97]; Fontana et al. 1998 [OR 1.5; 95% CI 0.4–5.8]; Hardell and Eriksson 1999 [OR 1.3; 95% 0.7–2.3]), or 2,4-dichlorophenoxypropionic acid (2,4-DP) and 2,4-dichlorophenoxybutyric acid (2,4-DB) (Kogevinas et al. 1995 [OR 1.11; 95% CI 0.46–2.65]).

A meta-analysis that evaluated the weight of evidence of the epidemiological studies of NHL did not find evidence that would support an association between exposure to 2,4-D and NHL (rate ratio 0.97; 95% CI 0.77–1.22) (Goodman et al. 2015).

Cocco et al. (2013) found no increased risk of lymphoma overall, b-cell lymphoma, or chronic lymphocytic leukemia and occupational exposure to phenoxy acids overall or 2,4-D in particular, in the EPILYMPH case-control study that involved 2,348 incident lymphoma cases and 2,462 controls from six European countries.

Hodgkin's Disease. No association was found between 2,4-D and Hodgkin's disease in case-control studies conducted in the United States (Hoar et al. 1986 [OR 0.8; 95% CI 0.5–1.2]) and Canada (Pahwa et al. 2006 [OR 0.96; 95% 0.67–1.37]), or in a case-control study in Italy that assessed combined exposed of 2,4-D and 2,4,5-T (ORs were not estimated) (Fontana et al. 1998). Among children of parents in Iowa participating in the AHS, Hodgkin's disease cases diagnosed at 0–19 years of age were elevated (OR 2.56; 95% CI 1.06–6.14) based on five cases observed and 1.96 expected (Flower et al. 2004). However, analyses for specific products showed that neither maternal ever-use of 2,4-D (n=3,009, OR 0.72 [95% CI 0.32–1.60]) nor prenatal paternal use of 2,4-D (n=8,769, OR 1.29 [95% CI 0.71–2.35]) was associated with childhood cancer (Flower et al. 2004).

Soft Tissue Sarcoma (STS). In the population-based, case-control study of Hoar et al. (1986), exposure to 2,4-D was not associated with STS; an OR was not provided in the publication. A study of 357 cases and 1,506 controls residents of one of six Canadian provinces found no significant association between exposure to 2,4-D and STS (OR 0.97; 95% CI 0.71–1.32) (Pahwa et al. 2006). Restricting the analysis to 156 farm/dwelling/working cases and 673 controls yielded an OR of 0.96 (95% CI 0.63–1.47). STS was not elevated among 17,357 children (0–19 years of age) of parents in Iowa participating in the AHS (SIR 1.11; 95% [CI 0.38–3.62]) (Flower et al. 2004). Neither maternal ever-use of 2,4-D (n=3,009, OR 0.72 [95% CI 0.32–1.60]) nor prenatal paternal use of 2,4-D (n=8,769, OR 1.29 [95% CI 0.71–2.35])

was associated with childhood cancer (Flower et al. 2004). A case-control study nested in a large international cancer mortality study of workers exposed to phenoxy herbicides, chlorophenols, and dioxins (Kogevinas et al. 1997) reported an increased risk of STS (OR 5.72; 95% CI 1.14–28.65) for workers exposed to 2,4-D/2,4-DP/2,4-DB based on 9 cases and 24 controls (Kogevinas et al. 1995). Stratification by exposure category (none, low, medium, and high) resulted in dose-related associations; respective ORs were 4.55 (95% CI 0.61–53.4), 6.13 (95% CI 0.33–129.7), and 13.71 (95% CI 0.90–309).

Multiple Myeloma. No association has been found between agricultural exposure to 2,4-D and multiple myeloma in the few studies that examined this possibility (Brown et al. 1993 [OR 1.0; 95% CI 0.6–1.6]; Mills et al. 2005 [no data presented]; Pahwa et al. 2006 [OR 1.21; 95% CI 0.89–1.68]).

Leukemia. Risk of leukemia was reduced (OR 0.55; 95% CI 0.15–2.06) among males in association with 2,4-D in a study of lymphohematopoietic cancers among farmers in California (Mills et al. 2005). In females, the risk was elevated (OR 3.73; 95% CI 0.77–18.0), although the prevalence of exposure to 2,4-D was only 15% in this study. Childhood leukemia was not associated with exposure to 2,4-D in house dust (OR 0.96; 95% CI 0.85–1.08) in a study of 269 cases and 333 healthy controls (Metayer et al. 2013). No association was reported between agricultural exposure to 2,4-D and leukemia (OR 1.2; 95% CI 0.9–1.6) in a case-control study of men in Iowa and Minnesota (Morris et al. 1990). The SIR for leukemia was not elevated (SIR 0.91; 95% CI 0.47–1.75) among 17,357 children (0–19 years of age) from parents in Iowa participating in the AHS (Flower et al. 2004). Neither maternal ever-use of 2,4-D (n=3,009, OR 0.72 [95% CI 0.32–1.60]) nor prenatal paternal use of 2,4-D (n=8,769, OR 1.29 [95% CI 0.71–2.35]) was significantly associated with childhood cancer (Flower et al. 2004).

Gastrointestinal Cancer. A few studies provided information regarding 2,4-D and cancer to the gastrointestinal tract; the findings have been mixed. A small study of 57 colon cancer cases diagnosed in Kansas during 1976–1982 and 948 controls selected from the general population evaluated phenoxy herbicides use and risk of colon cancer (Hoar et al. 1985). The OR based on six cases that reported use of 2,4-D was 2.0 (95% CI 0.6–6.3), and two of the six cases also reported exposure to 2,4,5-T. The AHS reported an inverse association between ever/never exposed to 2,4-D by pesticide applicators and risk of colorectal cancer (OR 0.7; 95% CI 0.5–0.9) (Lee et al. 2007). The investigators noted that the lack of a monotonic dose-response pattern with lifetime exposure weakened the argument for a true protective relationship.

A population-based, case-control study of 170 men and women diagnosed with stomach cancer or 137 diagnosed with esophageal cancer and 502 controls in eastern Nebraska did not find an association with ever-use of 2,4-D (OR 0.8; 95% CI 0.4–1.3 for stomach cancer; OR 0.7; 95% CI 0.4–1.2 for esophageal cancer) (Lee et al. 2004a). However, an earlier case-control study of gastric adenocarcinoma among Swedish workers in various occupations that included 567 cases and 1,165 controls reported an elevated risk after exposure to herbicides (OR 1.56; 95% CI 1.13–2.15) (Ekström et al. 1999). Further analysis showed that the majority of the cases had been exposed to a combination of 2,4-D and 2,4,5-T and only two cases and no controls were exposed to 2,4-D only. The investigators noted that despite the positive association with exposure to phenoxyacetic acids, there was no clear relationship with cumulative duration of exposure. Risk of gastric cancer was increased in a nested case-control study of Hispanic farm workers in California exposed to high levels of herbicides, including 2,4-D, and pesticides (Mills and Yang 2007). The study involved 100 cases and 210 controls. Working in areas with high use of 2,4-D was associated with an increased risk of gastric cancer (OR 1.85; 95% CI 1.05–3.25). However, in multivariate-adjusted analysis using unexposed (zero pounds of use) as the referent category, there was no clear relationship between ORs and pounds of use. Moreover, gastric cancer risk was elevated only for pounds of use (1–14 pounds) in the second quartile, but not for the third (15–86 pounds) or the fourth quartile (86–1950 pounds). The investigators noted that not collecting information on dietary habits, family history, smoking, or alcohol consumption may have confounded the results.

Breast Cancer. A nested case-control study of newly diagnosed cases was conducted within a cohort of Hispanic women farm workers in California who were members of the United Farm Workers (UFW) of America (Mills and Yang 2005). The study included 128 cases diagnosed in 1988–2001 and 640 cancer-free controls. Cases included all newly diagnosed invasive breast cancers diagnosed among past or present members of the UFW between 1987 and 2001. The women were exposed to multiple pesticides. ORs for risk of breast cancer associated with pounds of use of all chemicals combined showed increases in multivariate-adjusted analyses. Adjusted ORs for breast cancer in quartiles of pesticide used were 1.00, 1.30 (95% CI 0.73–2.30), 1.23 (95% CI 0.67–2.27), and 1.41 (95% CI 0.66–3.02). Analyses for individual chemicals stratified by year of diagnosis (early, 1988–1994; late, 1995–2001) showed an elevated risk only for high 2,4-D use in late-diagnosed cases (OR 2.14; 95% CI 1.06–4.32). No elevated risks were found for low (OR 0.61; 95% CI 0.20–1.86) or high use (OR 0.62; 95% CI 0.23–1.69) and early-diagnosed cases or for low use and late-diagnosed cases (OR 2.16; 95% CI 0.95–4.93). In the much larger AHS analyses of 309 cases and 30,145 non-cases, rate ratios for 2,4-D calculated using Poisson regression and controlling for confounding factors were not elevated (Engel et al. 2005). The rate ratio for wife's 2,4-D use among all wives in the cohort was 0.8 (95% CI 0.6–1.1) and for husband's 2,4-D use

among wives who never used pesticides was 0.9 (95% CI 0.6–1.4). No associations were also found in analyses of farmer's wives by state (OR 0.7; 95% CI 0.6–1.0) or by menopausal status at enrollment (OR 1.2; 95% CI 0.7–2.1).

Cancer of the Nervous System. Two studies provide information regarding exposure to 2,4-D and cancer of the nervous system. A case-control study of residents (251 cases, 498 controls) from 66 counties in eastern Nebraska reported an association between increased risk of glioma and ever living or working on a farm and/or the duration of farming (OR 3.9; 95% CI 1.8–8.6) (Lee et al. 2005). However, an increased risk was found with 2,4-D exposure only when the questionnaire assessing demographics, smoking and alcohol consumption, diet, family history of cancer, complete residential and occupational history, medical history, and other factors was completed by proxies (in most cases, spouses or first-degree relatives) (OR 3.3; 95% CI 1.5–7.2), but not cases themselves (OR 0.6; 95% CI 0.2–1.6). A similar study of 798 histologically confirmed primary glioma cases and 1,175 population-based controls (non-metropolitan residents of four Midwest states) reported an inverse association between use of 2,4-D and incidence of glioma (OR 0.64; 95% CI 0.47–0.88) (Yiin et al. 2012). No association was found when proxy respondents were excluded (OR 0.76; 95% CI 0.51–1.11). The limited information available does not support an association between exposure to 2,4-D and glioma.

Prostate Cancer. A few studies provide information regarding exposure to 2,4-D and prostate cancer. No association was found in the AHS (p-value for trend=0.53, adjusted for age and family history of prostate cancer) (Alavanja et al. 2003). In a much smaller study of Dutch chlorophenoxy herbicide manufacture workers, the hazard ratios (HRs) were elevated in the two factories examined (HR 2.93; 95% CI 0.61–14.5; and HR 2.68; 95% CI 0.48–14.85) based on six cases among exposed workers and two among non-exposed workers in one factory and four cases among exposed workers and two among non-exposed workers in the other factory (Boers et al. 2010). A cohort study of 1,256 workers involved in the manufacture of 2,4-D in Michigan, reported a risk deficit of prostate cancers among the workers compared to Michigan white males (SIR 0.74; 95% CI 0.57– 0.94) (Burns et al. 2011). A case-control study of British Columbia farmers with potential exposure to multiple chemicals reported an elevated OR among those ever exposed to 2,4-D compared to an unexposed group (OR 2.72; 95% CI 1.12–6.57) (Band et al. 2011). Because there were only 12 exposed cases, dose-response analyses were not performed. Significant inconsistencies between studies preclude making any statement about the possibility of hazard.

Other Cancers. A study of 1,256 male workers employed in the manufacturing of 2,4-D in Midland, Michigan, reported an excess risk of "other respiratory" cancers compared to Michigan white males (SIR 3.79; 95% CI 1.22–8.84) (Burns et al. 2011). Five cases were observed compared to 1.32 expected. The "other respiratory" category excluded cancers of the larynx, bronchus, trachea, and lung and included nasal cavity, accessory sinuses, pleura, and other sites. Four of the five cases were mesotheliomas, which the investigators noted is strongly associated with exposure to asbestos; however, the workers' detailed job histories were not available due to confidentiality agreements.

In the AHS, no association was found between ever/never use of 2,4-D among herbicide applicators and spouses and pancreatic cancer (OR 0.9; 95% CI 0.5–1.5) (Andreotti et al. 2009). In addition, ORs for pancreatic cancer showed no relation to intensity-weighted exposure to 2,4-D among applicators. ORs for never use, low-intensity exposure, and high-intensity exposure were 1.0, 0.8 (95% CI 0.4–1.6), and 0.9 (95% CI 0.5–1.7), respectively.

A case-control study in dogs examined malignant lymphoma in residences where 2,4-D herbicides were applied onto lawns by the dog's owner and/or by commercial lawn care companies (Hayes et al. 1991). It seems reasonable to assume that the main route of exposure to the herbicides was by dermal contact, although it is likely that some ingestion also occurred by the dogs licking their paws. Dogs have been shown to absorb 2,4-D from lawns treated with products containing 2,4-D by measuring urinary levels of 2,4-D at various times after application of the product (Reynolds et al. 1994). The study by Hayes et al. (1991) included 491 dogs with lymphoma matched on age to 479 tumor control dogs and 466 non-tumor control dogs. Exposure was assessed by self-administered owner questionnaire and/or telephone interview. The investigators found a weak, but significant association between exposure to 2,4-D and risk of canine malignant lymphoma (OR 1.3; 95% CI, 1.04–1.67). However, an evaluation of the study by a scientific review panel found that numerous limitations in the study design, the most significant of which was exposure quantification, may have led Hayes et al. (1991) to erroneous conclusions (Carlo et al. 1992). The review panel noted, for example, that when separate analyses were conducted for commercial lawn treatment only, owner application of 2,4-D only, and both groups combined, none of the associations showed statistical significance. It was also noted that no clear dose-response trends were observed for number of commercial lawn chemical applications per year, but a positive increasing lymphoma risk trend was reported with annual number of owner applications of 2,4-D. In a later publication, Hayes et al. (1995) addressed many of the criticisms raised regarding the original study and clarified the conclusions by noting that the small reported association was in the range that could be easily explained by bias or confounding. They also stated that the results should be interpreted with caution

given the relatively low exposure levels and the problems related to exposure assessment. Kaneene and Miller (1999) reanalyzed the data using a more restrictive exposure definition and found that the numbers of dogs in the various exposure categories were substantially different than the numbers reached in the original study. Based on this redistribution of dogs, Kaneene and Miller (1999) could not confirm a dose-response relationship between 2,4-D use and malignant lymphoma.

The potential carcinogenicity of 2,4-D has been examined in bioassays in rats, mice, and dogs, and in these three species, 2,4-D yielded negative results. In these studies, rats were exposed up to 150 mg 2,4-D/kg/day in the diet for 2 years (Charles et al. 1996a; EPA 1996a; Hansen et al. 1971), mice were similarly exposed to up to 300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1987a), and dogs were exposed up to 10 mg 2,4-D/kg/day for 2 years (Hansen et al. 1971).

2,4-D was not a promoter of liver tumors in rats initiated with diethylnitrosamine for 5 weeks followed by administration of a diet containing 0.05% 2,4-D (approximately 25 mg 2,4-D/kg/day) for 23 weeks (Abdellatif et al. 1990).

Based on the information available, the EPA has assigned 2,4-D to carcinogenicity Group D, “not classifiable as to human carcinogenicity” (EPA 2005a). The Department of Health and Human Services has not classified 2,4-D as to its carcinogenicity (NTP 2014). IARC recently classified 2,4-D as possibly carcinogenic to humans (Group 2B) based on inadequate evidence in humans and limited evidence in experimental animals (IARC 2018; Loomis et al. 2015).

2.20 GENOTOXICITY

2,4-D has shown mixed results for genotoxic activity in *in vivo* and *in vitro* tests with organisms ranging from bacteria to humans. Tables 2-4 and 2-5 present a cross-section of some of the genotoxicity data that are available for 2,4-D in *in vivo* and *in vitro* test systems.

Table 2-4. Genotoxicity of 2,4-D *In Vivo*

Species (test system)	Endpoint	Results	Reference
Mammalian cells:			
Human (occupational exposure/buccal cells)	Telomere length	+	Hou et al. 2013
Human (occupational exposure/peripheral blood leukocytes)	Telomere length	+	Andreotti et al. 2015
Human (occupational exposure/lymphocyte culture, urine)	Chromosome aberrations	–	Garry et al. 2001
Human (occupational exposure; peripheral blood lymphocytes)	Chromosome aberrations	+	Kaioumova and Khabutdinova 1998
Human (occupational exposure; peripheral lymphocytes)	Chromosome aberrations	–	Mustonen et al. 1986
Human (occupational exposure/blood and urine)	Micronuclei frequency	–	Figgs et al. 2000
Human (occupational exposure/blood and urine)	Lymphocyte proliferation	+	Figgs et al. 2000
Human (occupational exposure/peripheral lymphocytes)	Micronuclei frequency	–	Holland et al. 2002
Mouse (host-mediated assay using *Salmonella typhimurium* and *Saccharomyces cerevisiae* as indicators)	Mutation (host-mediated assay)	–	Zetterberg et al. 1977[a]
Mouse (gestational exposure, fetal deaths)	Mutation; dominant lethal assay	–	Epstein et al. 1972
Mouse (bone marrow, spermatocyte cells)	Chromosome aberrations; sperm-head abnormalities	+	Amer and Aly 2001
Mouse (bone marrow)	Chromosome aberrations	+	Venkov et al. 2000
Mouse (bone marrow)	Chromosome aberrations	–	Yilmaz and Yuksel 2005
Mouse (bone marrow, spermatogonial cells)	Sister chromatid exchange	+	Madrigal-Bujaidar et al. 2001
Mouse (hair follicle)	Hair follicle nuclear aberration test	+	Schop et al. 1990
Mouse (bone marrow)	Micronucleus test	–	Schop et al. 1990
Mouse (bone marrow)	Micronucleus test	–	Charles et al. 1999b
Rat (blood lymphocytes)	Sister chromatid exchange	–	Linnainmaa 1984
Rat (lymphocytes)	Sister chromatid exchange	–	Mustonen et al. 1989
Rat (primary hepatocytes)	Unscheduled DNA synthesis	–	Charles et al. 1999a
Rat (primary hepatocytes, white blood cells)	DNA damage	–	Kitchin and Brown 1988
Chinese hamster (bone marrow cells)	Sister chromatid exchange	–	Linnainmaa 1984

Table 2-4. Genotoxicity of 2,4-D *In Vivo*

Species (test system)	Endpoint	Results	Reference
Non-mammalian cells:			
Drosophila melanogaster	Somatic mutation and recombination (wing spot test)	(+)	Kaya et al. 1999
D. melanogaster	Somatic mutation (wing spot test)	+	Tripathy et al. 1993
D. melanogaster	Sex-linked recessive mutation	+	Tripathy et al. 1993
D. melanogaster	Sex-linked recessive mutation	(+)	Magnusson et al. 1977
D. melanogaster	Sex-linked recessive mutation	+	Rasmuson and Svahlin 1978
D. melanogaster	Sex-linked recessive mutation	(+)	Vogel and Chandler 1974

[a]Study conducted using 2,4-D sodium salt.

– = negative result; + = positive result; (+) = weak positive result; 2,4-D = 2,4-dichlorophenoxyacetic acid; DNA = deoxyribonucleic acid

Table 2-5. Genotoxicity of 2,4-D *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100, TA1535, TA1535, TA1537, TA1538 (Ames test)	Gene mutation	−	−	Charles et al. 1999a
S. typhimurium TA 98, TA100	Gene mutation	−	−	Kubo et al. 2002
S. typhimurium TA97, TA98, TA100, TA102; *Escherichia coli*	Gene mutation/SOS chromatid test	−	−	Mersch-Sundermann et al. 1994
S. typhimurium	Mutation (host mediated assay)	No data	−	Styles 1973
S. typhimurium TA1530, TA1535, TA1531, TA1583	Mutation	No data	−	Zetterberg et al. 1977[a]
S. typhimurium. uvrB, rec; E. coli; Bacillus subtilis rec	DNA damage	No data	+	Garrett et al. 1986
E. coli	Mutation (modified SOS microplate assay)	No data	−	Venkat et al. 1995
Saccharomyces cerevisiae strain *D7ts1*	Mitotic gene conversion; reverse mutation	No data	+	Venkov et al. 2000

Table 2-5. Genotoxicity of 2,4-D *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
S. cerevisiae strains D4, D5	Mitotic gene conversion; recombination	No data	+	Zetterberg et al. 1977[a]
S. cerevisiae strain RAD 18	Mitotic gene conversion; recombination	No data	+	Zetterberg 1978
Eukaryotic organisms:				
Human fibroblasts	Mutation (colony forming ability, single strand breaks)	No data	−	Clausen et al. 1990
Human fibroblasts	Mutation (colony forming ability, single strand breaks)	No data	+	Clausen et al. 1990[b]
Human lymphocytes	Sister chromatid exchange	No data	+	Korte and Jalal 1982
Human lymphocytes (whole blood and leukocyte cultures)	Sister chromatid exchange	No data	+	Soloneski et al. 2007
Human lymphocytes	Sister chromatid exchange	No data	+	Turkula and Jalal 1985
Human lymphocytes	Chromosome aberrations	−	−	Mustonen et al. 1986
Human lymphoma and leukemia cells	Chromosome aberrations	No data	+	Venkov et al. 2000
Human lymphocytes	Chromosome aberrations; micronucleus assay	+	+	Zeljezic and Garaj-Vrhovac 2004
Human lymphocytes	DNA damage	No data	+	Sandal and Yilmaz 2011
Chinese hamster (V79 cell culture)	Mutation	No data	+	Ahmed et al. 1977
Chinese hamster (CHO cells)	Chromosome aberrations	+	−	Galloway et al. 1987
Chinese hamster (CHO cells)	Sister chromatid exchange	−	+	Galloway et al. 1987
Chinese hamster (CHO cells)	Sister chromatid exchange	No data	+	González et al. 2005
Chinese hamster (CHO cells)	Sister chromatid exchange	−	−	Linnainmaa 1984
Chinese hamster (CHO cells)	DNA damage	No data	+	González et al. 2005
Rat (primary hepatocytes)	Unscheduled DNA synthesis	No data	−	Charles et al. 1999a
Syrian Golden hamster embryo (SHE cells)	Morphological cell transformation, DNA damage	No data	+	Maire et al. 2007

Table 2-5. Genotoxicity of 2,4-D *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Syrian Golden hamster embryo (SHE cells)	Morphological cell transformation	No data	−	Mikalsen et al. 1990

[a]Study conducted using 2,4-D-sodium salt.
[b]Study conducted using 2,4-D-ammonium salt.

– = negative result; + = positive result; (+) = weakly positive; 2,4-D = 2,4-dichlorophenoxyacetic acid; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; SHE = Syrian hamster embryo

In vivo Exposure Studies. Results from human *in vivo* exposure genotoxicity studies are mixed (Table 2-4). The association of occupational pesticide use and relative telomere length (shorter telomere length has been associated with increased risk of cancer) was investigated in a cohort of 1,234 cancer-free white male pesticide applicators in the AHS (Hou et al. 2013). Exposure to 2,4-D, as assessed through questionnaires, was significantly associated with a decrease in relative telomere length (p=0.004) after adjusting for age at buccal cell collection, state of residence, license type, use of chewing tobacco, and total pesticide-application days. Similar results were reported in a subsequent evaluation of leukocyte DNA from 568 cancer-free males in the AHS (p-trend=0.001) (Andreotti et al. 2015). Increased chromosomal aberrations in lymphocytes were reported in another occupational study that investigated the effect of 2,4-D and 2,4,5-T production on plant workers (Kaioumova and Khabutdinova 1998). However, because of limitations including the relatively small sample of only 19 participants, the apparent lack of control for confounders, suspected mixed exposure, and no measures of exposure, the results should be interpreted with caution. Negative results for chromosomal aberrations or micronuclei were found in additional occupational exposure studies (Figgs et al. 2000; Garry et al. 2001; Holland et al. 2002; Mustonen et al. 1986). Lymphocyte proliferation (replicative index) and micronuclei frequency were determined in urine specimens of 12 herbicide spraying applicators (Figgs et al. 2000). Proliferation index increased in the exposed group after first exposure (p=0.016) and was also greater among the exposed than among a control group of non-applicators (p=0.046). Urinary 2,4-D was associated with increased proliferation index after spraying; however, no statistically significant dose-response was observed. In a study by Garry et al. (2001), urinary levels of 2,4-D were measured in 24 herbicide applicators and 15 minimally exposed controls. With this limited sample size, urinary 2,4-D levels were not statistically correlated with frequency of chromosomal aberrations, and the amount of 2,4-D applied had no direct effect on urinary 2,4-D. Garry et al. (2001) noted that due to the relatively small sample size, the results need to be interpreted with caution. In another small study of only 19 forest workers exposed to 2,4-D and 15 controls, there was no increase in the incidence of chromosomal aberrations in

the lymphocytes of herbicide sprayers, as measured in blood samples taken after the spraying season (Mustonen et al. 1986). There was also no association between urinary 2,4-D and length of exposure in this study (9–11 days). The small number of subjects studied limits the interpretation of the results of this study.

In animal studies, oral exposure to 2,4-D has been found to cause chromosomal aberrations, sister chromatid exchanges, and sperm-head abnormalities in somatic and germ cells of mice (Amer and Aly 2001; Madrigal-Bujaidar et al. 2001; Venkov et al. 2000). Acute dermal exposure to 2,4-D increased the incidence of hair follicle nuclear aberrations in mice (Schop et al. 1990). Other studies reported negative findings for chromosomal aberrations and sister chromatid exchanges (SCEs) in bone marrow and lymphocytes following oral exposure in mice, rats, and Chinese hamsters (Linnainmaa 1984; Mustonen et al. 1989; Yilmaz and Yuksel 2005). Negative results were also reported in a dominant lethal mutation assay in mice (Epstein et al. 1972), in two mice micronucleus tests (Charles et al. 1999b; Schop et al. 1990), and in assays for unscheduled DNA synthesis and DNA damage in primary hepatocytes and white blood cells of rats following oral exposures (Charles et al. 1999a; Kitchin and Brown 1988). A host-mediated assay in mice was negative using *Salmonella typhimurium and Saccharomyces cerevisiae* as indicators for mutation following oral exposure to 2,4-D sodium salt (Zetterberg et al. 1977). *In vivo* 2,4-D exposure produced weakly positive results in a wing spot test (Kaya et al. 1999) and in sex-linked recessive mutation tests (Magnusson et al. 1977; Rasmuson and Svahlin 1978; Vogel and Chandler 1974) in *Drosophila melanogaster*. Positive results in these two tests in *Drosophila* were reported by Tripathy et al. (1993). It was suggested that binding of 2,4-D to DNA may induce conformational changes to the DNA molecule (Ahmadi and Bakhshandeh 2009).

In vitro Exposure Studies. As summarized in Table 2-5, 2,4-D was not mutagenic in *S. typhimurium* or *Escherichia coli* (Charles et al. 1999a; Kubo et al. 2002; Mersch-Sundermann et al. 1994; Venkat et al. 1995) and 2,4-D sodium salt was not mutagenic in *S. typhimurium* (Zetterberg et al. 1977). Negative results were also reported in an *in vitro* host-mediated assay in mice using *S. typhimurium* as an indicator for 2,4-D mutation (Styles 1973). In contrast, positive results were reported for DNA damage in *S. typhimurium, E. coli,* and *Bacillus subtilis* (Garrett et al. 1986). 2,4-D and the 2,4-D sodium salt also produced positive results for mitotic gene conversion and reverse mutations in *S. cerevisiae* (Venkov et al. 2000; Zetterberg 1978; Zetterberg et al. 1977).

A number of human cell lines have been tested with 2,4-D giving positive results without metabolic activation, resulting in DNA damage, increased micronuclei, chromosomal aberrations, and SCEs (Korte

and Jalah 1982; Sandal and Yilmaz 2011; Soloneski et al. 2007; Turkula and Jalal 1985; Venkov et al. 2000; Zeljezic and Garaj-Vrhovac 2004). In one study, the 2,4-D ammonium salt produced mutations in human fibroblasts; however, results for 2,4-D acid were negative in the same assay (Clausen et al. 1990). Negative results were also reported for chromosomal aberrations following exposure of human lymphocytes to 2,4-D (Mustonen et al. 1986). In this study, positive results for chromosomal aberrations were reported in the absence of metabolic activation using commercial 2,4-D, but negative results were obtained when purified 2,4-D was tested. The investigators suggested the different results may have been due to the commercial formulation containing an unidentified chlorophenol contaminant.

In vitro studies with other mammalian cells have demonstrated somewhat mixed results. Positive results were reported for mutation, chromosomal aberrations, SCEs, DNA damage, and morphological cell transformation in Chinese and Syrian hamster cell lines (Ahmed et al. 1977; Galloway et al. 1987; González et al. 2005; Maire et al. 2007). Negative results were reported for SCEs in Chinese hamster ovary cells (Linnainmaa 1984), unscheduled DNA synthesis in primary rat hepatocytes (Charles et al. 1999a), and morphological cell transformation in Syrian golden hamster cells (Mikalsen et al. 1990).

In summary, results of genotoxicity studies in humans, animals, and *in vitro* studies are mainly negative and do not provide strong support to the genotoxicity of 2,4-D. IARC (2018) summarized available genotoxicity results for 2, 4-D and concluded that the evidence for the genotoxicity of 2,4-D is “weak.”

2.21 MECHANISMS OF ACTION

2.21.1 Pharmacokinetic Mechanisms

Absorption. No information was located regarding specific mechanisms of absorption of 2,4-D through the gastrointestinal tract or the skin. Because 2,4-D and the simple salts exist predominantly in the ionized form at physiological pH, it does not readily move across the lipid bilayer of the cellular membranes. Therefore, active transport mechanisms of the parent anion must be involved in its entry into cells. Active transport translocation of 2,4-D has been demonstrated, for example, in studies with the choroid plexus from rabbits (Kim and O’Tuama 1981; Kim et al. 1983; Pritchard 1980), with renal cortical tissue from rats and rabbits (Berndt and Koschier 1973), and Chinese hamster ovary cells (Bergesse and Balegno 1995).

Distribution. Studies in animals have shown that once absorbed, 2,4-D is transported highly bound to proteins in plasma, particularly albumin, which is subject to saturable protein binding with large exposures. Although protein binding has not been directly shown in humans, Fang and Lindstrom (1980) reported that 2,4-D could bind *in vitro* to serum albumin from eight different mammalian species, including human serum albumin. The binding affinities varied among species. Affinity seemed to be the highest for human albumin followed by rat, horse, ovine, porcine, chicken, and guinea pig. Others have also reported binding of 2,4-D to bovine serum albumin (Haque et al. 1975; Kolberg et al. 1973) and to human serum albumin *in vitro* (Rosso et al. 1998). The latter investigators noted that the binding affinity of 2,4-D to human serum albumin was several times higher than the affinity found for common pharmaceutical compounds. An *in vitro* study showed that incubation of rat plasma with 0.5 mg 2,4-D resulted in 28.3% of the 2,4-D unbound to protein, which increased to 42% as the concentrations of 2,4-D in the medium was increased to 1.0 mg, suggesting saturation of the binding process under the conditions of the study (Tyynelä et al. 1990). In an *in vivo* study in male and female rats, determination of plasma protein binding at concentrations of 2,4-D of 6, 24, and 48 µg/mL showed that approximately 97% of the chemical was bound in both sexes (Griffin et al. 1997a). Another study reported that plasma protein binding values for rats dosed 5 or 50 mg/kg 2,4-D were 95.5 and 92.9%, respectively (van Ravenzwaay et al. 2003). The respectively values for dogs were 95.7 and 87.6%.

Metabolism. As indicated in Section 3.1.3, Metabolism, 2,4-D undergoes limited metabolism in humans and animals. There is no evidence that the limited metabolism of 2,4-D leads to the formation of toxic metabolites.

Excretion. 2,4-D is eliminated from the body mainly by excretion in the urine. Because of extensive protein binding in plasma over a wide range of concentrations (Griffin et al. 1997a; van Ravenzwaay et al. 2003), protein-bound 2,4-D is not readily filtered at the glomerulus, but it is actively secreted into urine by means of an OAT1 carrier protein located on the basolateral membrane of the renal proximal tubules. The carrier is saturable and the point of saturation varies between animal species, sex within species, and lifestage. In rat studies that employed single oral dosing with 2,4-D, saturation has been demonstrated at approximately 50 mg/kg/day in males (Gorzinski et al. 1987; Saghir et al. 2006, 2013a) and 25 mg/kg/day in females (Saghir et al. 2013a). Adult male rats express higher levels of OAT1 than adult female rats (Buist et al. 2002). The higher expression of OAT1 in the male rats is consistent with higher systemic concentrations of 2,4-D resulting in a greater delivered 2,4-D dose to proximal tubule cells in males compared to females (Marty et al. 2013). This would explain the increased sensitivity of male rat proximal tubule cells to 2,4-D toxicity (Marty et al. 2013; Saghir et al. 2013a). The differential

expression of OAT1 in male and female rats is also consistent with females showing a significantly lower rate of elimination from plasma, lower volume of distribution, and higher elimination half-life than males (Griffin et al. 1997a; see also Section 3.1.2 for higher distribution to tissues in female rats compared with male rats). The OAT1 carrier was also found to be developmentally-regulated, as expression increased 4-fold between PNDs 5 and 35 in both males and female rats (Buist et al. 2002). However, expression of more OAT1 messenger ribonucleic acid (RNA) in males than in females by PND 40 (Buist et al. 2002) could explain the findings of Saghir et al. (2013a) of lower renal clearance in females than in male pups on PND 35.

Comparative studies have shown that dogs have a slower renal clearance for 2,4-D and other organic acids than other species, including humans (Timchalk 2004). Following oral doses of 1–5 mg 2,4-D/kg, plasma half-life in dogs ranged from 31 to 92–106 hours. In contrast, plasma half-lives ranged from 0.8 to 12 hours in mice, rats, pigs, calves, and humans. Comparative analyses using allometric equations to scale between species based on body weight showed that volume of distribution, renal clearance, and elimination half-life increased linearly with body weight in all species tested except dogs. Renal clearance in dogs was slower than in other species and was not adequately described by scaling. Elimination half-life in dogs also was higher than in other species and was not well described by scaling. Timchalk (2004) proposed that the sensitivity of the dog to the toxicity of 2,4-D is primarily due to the dog's relatively low capacity to excrete organic acids and suggested that dogs might not be a relevant species for evaluation of human health risk.

2.21.2 Mechanisms of Toxicity

There is a limited amount of information on the mechanisms of toxicity. Several general modes of action have been proposed based on information on other chlorophenoxy herbicides as well as studies evaluating oxidative stress associated with 2,4-D exposure. Additionally, several studies have evaluated possible mechanisms associated with alterations in neurochemicals.

Bradberry et al. (2000) reviewed the toxicity of chlorophenoxy herbicides and suggested three modes of action that could be potentially involved, namely, effects associated with the plasma membrane, interference in cellular metabolic pathways involving acetylcoenzyme A (AcCoA), and uncoupling of oxidative phosphorylation as a result of disruption of cellular membranes by the herbicide. The paragraph below provides a brief summary of the information from Bradberry's review; the reader is referred to references cited therein for more detailed information. Support for alterations to plasma membranes

comes from studies showing chlorophenoxy herbicide-induced alterations to model membrane systems, *in vitro* human erythrocyte cell membranes, disruption of cell membrane transport mechanisms, and inhibition of ion channels. Because chlorophenoxyacetic acids are able to form analogues of AcCoA *in vitro*, the potential exists for such analogues to disrupt cellular metabolic pathways involving AcCoA, such as the synthesis of the neurotransmitter acetylcholine. The formation of a choline ester that could act as a false transmitter would affect muscarinic and nicotinic synapses. Similarly affected could be other metabolic pathways of AcCoA resulting in interference with energy metabolism and the citric acid cycle. Studies *in vitro* have shown that phenoxy herbicides can uncouple oxidative phosphorylation, thus compromising a variety of cellular activities, including the ability of the cell to maintain ionic gradients across membranes, DNA and protein synthesis, and polymerization of microtubules and microfilaments leading to disruption of the cytoskeleton and altering cell shape. Some effects reported in humans following poisoning with phenoxy herbicide formulations and in animals following exposure to high doses of 2,4-D, such as damage to the blood-brain barrier, myotonia, and muscle twitching, are consistent with modes of actions described above.

The role of oxidative stress in the toxicity of 2,4-D has been explored in a few studies. Lerro et al. (2017) evaluated possible associations between urinary 2,4-D and selected urinary markers of oxidative stress (malondialdehyde [MDA], 8-hydroxy-2′-deoxyguanosine [8-OHdG], and 8-isoprostaglandin-$F_{2\alpha}$ [8-isoPGF]) among 30 Iowa corn farmers who applied pesticides occupationally and 10 controls. Exposure to 2,4-D was associated with elevated levels of 8-OHdG (β=0.066; 95% CI 0.008–0.124) and 8-isoPGF (β=0.088; 95% CI 0.004–0.172). Twenty-five-day-old offspring from rats exposed to 100 mg 2,4-D/kg/day from PND 9 to 25 showed significant increases in reactive oxygen species in the midbrain, striatum, and prefrontal cortex (Ferri et al. 2007). Less marked effects were reported in the hippocampus and no effects were noted in the hypothalamus. The different sensitivities between tissues was attributed by the investigators to different enzyme activities profiles, different levels of copper or iron ions, which are involved in oxidative stress generation, and/or the high flux of reactive oxygen species generated during neurochemical reactions. Indicators of oxidative stress were increased and antioxidant enzyme levels were reduced in the liver from rats and their pups following maternal exposure to 126 mg 2,4-D/kg/day from GD 14 to PND 14 (Troudi et al. 2012a). Increased oxidative stress, decreased antioxidant enzyme activity, and decreased levels of non-enzymatic antioxidant levels were seen in hemolysate and bone homogenates from offspring from rats dosed in the same manner (Troudi et al. 2012b). In yet another study, exposure of rats to 100 mg 2,4-D/kg/day on GDs 1–19 resulted in increased levels of malondialdehyde and reduced levels of antioxidant enzymes in the liver of dams and fetuses on GD 20; this was partially prevented by treatment of the dams with vitamin E (Mazhar et al. 2014).

Treatment of mice with 2,4-D in drinking water in doses of up to 100 mg 2,4-D/kg/day on GDs 0–9 did not induce signs of oxidative stress in maternal blood collected on GD 9 (Dinamarca et al. 2007).

A series of studies have been conducted by Evangelista de Duffard and coworkers examining neurochemical alterations in the brain from both adult rats and from offspring of dams exposed to 2,4-D during gestation and lactation. In some of these studies, rats were treated orally and in other studies, rats were dosed by intraperitoneal injection. Doses tested were ≥50 mg 2,4-D/kg/day. A brief summary of the findings follows.

Exposure to 2,4-D induced behavioral alterations in adult rats through serotonergic and dopaminergic mechanisms and interacted with amphetamine to induce a 'Serotonergic Syndrome' (a behavioral response induced in rodents by stimulation of serotonergic receptors) and additional dopaminergic stimulation; female rats appeared to be more affected than males (Evangelista de Duffard et al. 1995). The behavioral alterations in the presence of amphetamine appeared to be due to increased content of serotonin and dopamine in the substantia nigra, ventral tegmental area, nucleus accumbens, striatum, midbrain, and cerebellum (Bortolozzi et al. 1998). The investigators hypothesized that the increase in serotonin and dopamine in amphetamine-challenged rats could occur because the neurons remain hyperactive after 2,4-D treatment and amphetamine initiates an immediate release of serotonin and dopamine to the extracellular fluid (Bortolozzi et al. 1998).

In another study, the investigators showed that rat offspring exposed to 2,4-D through the placenta and the dams' milk followed by direct exposure showed neurobehavioral alterations that seemed to disappear as adults (Bortolozzi et al. 1999). In offspring exposed during gestation and lactation, 2,4-D also induced neurobehavioral alterations, some of which could be unmasked with pharmacological challenges (Bortolozzi et al. 1999). Dopamine D_2 receptors appeared to be implicated in the stimulant-induced behavioral sensitization (Bortolozzi et al. 2002). Further studies showed that in 2,4-D-exposed rats, dopamine D2 receptors were increased in density by about 40% in the striatum of rats exposed perinatally and then directly, but were also increased in the prefrontal cortex and cerebellum; females appeared more affected than males (Bortolozzi et al. 2004).

Studies also showed that exposure to 2,4-D *in utero* and through lactation produced a permanent increase in serotonergic neurons in all mesencephalic nuclei from offspring (Garcia et al. 2001). However, perinatal exposure followed by direct exposure resulted in only an increase in serotonergic neurons from the dorsal raphe nuclei, suggesting an adaptable response of serotonergic neurons in the median raphe

nucleus. In addition, the immunocytochemically-detected glial reaction was different for the two exposure designs. Further studies showed that levels of dopamine and dopamine metabolites were decreased in the right side with respect to the left side in the striatum and nucleus accumbens in rats exposed perinatally and then directly, which seemed to provide support for the rotation motion exhibited by these rats (Bortolozzi et al. 2003). In subsequent studies of rat pups exposed via lactation, the investigators suggested that 2,4-D decreased tyrosine hydroxylase (enzyme that catalyzes the rate limiting step in this synthesis of catecholamines) immunoreactivity in the substantia nigra and ventral segmental area in the midbrain resulting in a significant diminution in serotonin fiber density (Garcia et al. 2004, 2006).

Injection of 2,4-D into various brain areas of adult rats showed different behavioral alterations possibly by exerting different types of interactions with the monoaminergic system depending on the location of the 2,4-D injection and dose and time period post-injection. Toxicity of 2,4-D appeared to differ between monoaminergic terminals, axonal fibers, and cell bodies (Bortolozzi et al. 2001).

Other studies from the same group of investigators showed that behavioral alterations could be related to induction of reactive gliosis in the hippocampus and cerebellum from rat pups exposed through maternal milk (Brusco et al. 1997), altering myelin deposition and ganglioside pattern in various brain areas from rat pups treated directly with 2,4-D (Rosso et al. 1997, 2000a) or through maternal milk (Duffard et al. 1996). They also showed that 2,4-D can disrupt microtubule assembly and disorganize the Golgi apparatus in cultured cerebellar granule cells *in vitro*, possibly leading to decreased neurite outgrowth (Rosso et al. 2000b).